

Antimicrobial activity of compounds from *Acanthospermum hispidum* DC and *Caesalpinia bonduc* (L.) ROXB: Beninese plants used by healers against HIV-associated microbial infections

Alban G. Houngbè¹, Habib M.Y. Ganfon¹, Sédami Medegan^{1,2}, Boniface Yèhouénou³, Bouraïma Bambola¹, Clément Gandonou¹, Fernand A. Gbaguidi^{1,2,*}

¹Laboratoire de Pharmacognosie/Institut de Recherche et d'Expérimentation en Médecine et Pharmacopée Traditionnelles (IREMPT) / Centre Béninois de la Recherche Scientifique et Technique (CBRST)/ UAC, 01 BP 06 Oganla Porto-Novo. ²Laboratoire de Chimie Pharmaceutique Organique, Ecole de Pharmacie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Campus du Champ de Foire, 01 BP 188, Cotonou, Bénin. ³Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA)/Ecole Polytechnique d'Abomey-Calavi/Université d'Abomey-Calavi, 01 BP 2009 Cotonou.

ARTICLE INFO

Article history:

Received on: 06/03/2015

Revised on: 09/04/2015

Accepted on: 11/05/2015

Available online: 03/08/2015

Key words:

Microbial infections, HIV-AIDS, Beninese plants, cytotoxic activity, antimicrobial activity, chromatographic methods.

ABSTRACT

In this study, we have tested alcoholic extracts (60%) from four Beninese plants: *Ocimum gratissimum* L., *Acanthospermum hispidum* DC, *Caesalpinia bonduc* (L) Roxb and *Calotropis procera* W. T. Aiton. They are used by the healers to prevent opportunistic diseases associated to HIV-AIDS; on six strains such as: *Escherichia coli* O 157H7, *Staphylococcus aureus* ATCC 25923 which resist to methicillin (MRSA), *Salmonella typhi*, *Klebsiella pneumonia*, *Candida albicans* ATCC 10231, and *Mycobacterium bovis* BCG 040812 which cause microbial infections associated with HIV-AIDS. The results show that all the extracts are bacteriostatic and fungistatic but only the hydro-ethanolic extracts of *Acanthospermum hispidum* (HE₂) and of *Caesalpinia bonduc* (HE₃) presented antibiotic power (respectively ap = 2 and ap = 4) on *Candida albicans* ATCC 10231. The *Mycobacterium bovis* BCG shown resistance to tested extracts (CMI > 250 µg/mL). The two fungicidal extracts HE₂ and HE₃ did not show harmful effects on the cells WI-38 with an IC₅₀ > 100 µg/mL for HE₂ and IC₅₀ = 50 µg/mL for HE₃. The successive bio-guided purifications of extracts HE₂ and HE₃ permitted isolation of three antibacterial compounds: Flavanone (M₁); stigmasterol (M₂); and quercetin (M₃). The three isolated compounds possess antibiotic power (ap 3±1) on tested strains and are not toxic on shrimp larvae (LC₅₀: 0.30 ± 0.17 mg/mL).

INTRODUCTION

Bacterial infections are a most common cause for morbidity among the patients infected by HIV with a major impact in the population (Bonnet *et al.*, 2007). Respiratory infections are tracts and lungs diseases that cause cough and/or a labored breathing. According to the World Health Organization (WHO), all infections with brutal beginning, affecting ears, nose, throat, larynx, trachea, windpipe, bronchus, the bronchioles or lungs are respiratory infections (Sanogo, 2010).

* Corresponding Author

Fernand Ahokanou GBAGUIDI, Laboratoire de Pharmacognosie/Institut de Recherche et d'Expérimentation en Médecine et Pharmacopée Traditionnelles (IREMPT) / Centre Béninois de la Recherche Scientifique et Technique (CBRST)/ UAC, 01 BP 06 Oganla Porto-Novo. Email: ahokannou@yahoo.fr

Skin problems are among the first symptoms indicating the presence of HIV in the organism, and about 90% of the seropositive people present symptoms of cutaneous eruption (generalized dermatitis, bacterial, fungal, viral and parasitic infections, skin tumors) during their illness (Verville, 2013). Moreover, tuberculosis causes a number of deaths in the world and represents a major public health issue (Pavan *et al.*, 2009). It is an infectious, contagious and endemic disease, with higher respiratory tropism caused by the *Mycobacterium tuberculosis* (or Kock bacillus), which is responsible of the death of two millions people per year (OMS, 2006).

The Human Immunodeficiency Virus (HIV) causing the Acquired Immunodeficiency Syndrome (AIDS) and tuberculosis are related (OMS, 2004). In Africa, tuberculosis is often the first manifestation of infection by the HIV, and appears to be the main cause of death (Grant *et al.*, 1997; Rana *et al.*, 2000).

The treatments for all these infections (urinary, respiratory, pulmonary, cutaneous and tuberculosis), which are related to HIV, are based on a regular taking of antibiotics coming up from western medicine (Fournet *et al.*, 2008). The lower efficiency and the high costs of these medicines associated to chemo-resistance problems often limit their under-spread application. This situation highlights the urgent need to develop from medicinal herbs new efficient and inexpensive a toxic drugs to treat microbial infections associated with HIV-AIDS. An ethnopharmacology study was recently performed by the Health Minister of Benin and shows that 13 Beninese plants were mostly used by endogenous medicine to fight against sexually transmitted infections associated to HIV-AIDS (Houngnihin *et al.*, 2010). Our study is focused on antimicrobial activity of these plants extracts on various strains associated to HIV-AIDS. The previous study realized by our team (Houngbeme *et al.*, 2014) has shown that only 4 of the hydro-ethanolic extracts of *Ocimum gratissimum* L; *Acanthospermum hispidum* DC, *Caesalpinia bonduc* (L) Roxb and *Calotropis procera* W.T.Aiton), presented activity against bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922).

The present work fits into the scope of our long-term continuing investigations and has for main purpose to evaluate antimicrobial activity of these hydro-ethanolic extracts on germs implied in microbial infections associated to HIV-AIDS in an effort to isolate the bioactive molecules and evaluate their toxicity.

MATERIAL AND METHODS

Material

The fresh samples of the plants used in this study (Table 1) were dried for about fourteen days in a room at constant room temperature (22°C). They were then carefully powdered using an electric grinder (of Flour mills Nigeria, EL MOTOR N°1827).

The microbial support used is made up of: *Escherichia coli* ATCC O: 157H7, *Salmonella typhi* (isolated), *Klebsiella pneumoniae* (isolated) *Staphylococcus aureus* ATCC 25923 resisting to methicillin (MRSA), *Candida albicans* ATCC 10231 and *Mycobacterium bovis* BCG 040812. These strains were provided by the National Laboratory of Public Health of the Health Ministry of Benin.

The brine shrimp larvae (*Artemia salina*, LEACH) are commercialized by the German company JBL GmbH & Co.KG and the cells WI-38 are used for assessing the toxicity tests.

Methods

Preparation of crude extracts (Houngbeme *et al.*, 2014):

Fifty grams (50 g) of each powdered plant were mixed with 500 mL of water-ethanol 95° (4:6; v/v). The mixture is stirred for (72h) and the steeping obtained is successively filtered three times on hydrophilic cotton. The filtrate was then evaporated to dryness at 40°C using a rotary evaporator (Heidolph Laborota 4000 efficient) coupled to a water chiller (Jubalo FL 300) to give the crude extracts and the yield was determined.

Test on bacteria and yeasts

The micro-dilution method in 96 wells microplates was used throughout (Houngbeme *et al.*, 2014). Two blank samples were performed: a negative one with extract and a positive one with bacterial suspension in order to control the growth of germs. The negative control (on the line A of the plate) is composed by various successive dilutions (two fold) from 100 µL of the main extract solution up to 20 mg/mL of DMSO (1%) and 100 µL of Mueller Hinton Broth (MHB) containing 0,02g/L of red phenol. The positive control (on line B) is performed in the same way as the negative control but the extract solution is replaced by 100 µL of bacterial suspension at 10⁶ colony forming units per milliliter (CFU / mL) (Density equal to Scale 2 of MC Farland). In the wells of lines C and D, the strains were incorporated into the extracts by adding 100 µL of microbial suspension to each well containing extract dilutions.

Cotrimoxazol (*Bio-Rad*) which have action on various bacteria was used as reference's antibiotic at 0.5 µg/mL for the stock solution. The microplate was recovered and connected to the sterilizer at 37°C for 24 h duration. We determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The knowledge of MIC and MBC were used to calculate the antibiotic power (ap) of the tested extract: ap = MBC/MIC. When ap is lower or equal to 4, this means that the tested extract shows some antibiotic power (Yèhouénou, 2012).

In vitro test on *Mycobacterium bovis* (*M. bovis*)

The method developed with success by Yemoa *et al.* (2011) to test the *Mycobacterium ulcerans* (pathogenic agent of the ulcer of Buruli) sensitivity was adapted herein on hydro-ethanolic extract of plants. It is a method of liquid spot microdilution in microplates of 96 wells, using the technical << Resazurin Microtiter Assay: REMA >> (Palomino *et al.*, 2002). Moreover, we also performed successive dilutions starting from 100 µL of the extract solution at 500 µg / mL and 100 µL of the spot 7H9 in the wells of columns 2 to 10 (line B to G). We also performed negative controls (200 µL of the middle 7H9 in the wells B₆, C₆, D₆) and positive controls (100 µL of bacterial suspension at 10⁸ CFU/ mL and 100 µL of the middle 7H9 in the wells E₆, F₆, G₆). 200 µL of sterilized distilled water were introduced into the external wells (columns 1, 7 to 12; lines A and H). Over the dilutions, the concentration of DMSO in the middle of the culture is lower than the threshold of 0.625 % (Cos *et al.*, 2006).

After dilution, 100µL of bacterial suspension of *M. bovis* at 1mg / mL containing approximately 10⁸ CFU / mL (Portaels *et al.*, 1998) were inoculated in the wells containing the extracts. The plates are then incubated at 31 ± 1 °C for 15 days and were added in the first well (positive control), 30 µL of aqueous solution of resazurin (Acros organic N.V) at 0,02% w/v (initially blue) prepared and stored at 4°C for two weeks before use. The plates are afterwards put in a sterilizer at 31 ± 1 °C. The development of

a color after 24 h evidences the growth of *M. bovis*. The whole plate is then revealed by adding 30 µL of resazurin in each well and assessed 24 h later. The results can be interpreted and are conclusive if and only if the negative controls remain blue and the positive controls remain pink. Based on the previous works, an extract is considered being active when $\text{CMI} \leq 128 \mu\text{g} / \text{mL}$ (Tosun *et al.*, 2004; Gu *et al.*, 2004) and not active, if the $\text{CMI} > 250 \mu\text{g} / \text{mL}$.

Larval toxicity assay (Houngbème *et al.*, 2014)

The test is performed against *Artemia salina* Leach by Fatondji *et al.* (2010); Sakirigui *et al.* (2012a, 2012b) and proposed in the literature as a simple bioassay method for assessment of preliminary toxicity of natural active products (Pelka *et al.*, 2000). The eggs of *Artemia salina* were incubated in sea water until hatching of young larvae (48 h). We prepared under various concentrations a series of solutions of each tested crude extracts. A defined number of larvae (16) were introduced in each solution. All solutions and control solutions without active component were put under stirring for 24 h. A counting of the dead larvae number (under a microscope) in each sample was performed to evaluate the toxicity of the solution. In case of death (during the control), the data were corrected by Abbott's formula: $\% \text{ death} = [(\text{test} - \text{control}) / \text{control}] \times 100$ (Carballo *et al.*, 2002). Data (dose-response) were subsequently transformed by logarithm and the LC_{50} were determined by linear regression (Mousseux, 1995). Tests were carried out in triplicate.

Cytotoxicity assay (Block *et al.*, 2004; Ganfon *et al.*, 2012)

The cytotoxicity of the extract on J774 and WI-38 cells was evaluated as described by Stevigny and *al.* (2002), using the tetrazolium MTT salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma)) colorimetric method based on the cleavage of the reagent by dehydrogenases in viable cells (Mosmann, 1983).

Camptothecin (Sigma) was used as positive cytotoxic reference's compound at $0.25 \mu\text{g}/\text{mL}$. Stock solution of compounds and active fractions were prepared in DMSO at $20 \text{ mg}/\text{mL}$. The solutions were diluted in order to provide 200, 100, 50, 25, 12.5 and $6.25 \mu\text{g} / \text{mL}$ concentrated solutions. The highest concentration of solvent which was shown to be non-toxic at which the cells were exposed was $\approx 1\%$. Each extract was tested in a series of six dilutions in 96-wells microtiter plate. Each experiment was duplicated to confirm the results.

Chromatographic methods

Thin layer chromatography (TLC)

The pure material was obtained by standard preparative TLC method using stationary phase: plate $10 \times 10 \text{ cm}$, TLC Silicagel F254S, Merk ®; mobile phase: toluene - ethyl acetate - formic acid (36-12-5, v/v/v). The bioautographic method (derived from analytic TLC) was used, in order to detect the presence or not of clear zones of inhibition for 24 h (Haouat *et al.*, 2013).

Fractionation on column at atmospheric pressure

This technique was used to achieve coarse fractions and to mark which fraction recovers the most active compounds after the biological tests. The operating conditions are:

- stationary phase: Silica frost, $0.062 \text{ mm}-0.2 \text{ mm}$ and the frost of polyamid SC6 MN.
- elution: 300 mL of eluant in the following order: n-hexan, dichloromethane, ethyl acetate, n-butanol and isopropanol

The MPLC (Medium pressure Liquid Chromatography)

The active samples obtained by fractionation on open column are put on the MPLC column in order to fractionate them again until the separation and the isolation of the most compounds using a glass column: omnifit (OM 6427 $15 \times 750 \text{ mm}$); stationary phase: Lichroprep Si RP-18.

Separation on dextran frost: sephadex® LH 20.

With this frost, the compounds are separated according to their molecular weight using stationary phase: sephadex LH20 and eluent: ethanol 95° .

RESULTS AND DISCUSSION

Antimicrobial activity of extracts

The Table 2 summarizes the values of different biological parameters measured for hydro-ethanolic extracts of plants. The biological screening took into account six bacterial strains implied in microbial infections associated with HIV-AIDS. The fourth tested extracts showed bacteriostatic and fungistatic activities on our studied strains; this result comes to reinforce our preliminary works for which these same extracts were revealed bacteriostatic and bactericidal on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (Houngbème *et al.*, 2014).

In this study, the *Mycobacterium bovis* (*M. bovis*) BCG 040812 remained non-sensitive to the extracts. *M. bovis* resistance's could be explained by the mycobacteria complex chemical composition. Indeed, they are rich in lipids and often permeable to less polar compounds (Connell and Nikaido, 1994). On the over hand, the well known property of *Mycobacterium* is the capacity of its cellular inner side to resist to bleaching by diluted acid and alcohol characteristic related to the high composition in lipid of the cellular inner side (Boukary *et al.*, 2011).

Two extracts are indeed fungicidal, precisely the hydro-ethanolic extracts of *Acanthospermum hispidum* (HE₂) and of *Caesalpinia bonduc* (HE₃) have some antibiotic power (respectively $\text{ap} = 2$ and $\text{ap} = 4$) on *Candida albicans* ATCC 10231. Comparing to the Cotrimoxazol, it can be assumed that there is no significant difference between, Cotrimoxazol ($\text{ap} = 1$) and HE₂ activities ($\text{ap} = 2$). Nevertheless, HE₃ activity ($\text{ap} = 2$) appears to be lower than Cotrimoxazol's. The activity demonstrated by the extract of *Caesalpinia bonduc* corroborates the previous works which emphasizes the antibacterial and

fungicidal activities of the plant (Singh and Raghav, 2012). From this result, it can be deduced that the hydro-ethanolic extracts of *Acanthospermum hispidum* (HE₂) and *Caesalpinia bonduc* (HE₃) can contribute to the slowing of candidoses which are highly associated to HIV-AIDS. The calculated selectivity index showed that the extracts are more toxic on the shrimp larvae than active because the SI is lower to 1 (Tiuman *et al.*, 2005). Only the HE₃ extract possesses a weak selectivity (SI=1.12) on *E. coli* O 157H7.

Cytotoxicity of HE₂ and HE₃ extracts

For this test, the results are expressed by the inhibitory concentration 50 % (IC₅₀) when superior to 100 µg / mL for the hydro-ethanolic extract of *Acanthospermum hispidum* (*A. hispidum*) (HE₂) against 0,4 (± 0,2) µg / mL for the witness (the comptothecin).

The IC₅₀ is equal to 50 µg / mL for the hydro-ethanolic extract of *Caesalpinia bonduc* (HE₃). The limit of toxicity being fixed at 20 µg / mL (Ganfon *et al.*, 2012), it can be concluded that the HE₂ and HE₃ extracts do not present any harmfulness on the cells WI-38. They can be considered therefore not toxic. This result reinforces the previous result on larval toxicity which allowed to highlight the non-toxic character of these extracts on the shrimp larvae (Houngbème *et al.*, 2014), and therefore, on Human cells of carcinoma and colon in accordance to the works of Pelka *et al.* (2000) and Carballo *et al.* (2002).

These results are similar to those of Ganfon *et al.*, when they evaluated in 2012, the *in vitro* cytotoxicity of acid aqueous extracts of *A. hispidum* on the same cell line WI-38. The two fungicidal and bacteriostatic extracts are not toxic, which motivated our submitting to successive fractionation in an effort to isolate some molecules.

Bio-guided purification of fungicidal extracts HE₂ and HE₃ Antimicrobial activities of obtained fractions

The two extracts are firstly submitted to a fractionation on column at atmospheric pressure and the different fractions obtained are tested on the previous strains. The results are summarized in the tables 3 and 4.

All fractions from the two extracts showed bacteriostatic and fungistatic actions on the different microbial strains. For the fractions from extract HE₂, the MIC vary from 0.3125 mg / mL to 10 mg / mL for *Escherichia coli* O 157H7, from 0.625 mg / mL to 5 mg / mL for both MRSA and *Salmonella typhi*, and to finish, from 1.25 mg / mL to 10 mg / mL for both *Klebsiella pneumoniae* and *Candida albicans*.

Concerning the values of MIC of the fractions from HE₃ extract, they vary from 0.3125 mg / mL to 10 mg/mL for both *E. coli* O 157H7 and *Salmonella typhi*, from 0.625 mg / mL to 10 mg / mL for MRSA; from 2.5 mg / mL to 10 mg / mL for *Klebsiella pneumoniae* and from 1.25 mg / mL to 5 mg / mL for *Candida albicans*. At the end of this biological screening, three fractions (FBut of HE₂, FBut and FHex of HE₃) appeared to be bactericidal

and fungicidal on three microbial strains: *E. coli* O 157H7 (gram-); MRSA (gram+) and *Candida albicans* (yeast). The difference between antibiotic power of fractions and positive reference wasn't significant for the hexanic fraction of HE₂ and the butanolic fraction of HE₃ respectively on MRSA and *C. albicans* (ap= 2). We became interested in these three fractions in order to isolate the molecules responsible of the antimicrobial activity.

Identification of active spots by bioautography

In this experiment, we have studied only three germs on which the fractions have presented activity. The results are presented at Table 5.

The diameter of inhibitory area varies from microbial strain to another one and according to the nature gram+ or gram- of the latters. Otherwise, there is no significant difference between the inhibition diameters of spots and the one of nystatin so those fractions could be good candidates for the elaboration of new fungicidal medicines.

Isolation of M₁, M₂ and M₃ compounds

-The n-butanol fraction (90 mg) of HE₂ extract of *A. hispidum* is put on the polyamid frost flown at atmospheric pressure glass in order to get rid of colours (chlorophyll). The obtained residue has undergone a first fractionation by MPLC in reverse phase using acetonitrile / ethyl acetate gradient (6-4 to 8-2, v/v) to give ten fractions. The sub-fraction Fb_{2,3} is separated on the Sephadex® LH20 with ethanol 95° to obtain the M₁ compound (9 mg) which is a white amorphous powder which absorbs at the UV at 366 nm and becomes yellow with the NEU/ PEG reagent on a silica slab CCM similar to a flavonoid.

-100 mg of hexanic fraction FHex₃ are split up on MPLC with silica gel 60 F254 nm, 15-25µm, Merck and a mixture composed of toluene-ethyl acetate (8-2 ; v/v). Then, we performed a preparative CCM followed by a filtering on SPE cartridge. The filtered product obtained is concentrated and separated by chromatography on sephadex gel LH20. We obtained a white amorphous powder called M₂. The M₂ compound is neither visible on CCM slab with eyes or with UV at 254 nm or on 366 nm without revelation by sulfuric anisaldehyde, which colors the compound M₂ in blue then it can be predicted a terpenoid or a steroid.

-The Fbut₃ fraction (85 mg) is submitted at a fractionation by MPLC in reverse phase (Lichroprep RP18; 40-60 µm, Merck) with acetonitrile / ethyl acetate (2-8; v/v) to have eight fractions (Fb_{3,1} à Fb_{3,8}). The sub-fraction Fb_{3,6} (47.52 mg) is then split up on MPLC with the same stationary phase and a mixture of acetonitrile / methanol (8-1;v/v). Then, the under fraction Fb_{3,6,3} (23.15 mg) is separated on Sephadex® gel LH20 with ethanol 95° to have 9.63 mg of a yellow amorphous powder named M₃ which absorbs in the visible and is coloured in yellow with NEU / PEG reagent; M₃ can be a flavonoid.

Table 1: List of Studied Plants (Houngbème *et al.*, 2014).

N°	Species	Local names	Family	Part used	Locality of crop
1	<i>Ocimum gratissimum</i> L.	Fon and Goun : <i>tchayo</i> ; Yoruba : <i>simonua</i>	LAMIACEAE	leaves	Danto/ Ouémé
2	<i>Acanthospermum hispidum</i> DC.	Fon : <i>kpononmi</i> ; Goun : <i>ahwanglon</i> ; Yoruba : <i>tchakatoun</i>	ASTERACEAE	A .P	Danto/ Ouémé
3	<i>Caesalpinia bonduc</i> (L.) Roxb.	Fon : <i>adjikwin</i> ; Yoruba : <i>ewé ago, itchè</i>	CAESALPINIACEAE	leaves	Adjohoun/ Ouémé
4	<i>Calotropis procera</i> (Aiton)	Fon and Goun : <i>amouman, kpento</i> ; Yoruba : <i>boum boum, kpaon</i>	ASCLEPIADACEAE	leaves	Abomey-calavi/ Atlantique

A .P: aerial parts

Table 2: Results of antimicrobial activity and toxicity of extracts.

Extracts	LC ₅₀ (mg/mL)	Strains											
		<i>E. coli</i> O 157H7				MRSA				<i>Salmonella typhi</i>			
		MIC	SI	MBC	ap	MIC	SI	MBC	ap	MIC	SI	MBC	ap
HE1	0.05	2.5	0.02	-	-	2.5	0.02	-	-	0.625	0.08	-	-
HE2	0.39	0.625	0.624	-	-	0.625	0.624	-	-	1.25	0.312	-	-
HE3	0.35	0.3125	1.12	-	-	1.25	0.28	-	-	2.5	0.14	-	-
HE4	0.02	2.5	0.008	-	-	1.25	0.016	-	-	1.25	0.016	-	-
Cotrimoxazol	-	0.125	-	0.125	1	0.0312	-	0.0312	1	0.125	-	0.125	1

Extracts	LC ₅₀ (mg/mL)	Strains											
		<i>Klebsiella pneumoniae</i>				<i>Candida albicans</i>				<i>M. bovis</i>			
		MIC	SI	MBC	ap	MIC	SI	MBC	ap	MIC	SI	MBC	ap
HE1	0.05	1.25	0.04	-	-	1.25	0.04	10	8	>250	-	nd	nd
HE2	0.39	1.25	0.312	-	-	1.25	0.312	2.5	2	>250	-	nd	nd
HE3	0.35	2.5	0.14	-	-	0.625	0.56	2.5	4	>250	-	nd	nd
HE4	0.02	1.25	0.016	-	-	0.625	0.032	10	16	>250	-	nd	nd
Cotrimoxazol	-	0.125	-	0.125	1	0.0625	-	0.0625	1	>250	-	-	-

MIC: Minimum Inhibitory Concentration (mg/mL but µg/mL for Cotrimoxazol and *Mycobacterium bovis*); MBC: minimum bactericidal concentration (mg/mL), LC₅₀: Lethal concentration 50%; ap: antibiotic power; HE: hydro-ethanolic extract, SI = LC₅₀ / MIC: Selectivity index; nd: no determining. The indices 1, 2, 3 and 4 indicate respectively *O.gratissimum*, *A.hispidum*, *C. bonduc* and *C. procera*.

Table 3: Results of antimicrobial activities and toxicity of he₂ fractions .

Fractions of HE ₂	LC ₅₀ (mg/mL)	Strains											
		<i>E. coli</i> O 157H7				MRSA				<i>Salmonella typhi</i>			
		MIC	SI	MBC	pa	CMi	SI	MBC	ap	MIC	SI	MBC	ap
F _{Hex}	0.12	0.3125	0.02	-	-	2.5	0.02	-	-	0.625	0.08	-	-
F _{DCM}	0.07	0.625	0.62	-	-	0.625	0.62	-	-	1.25	0.312	-	-
F _{ACE}	0.18	0.3125	1.12	-	-	1.25	0.28	-	-	2.5	0.14	-	-
F _{But}	0.78	2.5	0.008	10	4	2.5	0.02	5	2	5	0.016	-	-
F _{Ipr}	0.21	10	0.021	-	-	5	0.04	-	-	5	0.042	-	-

Fractions of HE ₂	LC ₅₀ (mg/mL)	Strains											
		<i>Klebsiella pneumoniae</i>				<i>Candida albicans</i>							
		MIC	SI	MBC	ap	MIC	SI	MBC	ap	MIC	SI	MBC	ap
F _{Hex}	0.12	1.25	0.04	-	-	5	-	0.024	-	-	-	-	-
F _{DCM}	0.03	1.25	0.312	-	-	2.5	-	0.012	-	-	-	-	-
F _{ACE}	0.18	2.5	0.14	-	-	1.25	-	0.144	-	-	-	-	-
F _{But}	0.78	2.5	0.016	-	-	2.5	-	0.312	5	-	-	-	-
F _{Ipr}	0.21	10	-	-	-	10	-	0.021	-	-	-	-	-

MIC: Minimum Inhibitory Concentration (mg/mL); MBC: minimum bactericidal concentration (mg/mL), ap: antibiotic power, HE: hydro-ethanolic extract, SI = LC₅₀ / MIC: Selectivity index; LC₅₀: Lethal concentration 50%; F_{Hex}: n-hexane fraction; F_{DCM}: dichloromethane fraction; F_{ACE}: ethyl acetate fraction ; F_{But}: n-butanol fraction ; F_{Ipr}: isopropanol fraction

Table 4: Results of antimicrobial activities and toxicity of he₃ fractions

Fractions of HE ₃	LC ₅₀ (mg/mL)	Souches microbiennes											
		<i>E. coli</i> O 157H7				MRSA				<i>Salmonella typhi</i>			
		MIC	SI	MBC	ap	MIC	SI	MBC	ap	MIC	SI	MBC	ap
F _{Hex}	0.12	0.3125	0.02	1.25	4	2.5	0.02	5	2	0.312	0.08	-	-
F _{DCM}	0.03	10	0.624	-	-	5	0.62	-	-	10	0.312	-	-
F _{ACE}	0.18	10	1.12	-	-	10	0.28	-	-	10	0.14	-	-
F _{But}	0.78	2.5	0.008	10	4	0.625	0.02	2.5	4	2.5	0.016	-	-
F _{Ipr}	0.21	10	0.021	-	-	5	0.04	-	-	10	0.021	-	-

Fractions of HE ₃	LC ₅₀ (mg/mL)	Souches microbiennes											
		<i>Klebsiella pneumoniae</i>				<i>Candida albicans</i>							
		CMi	SI	CMB	pa	CMi	SI	CMB	pa	CMi	SI	CMB	pa
F _{Hex}	0.12	5	0.04	-	-	1.25	0.04	5	4	-	-	-	-
F _{DCM}	0.03	10	0.312	-	-	5	0.312	-	-	-	-	-	-
F _{ACE}	0.18	10	0.14	-	-	5	0.56	-	-	-	-	-	-
F _{But}	0.78	2.5	0.016	-	-	2.5	0.032	5	2	-	-	-	-
F _{Ipr}	0.21	10	0.021	-	-	5	0.042	-	-	-	-	-	-

MIC: Minimum Inhibitory Concentration (mg/mL); MBC: minimum bactericidal concentration (mg/mL), ap: antibiotic power, HE: hydro-ethanolic extract, SI = LC₅₀ / MIC: Selectivity index; LC₅₀: Lethal concentration 50%; F_{Hex}: n-hexane fraction; F_{DCM}: dichloromethane fraction; F_{ACE}: ethyl acetate fraction ; F_{But}: n-butanol fraction ; F_{Ipr}: isopropanol fraction.

Table 5: Values of Inhibitory diameter of Spotlights.

Fractions spotlights / antibiotic	Diameter of inhibitory area (mm)		
	<i>E.coli</i> O 157H7	<i>MRSA</i>	<i>C. albicans</i>
M ₁	5	7	8
M ₂	5	7	7
M ₃	4	6	8
Nystatin (T ₁)	-	-	10
Bactrim (Triméthoprim-sulfaméthoxazole, T ₂)	11	-	-
Pénicilline G (T ₃)	-	12	-

M₁, M₂ and M₃ indicate the compounds that showed a inhibitory area respectively in *F_{But}* of HE₂, *F_{Hex}* and *F_{But}* of HE₃

Table 6: Result of biological activities of isolated compounds.

isolated molecules	LC ₅₀ (mg/mL)	Strains											
		<i>E. coli</i> O 157H7				<i>MRSA</i>				<i>Candida albicans</i>			
		MIC	SI	MBC	ap	MIC	SI	MBC	ap	MIC	SI	MBC	ap
M1	0.28	2.5	0.11	10	4	2.5	0.11	10	4	5	0.06	10	2
M2	0.13	0.625	0.21	2.5	4	2.5	0.05	5	2	0.625	0.21	2.5	4
M3	0.48	2.5	0.19	10	4	0.625	0.77	2.5	4	2.5	0.19	10	4

MIC: Minimum Inhibitory Concentration (mg/mL); MBC: minimum bactericidal concentration (mg/mL), ap: antibiotic power, HE: hydro-ethanolic extract, SI = LC₅₀ / MIC: Selectivity index; LC₅₀: Lethal concentration 50%; M₁, M₂ et M₃ indicate the three isolated molecules.

Structure of isolated compounds

The treatment of the information's provided by spectral techniques helps to define the molecular structure of isolated compounds.

Spectroscopic data of M₁ Compound

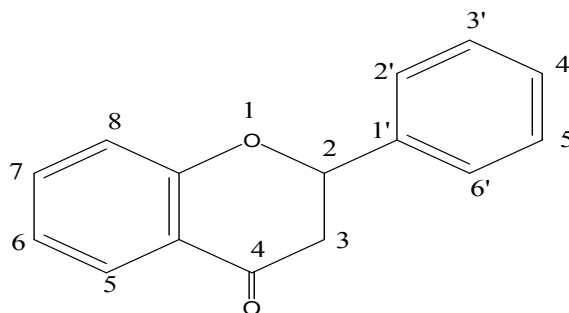
MS (m/z): 225.09073 ([M+H]⁺); 210 ([M+H]⁺-CH₃); 207 ([M+H]⁺-H₂O); 147 ([M+H]⁺-C₆H₆); 121([M+H]⁺- C₈H₈).

¹H NMR (DMSO-d₆, 400 MHz, δ en ppm): δ 2.82 (dd, H-3b); 3.25 (dd, H-3a); 5.5 (dd, H-2); 5.85 (d, H-5, H-8); 7.1 (dd, H-6, H-7); 7.50 (dd, H-3', 5'); 7.6 (d, H-2', 6').

¹³C NMR (DMSO, 100 MHz, δ en ppm): δ 191.55(C=O); 161.06 (C-9); 138.90 (C-1'); 136.27 (C-7); 128.50 (C-5); 128.54 (C-3', C-5); 126.61 (C-4'); 126.31 (C-2', C-6'); 121.45 (C-10); 120.65 (C-6); 118.03 (C-8); 78.80 (C-2); 43.5 (C-3).

The mass spectrum shows that the formula of M₁ compound is C₁₅H₁₂O₂ whose molecular mass is 224.09073 g / mol (calculated mass is 224.08376 g/mol) and corresponds to non-substituted flavanone. The presence of peaks between 7 and 8 ppm on the ¹H-NMR spectrum shows that the flavonoid possesses non substituted cycle (Mabry *et al.*, 1970). Two non equivalent protons at δ 3.10 ppm and 2.85 ppm correspond to H-3 protons of a flavanone (Mabry *et al.*, 1970). A peak at δ 5.43 ppm corresponds to H-2 (CH) proton of a flavanone (Mabry *et al.*, 1970). We find some aromatic protons at δ 7.6 ppm (d, J = 2.1 Hz), 7.50 ppm (dd, J = 8.3, J = 2.1 Hz) which are respectively attributed to H-2', 6' and H-3', 5'. On the other hand, we don't observe a peak between δ 12.5 et 13 ppm what shows the absence of phenolic proton in the compound (Harborne, 1993). The ¹³C-NMR spectrum shows thirteen peaks which correspond to thirteen carbons. The absence of peaks at δ 161.7 ppm and 160.6 ppm shows that it's a non

substituted cycle (Portet, 2007). Those pieces of information are in accordance with the flavanone non-substituted molecule (figure 1).

**Fig. 1:** Structure of Flavanone (M₁)

Spectroscopic data of M₂ compound

MS (m/z): 413, 3608 ([M+H]⁺); 435, 35924 ([M+Na]⁺)

¹H NMR (CDCl₃, 400 MHz, δ en ppm): δ 7.30 (s, OH-3); 5.2 (m, H-6); 4.9 (s, H-22); 5.1 (s, H-23); 3.51 (tdd, H-3); 1.21 (s, H-19); 1.17 (s, H-28); 1.03 (s, H-27); 0.99 (s, H-26); 0.97 (s, H-24); 0.90 (s, H-29).

¹³C NMR (CDCl₃, 100 MHz, δ en ppm): δ 140.76 (C-22); 138.33 (C-5); 129.28 (C-23); 121.72 (C-6); 76.71 (C-3); 56.87 (C-4); 55.96 (C-5); 51.25 (C-24); 50.40 (C-17); 42.30 (C-9); 42.22 (C-13); 39.69 (C-10); 37.27 (C-10); 36.52 (C-20); 31.9 (C-25); 31.66 (C-21); 28.94 (C-23); 25.43 (C-12); 24.36 (C-11); 21.23 (C-25); 21.11 (C-26); 19.41 (C-29)

The peak [M+H]⁺ at m/z = 413.3608 and the peak [M+Na]⁺ at m/z = 435.35924 correspond to brute formula C₂₉H₄₈O (calculated mass is 412.37074 g/mol) which is the one of stigmaterol. The H-3 proton appears in form of tripled splitted at δ 3.51 ppm (J = 4.5 and 1.0 MHz) and H-6 olefinic proton shows a multiplied at δ 5.2 ppm. Moreover, two olefinic protons appear at δ 5.16 ppm (m) and δ 5.14 ppm (m) corresponding respectively to

H-20 and H-21 protons of stigmasterol (Habib *et al.*, 2007; Jain *et al.*, 2009). Six methyl protons also appear at δ 0.90-1.21 ppm. The chemical shift values of carbons correspond well to the ones of stigmasterol carbons.

These spectrometric data are in accordance with the reported values in literature for the stigmasterol (Jain and Bari, 2010; Hartati *et al.*, 2008; Rajput and Rajput, 2012). On the basis of this, we can attribute to M_2 the structure of stigmasterol (figure 2).

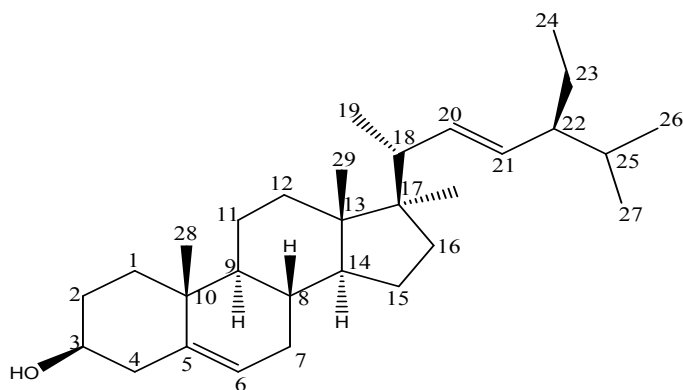


Fig. 2 : Structure Du Stigmasterol (M_2).

Spectroscopic data of M_3 compound

MS (m/z): 303.04967 ($[M+H]^+$); 325.03159 ($[M+Na]^+$); 285 ($[M+H]^+-H_2O$); 257 ($[M+H]^+-HCOOH$).

1H NMR (DMSO, 400 MHz, δ en ppm): δ 7.9 (*d*, H-2', H-6'); 6.9 (*d*, H-5'); 6.5 (*d*, H-8); 6.2 (*d*, H-6); 3.5 (*s*, OH-3',4',5,7); 12.5 (*s*, OH-3).

^{13}C NMR (DMSO, 100 MHz, δ en ppm): δ 175.8 (C-4); 163.84 (C-7); 160.68 (C-5); 156.09 (C-2); 147.66 (C-9); 146.75 (C-4'); 145.01 (C-3'); 135.70 (C-3); 121.92 (C-1'); 119.94 (C-6'); 115.57 (C-2'); 115.02 (C-5'); 102.97 (C-10); 98.14 (C-6); 93.31 (C-8).

The molecular ion $[M+H]^+$ at $m/z = 303.04967$ responds to the brute formula $C_{15}H_{10}O_7$, the one of the quercetin. The calculated mass is 302.0426 g/mol. On the 1H -NMR spectrum of M_3 compound, we observe two aromatic protons forming two doubled at δ 6.20 and 6.50 ppm ($J = 2.0$ Hz), characteristic of cycle A of a flavonoid substituted in C-5 and C-7 and can be attributed respectively to H-6 and H-8 protons (Markham, 1982). The presence of three aromatic protons at δ 7.54 ppm (*d*, $J = 2.1$ Hz), 7.50 ppm (*dd*, $J = 8.3$, $J = 2.1$ Hz), 6.92 ppm (*d*, $J = 8.3$ Hz) corresponding respectively to H-2', H-6' and H-5'. Moreover we notice the presence of a peak at 12.5 ppm which is a phenolic proton (Harborne, 1993).

The ^{13}C -NMR spectrum of M_3 compound shows ten quaternaries carbons, one carbonyl group ($C=O$, δ 175.8 ppm) and C-3 carbon characteristic of a flavonol (δ 135.7 ppm), and five aromatic CH (Mabry *et al.*, 1970; Nancy *et al.*, 1999). All these

information help us to identify the M_3 compound as quercetin (figure 3).

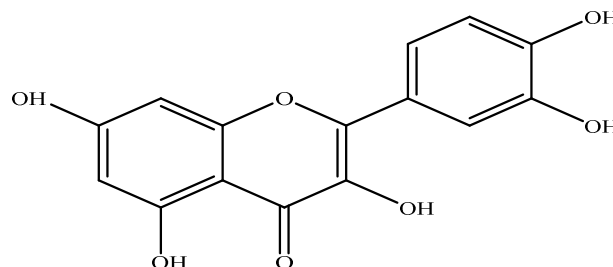


Fig. 3: Structure Of Quercetin (M_3)

Antimicrobial activities and toxicity of isolated compounds

We undertook to determine the MIC and the MBC of these molecules by microdilution. Their toxicity was also evaluated on the larvae of shrimps. The obtained results are summarized at Table 6. The three isolated compounds present antibiotic power on the studied strains. Their antibiotic power values whose vary between 2 and 4 were compared with the antibiotic power of Cotrimoxazol ($ap = 1$). There is no significant difference between the Cotrimoxazol activity's ($ap = 1$) and the Flavanone and the Stigmasterol activities whose present the same antibiotic power ($ap = 2$) on *C. albicans* and MRSA respectively. These results come to reinforce the ones shown by the inhibition areas. The antimicrobial activity of M_1 and M_2 compounds finds its justification in the fact that the flavonoids are used for a range of pharmacological activities as their antimicrobial actions (Sannomiya *et al.*, 2005; de Rijke *et al.*, 2006). The highest antibiotic power ($ap = 2$) is obtained on *Candida albicans* ATCC 10231, what suggests that the M_1 compound has affinity with chemical composition of the inner side of *C. albicans*. The compounds are not toxic on the larvae of shrimps ($LC_{50} > 0.1$ mg/mL).

CONCLUSION

The present study permitted to value the plants used by Beninese healers in the treatment of microbial infections associated with HIV-AIDS. The hydro-ethanolic extracts of *A. hispidum* and of *C. bonduc* are fungicidal on pathogenic yeast (*Candida albicans* ATCC 10231). Two flavonoids (flavanone and quercetin) and one steroid (stigmasterol) are identified from these extracts using chromatographic and spectroscopic methods. The three isolated molecules of these extracts possess antibiotic power on the tested strains (*Candida albicans* ATCC 10231, *E. coli* O 157H7, MRSA), what justify the derived fractions antimicrobial activity. These molecules are not toxic on some human cells like the carcinome and colon. Based on these results, we expect to be able to conceive formulae of new medicines and imprint new progresses to phytotherapy of microbial infections associated to HIV-AIDS. It is essential how to go further in this work by studying the mechanism of these molecules.

ACKNOWLEDGEMENT

We sincerely thank Dr Boniface Yèhouénou for their technical support during the biological tests.

REFERENCES

- Block S, Baccelli C, Tinant B, Rozenberg LVMR, Jiwan J-LH, Llabre G, De Pauw-Gillet M-C, Quetin-Leclercq J. Diterpenes from the leaves of *Croton zambesicus*. *Phytochemistry* 2004; 65: 1165–1171.
- Bonnet F, Chène G, Thiébaud R. Trends and determinants of severe morbidity in HIV-infected patients: the ANRS CO3 Aquitaine Cohort, 200–2004. *HIV Med*, 2007; 8: 547–554.
- Boukary A.R., Thys E., Mamadou S., Rigouts L., Matthys F., Vias Franck S.G., Gamatie D., Yenikoye A., Saegerman C. La tuberculose à *Mycobacterium bovis* en Afrique subsaharienne. *Ann. Méd. Vét.*; 2011; 155: 23–37
- Carballo JL, Hernandez-Inda ZL, Pérez P and Garcia-Grávalos MD. A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnology*, 2002; 2:17.
- Connell ND, Nikaido H. 1994. Membrane permeability and transport in *Mycobacterium tuberculosis*. In: Bloom BR, editor. *Tuberculosis: pathogenesis, protection and control*. Washington: American Society of Microbiology. p. 333–452
- Cos P, Vlietinck AJ, Berghe DV, Maes L. Anti-infective potential of natural products: how to develop a stronger *in vitro* 'proof-of-concept'. *J Ethnopharmacol*, 2006; 106: 290–302
- de Rijke E, Out P, Niessen WM, Ariese F, Gooijer C, and Brinkman UA. Analytical separation and detection methods for flavonoids. *Journal of Chromatography A*, 2006; 11 (12): 31–63.
- Fatondji HR, Gbaguidi F, Kpoviessi S, Sonounameto E, Lagnika L, Ambaliou S, Moudachirou M, Poupaert J, Accrombessi G. Synthèse, caractérisation et étude de propriétés antimicrobiennes de la semicarbazone et de la thiosemicarbazone de la carvone. *J.Soc.ouest-Afr.Chim*, 2010; 030:11–17
- Fournet ARFM, Lagoutte D, Poupon E, Soriano-Agaton F. Utilisation de la cantifln-6-one et ses analogues dans le traitement des pathologies liées aux mycobactéries. *Espace net, Registre européen des brevets*. 2008.
- Ganfou H, Bero J, Tchinda TA, Gbaguidi F, Gbenou J, Moudachirou M, Frédéric M, Quetin-Leclercq J. Antiparasitic activities of two sesquiterpenic lactones isolated from *Acanthospermum hispidum* D.C. *Journal of Ethnopharmacology*, 2012; 141 (1): 411–417.
- Gbaguidi F. 2005. Identification, purification, isolement et quantifications de principes actifs de *Mitracarpus scaber* Zucc. et évaluation de leurs activités antimicrobiennes sur *Dermatophilus congolensis*. Thèse de doctorat. Université d'Abomey-Calavi. Bénin. p 176
- Grant AD, Djomand G, De Cock KM. Natural history and spectrum of disease in adults with HIV/AIDS in Africa. *AIDS*, 1997; 11: 43–54.
- Gu JQ, Wang Y, Franzblau SG, Montenegro G, Yang D, Timmermann BN. Antitubercular constituents of *Valeriana laxiflora*. *Planta Med*, 2004; 70: 509–514.
- Habib MR, Nikkon F, Rahman M, Haque ME, Karim MR. Isolation of stigmaterol and beta-sitosterol from methanolic extract of root of bark of *Calotropis gigantea* (Linn.). *Pak. J.Biol. Sci.*, 2007; 10: 4171–4176.
- Haouat AC, Sqalli H, Farah A, Haggoud A, Iraqui M. Activité antimycobactérienne des extraits de deux espèces marocaines du genre *Cistus*. *Phytothérapie*, 2013; 11 (6): 365–372.
- Harborne JB. 1993. *The flavonoids: advances in research since 1986*. Chapman & Hall / CRC. London.
- Hartati S, Kadono LBS, Kosela S, Harrison LJ. A new pyrano Xanthone from the stem barks of *Garcinia tetrandra* (Pierre). *J. Biol. Sci.*, 2008; 8: 137–142.
- Houngbème AG, Gandonou C, Yehouénou B, Kpoviessi SDS, Sohounhloué D, Moudachirou M and Gbaguidi FA. Phytochemical analysis, toxicity and antibacterial activity of benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with hiv/aids. *Int J Pharm Sci Res*, 2014; 5(5): 1739–1745.
- Houngnihin RA, Gbaguidi F, Agbani OP, Glo V, Dossou P, Dissou F, Medegan-Fagla VK, Trougnin V. 2010. Monographie de cent (100) plantes utilisées en Médecine traditionnelle pour la prévention et le traitement des affections opportunistes du VIH-SIDA, 1ère Edition, Bénin; 104p.
- Jain PS and Bari SB. Isolation of lupeol, stigmaterol and campesterol from petroleum ether extract of Woody stem of *Wrightia tuictoria*. *Asian J. Plant. Sci.*, 2010; 9: 163–167.
- Jain PS, Bari SB and Surana SJ. Isolation of stigmaterol and γ -sitosterol from petroleum ether extract of Woody stem of *Abelmoschus manihot*. *Asian J. Biol. Sci.*, 2009; 2: 112–117.
- Mabry TJ, Markham KR, and Thomas MB. 1970. The systematic identification of flavonoids. Springer-Verlag. Berlin
- Markham KR. 1982. Techniques of Flavonoid Identification. Academic Press. London.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 1983; 65, 55–63.
- Mousseux M. 1995. Test de toxicité sur larves d'*Artemia salina* : entretien d'un élevage de balanes. Nouméa, Centre Universitaire de Nouvelle Calédonie. 20 p.
- Nancy G, Vincent G, Jacque D, Patricia B, Monique T, Luc A. Anti-inflammatory and immunological effect of *Centaurea cyanus* flowers-heads. *Journal of Ethnopharmacology*, 1999; 68: 235–241.
- OMS. The Global Plan to Stop TB, 2006–2015. Actions for life: towards a world free of tuberculosis. *Int. J. Tuberc LungDis.*, 2006; 10: 240–241.
- OMS. 2004. World Health Organization Office for the African Region, Harare, Zimbabwe October 2004/TB/HIV Control Strategy for the African Region. 22 p.
- Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother*, 2002; 46: 2720–2722
- Pavan FR, Sato ND, Higuchi TC, Santos CBA, Vilegas W, Leite QFC. *In vitro* anti-*Mycobacterium tuberculosis* activity of some Brazilian "Cerrado" plants. *Brazilian Journal of Pharmacognosy*. 2009; 19(1B): 204–206
- Pelka M, Distler W, Petschelt A and Dent J: A new screening test toxicity testing of dental materials. *J Dent*. 2000; 28(5):341–5.
- Portaels F, Traore H, de Ridder K, Meyers WM. *In vitro* susceptibility of *Mycobacterium ulcerans* to clarithromycin. *Antimicrob Agents Chemother*. 1998; 42: 2070–2073.
- Portet B. 2007. Recherche bioguidée de molécules antipaludiques d'une plante guyanaise : *Piper hostmannianum* var. *berbicense*. Thèse de doctorat. Université de Toulouse.
- Rajput AP and Rajput TA. Isolation of stigmaterol and beta-sitosterol from chloroform extract of leaves of *corchorus fascicularis* Lam. *International Journal of biological chemistry*, 2012; 6 (4): 130–135
- Rana FS, Hawken MP, Mwa-chari C. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. *J Acquir Immune Defic Syndr*. 2000; 24, 23–29.
- Sakirigui A, Kpoviessi DSS, Gbaguidi F, Kossouh C, Poupaert J, Moudachirou M, Accrombessi CG. Antitrypanosomal activity and toxicity of substituted citronellal semicarbazones and thiosemicarbazones hemi-synthesized *in situ* in the essential oil of *Eucalyptus citriodora*. *Journal of Chemical and Pharmaceutical Research*, 2012; 4(9):4373–4380.
- Sakirigui A, Kpoviessi salomé DSS, Gbaguidi F, Kossouh C, Bero J, Quetin-Leclercq J, Moudachirou M, Poupaert J, Accrombessi CG. Selective trypanocide activity of some substituted thiosemicarbazones of citral from benin *cymbopogon citratus* essential oil and their toxicity against *Artemia salina* Leach. *International Journal of Research and Reviews in Applied Sciences*, 2012; 12 (3):454–462
- Sannomiya M, Montoro P, Piacente S, Pizza C, Brito A, and Vilegas W: Application of liquid chromatography/electrospray ionization

tandem mass spectrometry to the analysis of polyphenolic compounds from an infusion of *Byrsonima crassa* Niedenzu. Rapid Communications in Mass Spectrometry. 2005; 19 (16): 22-44.

Sanogo B. 2010. Etude des infections respiratoires aiguës en milieu communautaire chez les enfants de moins de 5 ans dans les régions de kayes, sikasso, segou et mopti. Thèse de doctorat en Médecine. Université de Bamako ; 72 p

Singh V, Raghav KP. Review on pharmacological properties of *Caesalpinia bonduc* L. Int. J. Med. Arom. Plants, 2012; 2 (3): 514-530.

Stevigny C, Block S, Pauw-Gillet M-C, de Hoffmann E, Llabres G, Adjakidje V, Quetin-Leclercq J. "Cytotoxic aporphine alkaloids from *Cassytha filiformis*." *Planta Medica*, 2002; 68: 1042-1044.

Tiuman TS, Ueda-Nakamura T, Garcia Cortez DA, DiasFilho BP, Morgado-Diaz JA, de Souza W, Nakamura CV: Antileishmanial activity of parthenolide, a sesquiterpene lactone isolated from *Tanacetum parthenium*. Antimicrobial agents and Chemotherapy. 2005; 49: 176-182.

Tosun F, AkyüzKizilay C, Sener B, Vural M, Palittapongarnpim P. Antimycobacterial screening of some Turkish plants. *J Ethnopharmacol*. 2004; 95: 273-275.

Verville J. 2013. Éruptions cutanées et autres problèmes de la peau associés au VIH/sida.

Yèhouénou BB. 2012. Composition chimique et activités antimicrobiennes d'extraits végétaux du Bénin contre les micro-organismes pathogènes et altérants des denrées alimentaires. Thèse unique de doctorat. Université d'Abomey-Calavi. Bénin. 221p

Yemoa A, Gbenou J, Affolabi D, Moudachirou M, Bigot A, Anagonou S, Portaels F, Quetin-Leclercq J, Martin A. Buruli Ulcer: A Review of In Vitro Tests to Screen Natural Products for Activity against *Mycobacterium ulcerans*. *Planta Med*, 2011; 77: 641-646.

How to cite this article:

Alban G. Houngbèmè Habib M.Y. Ganfon, Sédami Medegan, Boniface Yèhouénou, Bouraïma Bambola, Clément Gandonou, and Fernand A. Gbaguidi. Antimicrobial activity of compounds from *Acanthospermum hispidum* DC and *Caesalpinia bonduc* (L.) ROXB: Beninese plants used by healers against HIV-associated microbial infections. *J App Pharm Sci*, 2015; 5 (08): 073-081.