Phytochemical Screening and Biological Activities of *Combretum adenogonium* Leaves Extracts

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Abstract

Combretum adenogonium is beniniens pharmacopoeia medicinal plant used for the treatment of various diseases. This work aims to study the phytochemical and assess some biological activities of *C. adenogonium* leaves extracts. The phytochemical analysis (qualitative et quantitative) was conducted by standard analytical chemistry method. Antioxydant activity was evaluated by the DPPH method. Antibacterial activity was evaluated *in vitro* with 10 references strains , 10 *Staphylococcus* strains isolated from

meat products and 10 clinical *Staphylococcus aureus* strains isolated from Buruli ulcer lesions and pus. The Minimum Inhibitory Concentration (MIC) and Bactericidal (CMB) were determined by macrodilution method. The extracts cytotoxic effect was evaluated with *Artemia salina* larvae. The phytochemical screening revealed the presence of flavonoids, tannins, anthocyanins, saponin and triterpenoids. The methanolique extract present the higest content (450.66 ± 0.004 µg EAG/mg) of total polyphenolic compound. The results showed the good antioxidant activity. The inhibitory diameter zone vary ($p < 0.001$) according to the strains. The largest medium inhibitory diameter (21.85 ± 0.17 mm) was obtained with the ethanolic extract, while the lowest (6.00 ±0.00 mm) were recorded with water-ethanol extract. The variation between CMI and CMB is not significant ($p > 0.05$). The higher LD_{50} (27.66 mg/ml) was recorded with methanol extract. The results of this study confirm some use of *C. Adenogonium* extracts. But; these extracts must be use with moderation.

Keywords: *C. adenogonium*, phytochemistry, biological activities, cytotoxicity, Benin

Introduction

The use of natural products, known as sources of antioxidants, is becoming more and more rare, and many diseases related to the phenomenon of oxidative stress have emerged. The latter is involved in various pathologies such as cardiovascular disease, cancers, diabetes, dementia (Alzheimer, Parkinson) and the aging process (Aruoma, 2003). Thus, human health became precarious. In this situation, the cell can't control the excessive presence of toxic oxygen radicals in our body, made by human food practice change, pollution, more and more growing in our world (Defraigne and pass, 2007).

Scientific advances were able to show that natural products like plants can be huge sources of antioxidants and allow to counteract the pathological disorders associated with oxidative stress in the body. Cells use many antioxidant strategies and consume a lot of energy to control their level of reactive species of oxygen (Bouabdallah, 2014). Excess of reactive species of oxygen-related diseases are very common and there is a particular interest, through the search for new sources of antioxidant molecules. This interest is all the more remarkable in regard to the infectious diseases. They are a major public health problem in the world and particularly in Africa. They are responsible for 45 percent of deaths in countries with low income and almost a premature mortality in the world (Konan et al., 2014). Among infectious diseases, those caused by bacterial infections account for 70% of cases of mortality (Walsh, 2003). However, because of uncontrolled use,

inadequate and misuse of antibiotics in human and veterinary health, now there is the emergence of Multiresistant bacteria (Savard, 2003). The emergence and dissemination of these Multiresistant bacteria in human populations became public health issues of serious concern (Lozniewski and Rabaud 2010). The progression of the multi-resistance and the absence of real prospects for the discovery of new antibiotics in the years to come, led us to study the effectiveness of medicinal plants in order to isolate the active principles.

Traditional medicine remains the main use of a large majority of the population to solve their health problems (Kone, 2009). The who estimated in 2007 that about 80% of the population of developing countries have recourse to traditional medicine for their primary health need. Today, everywhere in Africa, the problem of costs and / or access to conventional medicines in rural areas, people turn more and more to the traditional, inexpensive and sometimes more effective medicine according to the popular belief. In Benin, many plants are used by the Beninese people to combat the diseases. Adjanohoun and *al*. (1989) have identified nearly 501 species used in traditional medicines in Benin. Also, Sinsin and al. (2000) identified 814 species belonging to 130 botanical families with medicinal virtues. *C. adenogonium* is one of the 140 species of the genus Combretum, native to tropical Africa and belonging to the family of the Combretacees. It is known for its digestive, insecticidal, antifungal, antiproliferative and cytotoxic properties in West Africa (Maregesi et al., 2008).

Given the complications and difficulties in the treatment of diseases and endogenous knowledge acquired from the traditional healers on *C. adenogonium* in the treatment of various ailments, several scientists have worked on this plant to determine her principle active and its mode of action in order to confirm or disprove these endogenous knowledge on the one hand and relieve patients on health and financial other hand. Moreover, few data are available on Antimicrobial, antioxidant, cytotoxic activity and the mechanism of cellular action of this plant. This work aims to study the phytochemistry and evaluate some biological activity of *C. adenogonium* leaves extracts.

Material and methods

Biological material

It consists on the one hand of the extracts obtained with *Combretum adenogonium* leaves. Moreover, The tested microrganisms include both bacteria (Gram positive and Gram negative) and yeast. These microorganisms are composed of:

Ten references strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis*T22695,

Pseudomonas aeruginosa ATCC 27853, *Proteus mirabilus* A24974, *Micrococcus luteus* ATCC10240, *Proteus vulgaris* A25015, *Streptococcus oralis*, *Enterococcus foecalis* ATCC 29212 and *Candida albicans* MHMR).

Ten *Staphylococcus* species (*S. hominis, S. chromogenes, S. sciuri, S. simulans S. xylosus, S. cohnii, S. aureus, S. auricularis, S. capitis* and *S. coprae*) isolated from meat products in Ivory Coast by Attien et al. (2013) and stored in the Laboratory of Biology and Molecular Typing in Microbiology (University of Abomey-Calavi, Benin).

Collection of plants material

The *C. adenogonium* leaves were collected in Tanguiéta and Kalale in March 2016. After harvest, the leaves were washed with water and dried in the laboratory (25 ± 2 ^{o}C) for 15 days. They were then powdered using a grinder Retsch type SM 2000/1430/pUm/Smfand. The powders were used for different extractions.

Phytochemical screening

The qualitative phytochemical screening a chemical analysis is performed based on colouring or precipitation reactions. It is made directly on the powder of the *C. adenogonium* leaves according to Houghton and Raman (1998).

Preparation of aqueous, ethanol ethyl acetate, hexanic and methanolique extracts

The extract were obtained according to the method describe by Dah-Nouvlessounon et *al*. (2015) and N'Guessan et *al*. (2007). Briefly, the leaf powder (50 g), was macerated into 500 ml of each solvent (water, ethanol, ethyl acetate and dichloromethane**)** under agitator for 72 h at room temperature. The each homogenate was then filtered two times on absorbent cotton and once on Whatman N°1 paper (125 mm ø, Cat No 1001 125). For the aqueous extract the filtrate was dried in the oven at 40°C. While for the organic (ethanol, ethyl acetate and dichloromethan) extract the filtrate were concentrated in vacuum using a rotary evaporator (Heidolph Instruments GmbH & Co. KG No: 591-28000-00-1) to obtain the extract. All extracts were stored in labeled sterile bottles and kept at -20°C until further use.

Determination of extracts yield

The performance of the crude extract is defined as the ratio between the mass of the dry extract obtained and the mass processed plant material (Harborne, 1998). This yield is calculated using the following formula:

$$R(\%) = \frac{\text{Extract weight}}{\text{Powder weight}} \times 100$$

Determination of the phenolic compounds

Total polyphenols were determined by using adapted Folin-Ciocalteu method as described by Singleton et al. (1999). Briefly, the methanolic solution of each extract (10 mg/ml) was diluted to 1/100 with distilled water. 125µl of this solution was then mixed with 625 µl of Folin-Ciocalteu reagent (10%). After 5 min, 500 µl of aqueous sodium carbonate (Na_2CO_3 ; 75 g/l) were added. After 2h of incubation in dark at the room temperature, the absorbances were measured in triplicate at 760 nm against a blank (0.5 ml Folin-Ciocalteu and 1 ml of Na_2CO_3) with spectrophotometer (BIOMATES 3S). The total phenolics content was determined using the plotted standard calibration curve with gallic acid (0-10 mg/ml).

Total flavonoïds content

The total flavonoids were determined according to the adapted method of Kim et *al*. (2003) Aliquots were prepared by mixing of 500 µl of extract solution (10 mg/ml), 500 µl of méthanolic solution of $AlCl_3$ (2%) and 3 ml of methanol. After 10 min of incubation, the absorbances were measured at 415 nm against a blank (500 µl d'$AlCl_3$ and 3.5 ml of méthanol) by using spectrophometer. A standard calibration curve ($R^2 = 1$) was plotted using rutine (0-150 µg/ml). The data obtained were the means of three determinations. The amounts of flavonoids in the bark and leave extracts were expressed as µg of rutine equivalents (RE)/mg of extract.

DPPH radical-scavenging essay

This test was evaluated using the procedure described by Lamian-Médard et al. (2008) it is based on the reduction of the stable free radical DPPH in the presence of a radical donor H^+ to the 517 nm wavelength. Briefly, a mass of 1 mg of the extract was dissolved in 1 ml of methanol (C = 1 mg/ml). The resulting solution is then diluted to $1/100^{th}$. To prepare the solution of DPPH, 4 mg of powder of DPPH was dissolved in 10 ml of methanol to have a mass concentration of 0.4 mg/ml. Then 1.5 ml of the extract (diluted) were mixed with 3 ml of DPPH methanolique solution . The mixture was incubated for 15 minutes in darkness at room temperature; the absorbance is read at 517 nm against a constituted white DPPH and methanol. The extracts anti-radical activity was determined using a calibration curve established with vitamin C (0 - 10 mg/ml). Each test was conducted in triplicatas.

Anti microbial activity assessment methods

Sensitivity test

It was done according to the disc method inspired from the one described by Bauer et al. (1966). Brieflt, 1 ml of pre-culture of 18-24 h (10^6

UFC/ml) enabled planting a box of Petri dishes containing agar Mueller Hinton by flood. After seeding, the sterile Whatman paper discs (5 mm de diameter) were deposited with sterile pince. These discs have been carefully impregnated with 30 µl of plant extract (20 mg/ml . The dishes were kept for 15-30 min at room temperature before incubation at 37°C. The inhibition zones diameters were measured after 24 to 48 hours (Adesokan et al., 2007) using a ruler graduated. For each extract, the experiment was performed in duplicate.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC has been determined by macrodilution method (Delarras, 1998) with Visual assessment of the growth of microorganisms. Briefly, nine concentrations (10 000, 5 000, 2 500, 1 250, 625, 312.5, 156.25, 78.12 and 39.06 µg/ml) was performed in screw tube. To 1 ml of the above concentrations was added 1 ml of the bacteria inoculum (10^6 UFC/ml). After 24 h of incubation turbidity tubes was examined relative to the control tube containing distilled water and the inoculum (10^6 UFC/ml).

Determination of the Minimum Bactericidal Concentration (MBC)

The MBC was determined by solid medium culture of all of the tubes from the MIC to high concentrations. These dishes were incubated at 37 ° C for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC (Farshori et al., 2013)

Cytotoxicity activity of *C. adenogonium* extracts

This test was evaluated according to the adaptation of the method described by Kawsar et al., (2008). The tests were conducted on larvae obtained by outbreak of 10 mg of *Artemia salina* (ARTEMIO JBL GmbH D to 67141 Neuhofem) under continuous stirring in 1 l of seawater for 72 hours. A 1 ml of each dilution in geometric series of reason ½ extract prepared from a stock solution of 20 mg/ml, in sea water, 1 ml of seawater containing 16 larvae has been added. The number of surviving larvae was counted after 24 h of incubation. The DL_{50} has been determined from the regression line obtained from the representative curve in the number of surviving larvae based on the concentration of extracts. Each test was performed in duplicate.

Data treatment and analysis

The spreadsheet Microsoft Excel version 2010 has been used for the capture and encoding the data. Minitab (version 17) software was used for the variance analysis (ANOVA). Finally a structuring of the medium was made which allowed us to compare and identify the excerpt (s) most active

on the various parameters through Student Newman and Keuls (SNK) test on the threshold of 5% of significance.

Results

The extraction performance

Table 1 shows the yield of the extracts. Methanol and ethanol gave the highest yield respectively 12.8% and 12.49%. While the lowest yield (0.5%) was recorded with the hexanique extract. The extraction of each solvent capacity analysis shows that the largest number of compound may have biological activities is extracted by methanol.

Table 1: Extracts yield from *C. adenogonium* leave according to the solvent

Types of extracts	Aqueous	water-ethanol	Ethanol	Hexanique	Methanol	Ethyl acetate
Powder mass (g)	200	100	200	40	25	40
Extracts mass (g)	12.67	6.6	24.98	0.2	3.2	1.4
Yield (%)	6.35	6.6	12.49	0.5	12.8	3.5

Phytochemical screening

The phytochemical screening shows that *C. adenogonium* leaves powder is rich in natural chemicals such as flavonoids, tannins, anthocyanins, leucoanthocyanes and the triterpenoids. While some secondary metabolite such as alkaloids, steroids, cyanogeniques derivatives and O-glycosides were absent (Table 2).

Table 2: Phytochemicals contituents of *C. adenogonium* leaves powder .

Chemical compound	leaf
Alkaloids	–
Tannins	+++
Saponins (MI)	+++ (167)
Anthocyanins	+++
Leuco-anthocyanins	+++
Flavonoids	+++
Steroids	–
Triterpenes	+++
Coumarin	+++
Reducing compound	+++
Glycosids	+
O-Hétérosids	-
Cyanogenic derivate	–

+: Low color intensity, +++: high color intensity - : absence of coloration; MI : index moss

Antioxidant capacities (DPPH radical scavenging)

Equivalent Ascorbic acid content in each extract are presented in figure 2. Ethyl acetate extract shows high value (6.5±0.11 mM) while the lowest (18.2±0.01 mM) was recorded with aqueous extract.

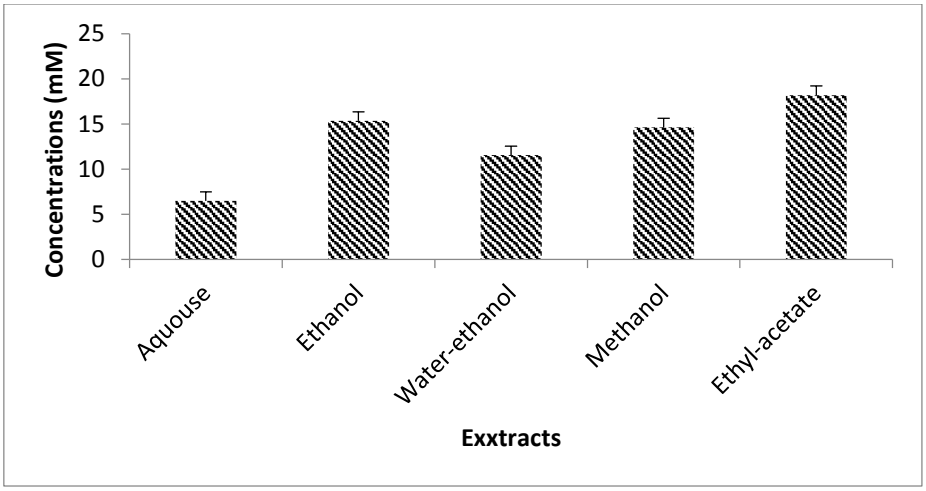

Figure 2: Equivalent Ascorbic acid content of *C. adenogonium* extracts

Total polyphenols and flavonïdes contents

Concentrations of total polyphenols and flavonoïds are determined from the calibration right ($y = 0.0787 x – 0.0379$; $R^2 = 0.996$) drawn using as standard the Gallic acid and rutin respectively. The results are presented in table 2. The methanolique extract has the highest (450.66 ± 0.004 µg EAG/mg of extract) total polyphenols contents while ethyl acetate extract have the lowest (97.93 ± 0.005 µg EAG/mg of extract).

Table 2: Total polyphenols and flavonoïds content of *C. adenogonium* extracts

Extracts	Total Polyphenols (µg EAG/mg)	Total Flavonoïds (µg ER/mg)
Aqueous	204.25 ± 0.001	492.25 ± 2.52
Ethanol	164.00 ± 0.005	615.31 ± 4.91
Water-ethanol	168.88 ± 0.004	485.42 ± 0.93
Methanol	450.66 ± 0.004	537.14 ± 2.24
Ethyl acetate	97.93 ± 0.005	662.20 ± 2.52

EAG: Equivalent Gallic acid; ER: Equivalent rutin

Antibacterial activity of *C. adenogonium* extracts on reference strains

Table 3 shows antibacterial profile of *C. adenogonium* leaves extracts with the references strains. The analysis of these results shows that only the hexanique (20 mg/ml) did not inhibit the growth of these strains. For this purpose, only hexane extract has not taken into consideration for the antibacterial tests. all of the reference strains are sensitive to different extracts except the hexanique extract. The aqueous extract of the leaves has no effect on the strains tested at the dose of 20 mg/ml but at a dose of 50 mg/ml, strains such as *P. vulgaris, S. oralis, E. foecalis, E. coli* are resistant to its activity. At the dose of 20 mg/ml, only hexanique and aqueous extracts.

Table 3: Antibacterial activity of *C. adenogonium* extracts with reference strains

Reference strains	EA	EE	EHE	EM	EH	EAT
S. aureus ATCC 29213	+	+	+	+	-	+
P. aeruginosa ATCC 27853	+	+	+	+	-	+
P. mirabilis A24974	+	+	+	+	-	+
M. luteus ATCC10240	+	+	+	+	-	+
S. epidermidis T22695	+	+	+	+	-	+
P. vulgaris A25015	-	+	+	+	-	+
S. oralis	-	+	+	+	-	+
E. foecalis ATCC 29212	-	+	+	+	-	+
E. coli ATCC 25922	-	+	+	+	-	+
C. albicans MHMR	+	+	+	+	-	+

+: inhibiton; -: no inhibition; EA: Aqueous extract; EE: Ethanol Extract; EHE: Water-Ethanol extract; EM: Methanol extract; EH: Hexane Extract; EAT: Ethyl acetate extract.

The extracts inhibitory diameter zone with the reference strains

The inhibitory diameter zone of the sensitive strains vary ($p < 0.001$) according to the extracts (Figure 3). For the aqueous extract (Figure 3A) the larger diameter (12.5 mm) was obtained with *P. aeruginosa* and the lower diameter (8.5 mm) was obtained with *S. aureus*. The ethanol extract largest diameter (22 mm) was obtained on *S. oralis* , and the lowest (9 mm) with *P. vulgaris* (Figure 3B). *C. albicans* is the reference strains who present the largest inhibitory zone (13 mm) with the ethyl acetate extract (Figure 3C). For the water-ethanol (D), *P. vulgaris* strain shows the high sensitibility (13.9 mm) to the extracts, while *E. coli* shows more resistance (6 mm) against this extracts.

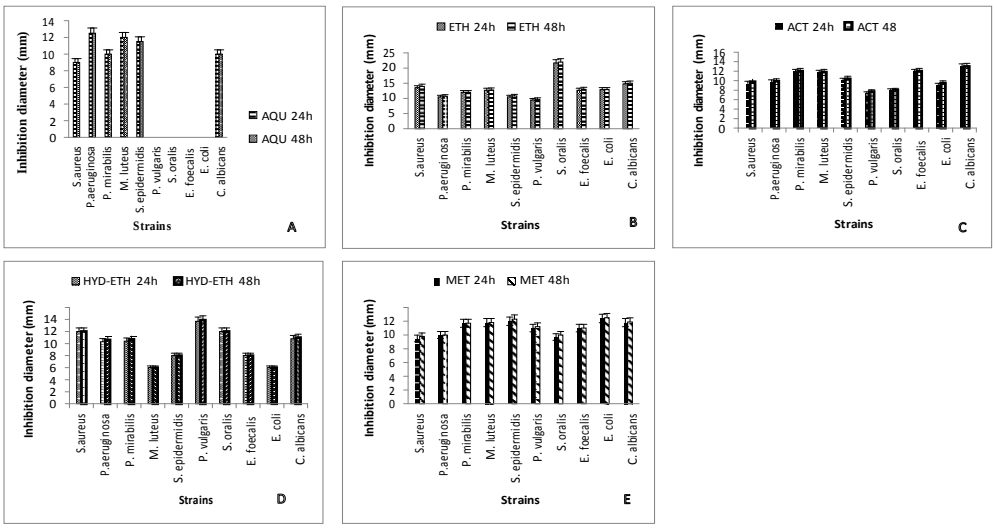

Figure 3: Diameters of inhibition of aqueouse extracts (A) ethanol (B), acetic (C) water ethanol (D) and methanolic (E) *C. adenogonium* one the strains of reference (24 h et 48 h)

Antibacterial activity of *C. adenogonium* leave extracts against the meat isolated *Staphylococcus* strains

Among the differents extract, only the aqueous extract have the lower rate (40%) of the tested strains inhibition. Figure 5 show that the inhibitory zone diameter vary according to the strains and the extracts. The Analysis of variance show that these diameter variation were not significant ($p > 0.05$) in the time (24 h and 48 h). On the contrary, the difference of the effect between the extracts type was very highly significant ($p < 0.001$).The greatest inhibitory diameters (17.2 mm) were obtained with the ethanol extract against the strain *S. coprae*. It was following to the methanol extract (17 mm, with *S. xylosus*). The low inhibitory diameter (7 mm) was registred with aqueous extract (*S. conhii*). The aqueous extract had no effect on the meat isolated *Staphylococcus* strains such as: *S. hominis, S. chromogenes, S. xylosus, S. aureus, S. auricularis* and *S. capitis.*

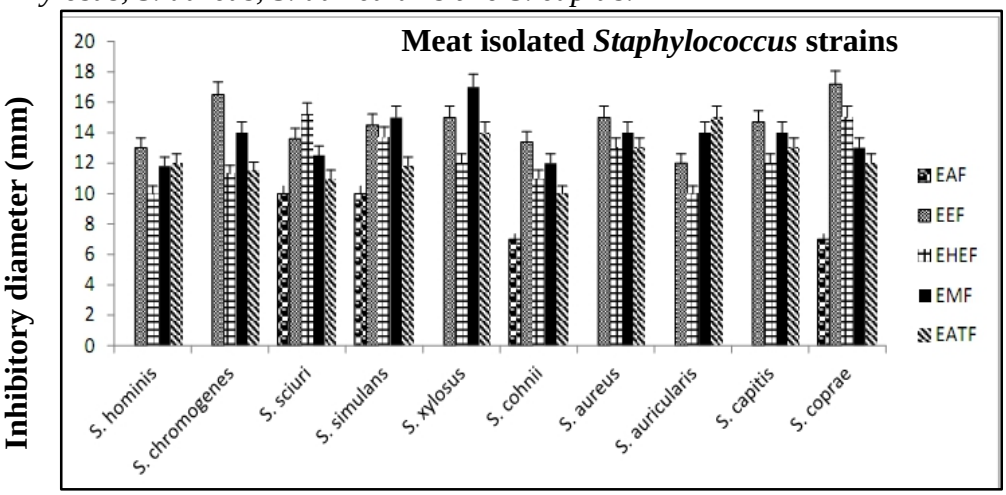

Figure 5: Diameter of inhibition: EAF: Aquoues extract, EEF: Ethanol extract; EHEF:Water-ethanol extract; EMF:Methanol extract; EATF: Ethyl-acetate extract.

Minimum Inhibitory Concentrations of *C adenogonium* extracts on reference strains

Minimal Inhibitory Concentrations of the extracts with reference strains are given in table 7. This table analysis shows that all extract have the MIC lower than the starting concentration (20 mg/ml). Smallest MIC (1.56 mg/ml) were obtained at least on a reference strains with all the extracts except the water-ethanol extract of which smallest MIC was 2.5 mg/ml. In addition, among the sensitive strains, only *S. epidermidis* and *E. coli* strains were recorded smallest MIC (1.56 mg/ml) of two types of extract at the same time. Nevertheless, it is necessary to notice that only the methanol extract had at the same time, smallest MIC with three reference strains (*S. epidermidis, E. coli* and *C. albicans*).

Table 7: Minimum Inhibitory Concentration (mg/ml) of the extracts on the studied reference strains

Reference strains	MIC (mg/ml)				
	EA	EE	EHE	EM	EAT
S. aureus ATCC 29213	6.25	5	2.5	2.5	5
P. aeruginosa ATCC 27853	12.5	2.5	10	10	2.5
P. mirabilis A24974	1,562	2.5	5	5	10
M. luteus ATCC10240	3.125	2.5	10	10	5
S. epidermidis T22695	3.125	2.5	5	1.25	1.25
P. vulgaris A25015	-	10	10	5	1.25
S. oralis	-	5	2.5	5	2.5
E. foecalis ATCC 29212	-	5	2.5	2.5	2.5
E. coli ATCC 25922	-	1.25	2.5	1.25	5
C. albicans MHMR	12.5	2.5	5	1.25	10

EA: aqueous extract; EE: Ethanolic extract; EHE: Water-ethanol extract; EM: Methanolic extract; EH: Henanic Extract; EAT: Ethyl acetate extract

Minimum Bactericidal Concentration (mg/ml) of the extracts on the reference strains

Minimum Bactericidal Concentrations vary depending on the strains and types of extracts (table 8). The analysis of the table shows that, except the MBC (10 mg/ml) obtained with the water-ethanol extract on *P. vulgaris* strain, no extract presented a MBC equal to the MIC although the strain tested. The lowest MBC (5 mg/ml) was obtained with the ethanol extract on *E. coli* strain. The ethanol extract proved to be most active while the aqueous extracts, methanolic and ethyl acetate extract presented the concentrations superior at those of starting (MBC > 20 mg/ml).

Table 8: Minimum Bactericidal Concentrations of extracts with reference strains

SRF	Minimum Bactericidal Concentration (mg/ml)				
	EA	EA	EHE	EM	EAT
***S. aureus* ATCC 29213**	> 20	20	> 20	> 20	> 20
***P. aeruginosa* ATCC 27853**	> 20	20	> 20	> 20	> 20
***P. mirabilis* A24974**	> 20	> 20	10	> 20	> 20
***M. luteus* ATCC10240**	> 20	> 20	> 20	> 20	> 20
***S. epidermidis* T22695**	> 20	> 20	> 20	> 20	> 20
***P. vulgaris* A25015**	-	> 20	10	> 20	> 20
S. oralis	-	20	> 20	> 20	> 20
***E. foecalis* ATCC 29212**	-	10	10	> 20	> 20
***E. coli* ATCC 25922**	-	5	> 20	> 20	> 20
***C. albicans* MHMR**	> 20	20	> 20	> 20	> 20

EA: aqueous extract; EE: Extract Ethanolique; EHE: Water-ethanol extract; EM: Methanolique extract; EH: Henanique extract; EAT: Ethyl acetate extract;

Cytotoxic activity of *C. adenogonium* extracts

Table 9 shows the average lethal dose (DL $_{50}$) according to the extracts as well as correlation coefficients. There is a variation of the DL_{50} from the regression line obtained from the representative curve in the number of surviving larvae based on the extracts concentration. The higher lethal concentration (27.66 mg/ml) of 50% of the larvae (DL $_{50}$) was obtained with methanol extract, while the lowest (2.87 mg/ml) was recorded with ethanol extract.

Table 9: *C. adenogonium* extracts lethality dose to the *Artemia salina* larvae

Extracts	DL_{50} **(mg/ml)**	R^2
Aqueous	4.39	0,54
Ethanol	2.87	0,91
Water-ethanol	3.04	0.91
Methanol	27.66	0.75
Ethyl acetate	3.29	0,78

Discussion

The research for bioactives molecules resulting from the plants in the treatment of various diseases becomes increasingly necessary for the world of research. In the present study carried out on the *C. adenogonium* leave, the extraction method showed an yield more high (12%) with methanol. The hexane on the other hand gave the lowest (0.5%). The lowest yield obtained with hexane shows that this plant leave do not contain many compound of lipidic nature. The variability observed in relation to the yield extraction with the other solvents would be probably related to the chemical composition of the leave used but also of the physiological state of the plant to harvest. In the same way, the difference in nature of solvents can be also explain this variation because Dah-Nouvlessounon et al. (2015) showed that the capacity of solvent extraction depends on the one hand on the affinity of this solvent with the phytomolécules and on the other hand of the polarity of this solvent.

Many secondary metabolites such as: tanins, flavonoïds, terpen, anthocyanin and saponines were detected in the extracts. This is conforming to the results of Novatus et *al*. (2012), which revealed the presence of these secondary metabolites in the hydro-ethanol extract of *Combretum adenogonium*. These metabolites were known for these biological activities such as: antiviral, antibacterial, anti-inflammatory and analgesic activity (Tsuchiya et *al*. 1996 and Ojewole, 2008). Yahaya et al. (2012) also revealed the presence of flavonoids, , tannins, glycosides, derivatives quinoniques and alkaloids in crude extract of a plant of the same family (*Combretum glutinosum*). But in this study, only the alkaloids have not been found in the *C. adenogonium* leaves extracts. It could be explained by factors influencing the plant photosynthesis. The absence of cyanogeniques derivatives in our

results reinforces the importance of our plant because they are the causes of toxicity due to the production of cyanide ions and manifest by the acceleration and the amplification of respiratory rate, respiratory depression, dizziness, headache, disorder of consciousness, coma deep etc (Bruneton, 1999). The presence of mucilages in the extracts confirms the presence of polysaccharides in the plant (Macdonald, 2010). Polysaccharides are beings alive, a large number of vital functions (breathing, energy metabolism, nutrition). polysaccharides may also be slow release of glucose forms, that can intervene in the regulation and the release of glucose in the diabetic (Macdonald, 2010).

The results show that the largest amount of total phenols was detected in the methanolic extract with an equivalent of Gallic acid up to two times higher compared to the fraction other excerpts. Similar results have been reported by Itoro et al. (2016) with methanolic extract of *Combretum platypetalum*. Indeed, this high content was positively correlated with its antioxidant capacity, in terms of neutralization of the DPPH radical with equivalent content of 14.63 mmolaire Ascorbic acid per milligram of extract. Similar observations were noted by Zakariat et al. (2011) for the leaves of *Melastoma malabathricum* where the highest content of the methanolic fraction in polyphenols has been associated with a maximum antioxidant capacity. The results shows that the total polyphenols of ethanol extract content is greater than that of the ethyl acetate extract and is small compared to the total aqueous extract polyphenols content. These results can be explained by the fact that the solvent plays an important role in the solubility of the chemical constituents (Mada, 2013). The production of free radicals in living organisms is a vital phenomenon for the cell regulated through various biochemical or enzymatic detoxification process (Salem, 2009). These free radicals are involved in many serious illnesses and constitute even aggressive form factors to DNA (Boumaza, 2009). Indeed, potential sources of antioxidant compounds have been searched in several types of plant materials such as vegetables, fruits, leaves, barks, roots and crude plant drugs. These antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress (Ozoy et al., 2008). The results show that *C. adenogonium* leaves are the natural potential source of antioxidant.

On the antimicrobial activity, four types of extracts (ethanol, water-ethanol, methanolic and ethyl acetate) from *C. adenogonium* leaf revealed their efficiencies at 20 mg/ml. On the other hand, with the same concentration, the aqueous extract has proved ineffective. The possible explanation to the difference of activity between the extracts may be the ability of solvent to solubilize and extract some phyto-molecules. Thus, according to Indabawa and Arzai (2011), during the liquid-liquid extraction,

phyto-molecules are distributed between the solvents according to their polarity and solubility. It can be thus concluded that the active antimicrobial compound contained in the *C. adenogonium* leaf are more soluble in these solvents (ethanol, water-ethanol, methanolic and ethyl acetate).

For used plant extracts, mortality of the *Artemia salina* is observed in the DL_{50} of 27,66 mg mg/ml; 4.39 mg/ml; 3.29 mg/ml; 3.04 mg/ml and 2.87 mg/ml respectively for methanolic, aqueous, ethyl acetate, water-ethanol and ethanol extracts. All samples tested, the ethanol extract proved the more toxic to a concentration of 2.87 mg/ml against the methanolique snippet which showed the lowest rate of toxicity to a concentration of 27, 66 mg.ml. these results confirm those of Novatus et al. (2012) which showed that the hydro-ethanol extract of leaves have a slight toxicity to brine shrimp with DL_{50} values of 76.965 mg/ml. This test has provided us with evidence that different extracts of leaves of *C. adenogonium* have a topical cytotoxic effect in this model of toxicity at the *Artemia salina*. Based on the correlation linking the degree of toxicity proposed by researchers Moshi et al. (2004) who affirmed that the extracts are non-toxic to the $DL_{50} \geq 100$ µg/ml, *C. adenogonium* is non-toxic regardless the concentrations determined in our study. The mechanism of toxicity may be related to inhibition of the hydrolytic enzymes (proteases and the carbohydrolases) or other interactions to inactivate microbial adhesins, protein transport and cell envelope (Cowan, 1999). Ultimately speaking of biological activity of *C. adenogonium*, acetatique extract is the best choice by its action both on the strains on the research of bioactive molecules.

Conclusion

It may be concluded that *C. adenogonium* extracts have many secondary metabolites and showed the interesting biological activities. Quantitative screening showed that methanol extract which contain highest amount of phenolic compounds and appreciable amount of flavonoids and ethyl acetate which contain highest amount of flavonoïds exhibited the greatest reducing power as Equivalent Ascorbic acid content. These effects have been correlated to the flavonoid and total phenolic contents of the leaf, indicating that phenolic compounds could be the major contributors to these activities. Additional work is need to determine whether these phenolic constituents are responsible for the antioxidant activity of *C. adenogonium* extracts. Though the extracts have a good inhibitory diameter, the most of them have MBC > 20mg/ml so don't have a good antimicrobial activity. Considering the traditional use and knowledge with present scientific investigation, it would be interesting to study other biological activities such as the anti-inflammatory activity, antiviral, antifongic, antidiuretic activity for the health-promoting properties and pharmaceutical applications.

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