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PHYTOCHEMICAL ANALYSIS, TOXICITY AND ANTIBACTERIAL ACTIVITY OF BENIN MEDICINAL PLANTS EXTRACTS USED IN THE TREATMENT OF SEXUALLY TRANSMITTED INFECTIONS ASSOCIATED WITH HIV/AIDS

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ABSTRACT: Phytochemical screening of thirteen medicinal plants used in the treatment of sexually transmitted infections (STI), HIV/AIDS in Benin, showed that mucilage, gallic tannins and anthocyanins were the most common chemical groups. Water-ethanol (HE): 4/6 *v/v*, aqueous (A) and dichloromethane (DCM) extracts were prepared for each plant and tested for toxicity against shrimp larvae (*Artemia salina* Leach). Twenty two extracts were found not toxic (LC_{50} ≥ 0.1 mg / mL) with A13 aqueous extract of *Vitex doniana Sweet* (LC_{50} = 1.35 mg / mL) as the most selective one. Antibacterial tests of extracts showed bacteriostatic activity on both strains tested (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) with the minimum inhibitory concentrations (MIC) ranging from 0.625 mg / mL to 5 mg/mL on *E. coli* and 0.078 to 1.25 mg/ml on *S. aureus*. Only the hydroethanolic extract of *Acanthospermum hispidum* (HE7) showed bactericidal activity against both strains with minimum bactericidal concentrations (MBC) values of 2.5 mg/mL and 0.625 mg/mL on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 respectively, while the hydro ethanolic extracts of *Ocimum gratissimum* (HE3), *Caesalpinia bonduc* (HE9) and *Calotropis procera* (HE10) were active against *S. aureus* ATCC 25923 with MBC values of 0.3125 mg/mL, 0.625 mg/mL and 1.25 mg/mL, respectively.

INTRODUCTION: Sexually transmitted infections have become nowadays a most significant public health concern and can lead to very serious complications in the absence of effective treatment.

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In a context of high HIV-AIDS, any strategy that aims at improving access to effective treatments must take into account the priority communities (especially rural) with limited access to health services because of their difficult economic status.

Health systems in Africa are facing nowadays many challenges due to the poor performance of available preventive and curative services and to the high cost of services in hospitals [1]. Faced with these difficulties, Traditional Medicine remains the primary source of medical care to the health needs of increasingly growing population when we know that 80% of the African population makes

increasingly use of medicinal plants to treat themselves [2]. This work aims at exploring scientifically the Beninese plants identified to treat sexually transmitted infections (STI) often associated to HIV/AIDS [3] in order to confirm their antimicrobial properties. In this study, the antibacterial potency of extracts of Beninese plants most commonly used against STI by traditional healers [3] will be evaluated on different strains involved in STI HIV/AIDS, their phytochemical and their general toxicity will also be assessed.

MATERIALS AND METHODS:

Material: Thirteen plants **(Table 1)** selected by an ethnobotanical survey in Benin in December 2010 [3] used by traditional healers for the treatment of STI of HIV/AIDS, were harvested and dried for ten days in a room at constant temperature (air conditioning) and carefully powdered with an electric grinder (of Flour MILLS NIGERIA, El MOTOR No 1827). Eggs of Shrimp larvae (*Artemia salina* Leach) commercialized by German company JBL GmbH & Co.KG, were used as biological material for assessing the toxicity of plant extracts. Reference strains (American Type Culture Collection): *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* 25922 were purchased from the company Biomerieux SA in France and used to evaluate the antimicrobial activity of extracts. These strains were maintained by subculture on nutrient agar supports growth [4].

TABLE 1: LIST OF STUDIED PLANTS

S. No.	Species	Familly	Part used	Lieu de récolte
1	*Jatropha curcas L.*	Euphorbiaceae	leaves	Danto
2	*Momordica charantia L.*	Cucurbitaceae	leafy stem	Sèmè-Podji
3	*Ocimum gratissimum L.*	Lamiaceae	leaves	Danto
4	*Senna alata syn. Cassia alata L.*	Caesalpiniaceae	leaves	Adjohoun
5	*Crateva religiosa Forst.*	Capparaceae	leaves	Adjohoun
6	*Centella asiatica(L.)Urban*	Apiaceae	leaves	Sèmè-Podji
7	*Acanthospermum hispidum DC.*	Asteraceae	A .P	Danto
8	*Amaranthus graecizans L.*	Amaranthaceae	leaves	Sèmè-Podji
9	*Caesalpinia bonduc (L.) Roxb.*	Caesalpiniaceae	leaves	Adjohoun
10	*Calotropis procera (Aiton) W.T. Aiton*	Asclepiadaceae	leaves	Abomey-calavi
11	*Khaya Senegalensis (Desr.)A. Juss.*	Meliaceae	leaves	Abomey-calavi
12	*Lantana camara L.*	Verbenaceae	leaves	Danto
13	*Vitex doniana Sweet*	Verbenaceae	leaves	Adjohoun

A .P: aerial parts

Phytochemical screening: The phytochemical screening of the extracts was performed according to the standard procedures : Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars, Fehling's test for glycosides, Liebermann-Burchard's test for triterpenoids, Liebermann-Burchard's test for steroids, frothy test for saponins, Shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard's test for free cyanogenetics derived and Borntrager's test for free anthraquinones [5].

Preparation of crude extracts: Fifty grams (50 g) of each powdered plant were mixed with 500 mL of solvent [distilled water (A), water-ethanol: 4/6, v/v (HE) and dichloromethane (DCM)]. The mixture was macerated for 72 hours and filtered three times successively. Then the filtrate was evaporated to dryness at 40°C using a rotary evaporator (Heidolph efficient Laborota 4000) coupled to a water chiller (Julabo FL 300) to give the crude extracts.

Larval toxicity test: The test is performed against *Artemia salina* Leach by the method of Fatondji *et al* [6], Sakirigui *et al* [7, 8] and proposed in the literature as a simple bioassay method for assessment of preliminary toxicity of natural active products [10]. The eggs of *Artenia salina* were incubated in sea water until hatching of young larvae (48 hours). Then, series of solutions of each tested crud extract at varying and progressive concentrations were prepared. A defined number of larvae (16) were introduced into each solution. All solutions and control solution containing no active substance were left under stirring for 24 hours.

Counting under a microscope the number of dead larvae in each solution was used to evaluate the toxicity of the solution. In the case, where there was death in the control medium, the data was corrected by Abbott's formula:

% death = [(test - control) / control)] x 100 [11]

Data (dose-response) are transformed by logarithm and the LC_{50} were determined by linear regression [12]. Tests were carried out in triplicate.

***In vitro* antibacterial test:** The evaluation of the sensitivity of microorganisms *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 to different extracts prepared was carried out by the technique of broth dilution microplate (96 wells) coupled with the spread on solid medium such that it was described by Moussaid *et al* [4] and successfully tested by Kpadonou *et al* [9]. The test is based on the determination of minimum inhibitory and bactericidal concentrations (MIC and MBC) and to calculate of the antibiotic potency (a.p) of the tested extracts. For this, a stock solution of 20 mg/mL of DMSO (1%) of each extract was prepared and filtered through Millipore membranes of 0.2 microns (Acrodisc, USA). After identification and isolation of different strains, a microbial suspension 10^6 colony forming units (CFU/mL) was prepared according to NCCLS McFarland Scale 2, 2002 by dissolving in a sterilized tube colony of germs in 5 mL mid Mueller Hinton Broth (MHB) and then with the whole in an oven at 37°C for 2 hours.

Two blank tests were performed during this test: negative control containing extract (successive dilutions of 100 µL of extract with 1% DMSO) and a positive control for controlling the growth of germs on the first two rows of the microplate. Then on the third line, 100 µL of microbial suspension is introduced into 100 µL of each of the ten dilutions of extract (successive twofold dilutions with 1% DMSO) and the plate is covered and incubated in an oven at 37°C. Twenty-four hours after incubation, we determined macroscopically the minimum inhibitory concentration (MIC) [15].

The MBC was determined following the MIC by transplanting dilutions whose concentration is greater than or equal to the IJC on the solid medium Mueller Hinton agar 38 g/L poured into sterile boxes kneaded and the antibiotic potency (ap) of the extracts was calculated by ap = MBC / MIC. When ap is less than 4, this means that the tested extract has antibiotic power.

RESULTS AND DISCUSSION:

Phytochemical screening: Phytochemical screening results were summarized in **Table 2**. Among these plants, eleven (11), ten (10) and nine (9) contained mucilage, gallic tannins and anthocyanins, respectively.

TABLE 2: RESULTS OF PHYTOCHEMICAL SCREENING

Plants	Al	TC	TG	Fl	Ant	Leu	Qn	SP	Tp	St	Cy	Mu	Cm	Cr	Hl	O-H	C-H	H-C
Jatropha curcas	-	-	++	-	++	++	-	++	++	-	-	++	-	++	-	-	-	-
Momordica charantia	-	-	-	+*	+	-	-	-	-	-	-	++	-	+	-	-	-	-
Ocimum gratissimum	-	-	++	-	++	-	-	-	-	-	-	++	-	-	-	-	-	-
Senna alata	-	++	-	+*	++	+ /-	-	-	-	-	-	++	-	++	-	-	-	++
Crateva religiosai	-	-	++	++	-	-	-	-	++	-	-	++	-	-	++	-	-	-
Centella asiatica	-	++	++	-	++	++	-	-	++	++	-	++	+	++	-	-	-	-
Acanthospermum hispidum	+	-	++	+*	-	-	-	-	+	+	-	+	-	-	-	-	-	-
Amaranthus graecizans	-	-	++	-	-	-	-	++	++	++	-	++	-	-	-	-	-	-
Caesalpinia bonduc	-	-	++	-	-	-	-	++	++	-	-	++	-	-	-	-	-	-
Calotropis procera	-	-	++	+*	++	-	-	++	++	++	-	++	-	++	-	-	-	-
Khaya Senegalensis	-	+	-	+*	+	+	-	+	-	+	-	-	+	+	+	-	-	-
Vitex doniana	-	-	+	+ /-	+	-	-	-	-	-	-	+	-	+	+	+	-	-
Lantana camara	-	++	++	-	++	++	-	++	++	-	-	-	++	++	++	-	-	-
Totaux[¤]	1	4	10	6	9	4	0	6	8	5	0	11	3	8	4	1	0	1

- (absent or not reveled); +/- (doubtful presence) + (present) ; ++ (abundant) ; ¤: Number of plants containing a given chemical group * orange color. Al: alkaloids, TC: catechin tannins, TG: gallic tannins, Fl: flavonoids, St: Steroids, Tp: terpenes, Leu Leucoanthocyanes, Ant: Anthocyanins, Mu: Mucilage, O-H: O-heterosides, C-H: C-heterosides; Hl: free glycosides, Cr: reducing compounds; Cm: Coumarins, Hc: cardiac heterosides; Qn: Quinones, Sp: saponins, Cy cyanogenic derivatives.

Thus, these three chemical groups (mucilage, gallic tannins and anthocyanins) were the most common ones in the studied plants. They contained at least one of the chemical groups such as alkaloids, anthocyanins, leuco anthocyanin, reducing compounds, mucilage, terpenes and steroids which possessed various pharmacological properties including anti-edematous, anti-inflammatory, antibacterial, antiviral, antifungal, antiseptic, anti-tumor, healing. These results could explain in part, the use of these plants in the treatment of STIs related to HIV-AIDS. But the presence of cardiac glycosides compounds in the leaves of *Senna alata* indicates that this plant should be used with caution.

Larval toxicity and antibacterial activity of the extracts: LC_{50} values, minimum inhibitory and bactericidal concentration and antibiotic power of tested extracts were summarized in **Table 3.**

TABLE 3: VALUES OF LETHAL CONCENTRATIONS 50% (LC_{50}), MINIMUM INHIBITORY OR BACTERICIDAL CONCENTRATION, SELECTIVITY INDEX AND ANTIBIOTIC POWER OF THE TESTED EXTRACTS.

Extracts	LC 50 (mg/ml)	Strains							
		Escherichia coli				*Staphylococcus aureus*			
		MIC	SI	MBC	p.a	MIC	SI	MBC	p.a
DCM1	0.73	0.625	1.17	-	-	0.625	1.17	-	-
DCM2	5.4 10^{-5}	2.5	2.16 10-5	-	-	0.625	8.64 10-5	-	-
DCM3	0.04	0.625	0.07	-	-	0.625	0.06	-	-
DCM4	0.04	0.625	0.07	-	-	0.3125	0.13	-	-
DCM5	0.19	0.3125	0.61	-	-	0.3125	0.61	-	-
DCM6	0.07	5	0.02	-	-	1.25	0.06	-	-
DCM7	0.41	1.25	0.33	-	-	0.3125	1.31	-	-
DCM8	0.03	2.5	0.01	-	-	0.1562	0.19	-	-
DCM9	0.14	2.5	0.06	-	-	0.625	0.22	-	-
DCM10	0.07	0.625	0.11	-	-	0.3125	0.22	-	-
DCM11	1.26	1.25	1.08	-	-	1.25	1.01	-	-
DCM12	0.17	1.25	0.14	-	-	0.3125	0.54	-	-
DCM13	0.59	0.3125	1.88	-	-	1.25	0.47	-	-
HE1	0.04	0.625	0.07	-	-	0.0781	0.51	-	-
HE2	0.02	5	0.01	-	-	0.625	0.03	-	-
HE3	0.05	2.5	0.02	-	-	0,3125	0.16	0.3125	1
HE4	0.19	2.5	0.08	-	-	1.25	0.15	-	-
HE5	0.19	1.25	0.15	-	-	1.25	0.15	-	-
HE6	0.04	2.5	0.02	-	-	1.25	0.03	-	-
HE7	0.39	1.25	0.31	2.5	2	0.3125	1.25	0.625	2
HE8	0.05	2.5	0.02	-	-	0.3125	0.16	2.5	8
HE9	0.35	1.25	0.28	-	-	0.625	0.56	0.625	1
HE10	0.02	1.25	0.02	-	-	1.25	0.02	1.25	1
HE11	0.10	2.5	0.04	-	-	1.25	0.08	-	-
HE12	0.23	1.25	0.18	-	-	1.25	0.18	-	-
HE13	0.08	1.25	0.07		-	1.25	0.06	-	-
A1	0.01	5	0.002	-	-	0.625	0.16	-	-
A2	0.02	2.5	0.01	-	-	0.3125	0.06	-	-
A3	0.19	5	0.04	-	-	1.25	0.15	-	-
A4	0.06	2.5	0.02	-	-	1.25	0.05	-	-
A5	0.13	0.625	0.21	-	-	0.1562	083	-	-
A6	0.04	5	0.01	-	-	0.625	0.06	-	-
A7	0.28	1.25	0.22	-	-	1.25	0.22	-	-
A8	0.59	5	0.11	-	-	0.625	0.94	-	-

A9	0.14	2.5	0.05	-	-	0.3125	0.44	-	-
A10	0.23	5	0.04	-	-	0.625	0.36	-	-
A11	0.7	2.5	0.28	-	-	0.625	1.12	-	-
A12	0.19	0.625	0.30	-	-	1.25	0.15	-	-
A13	1.35	0.3125	4.35	-	-	0.1562	8.64	-	-

MIC: Minimum Inhibitory Concentration (mg/mL) MBC: minimum bactericidal concentration (mg/mL), pa: antibiotic power; DCM: dichloromethane extract, A: aqueous extract, HE: hydro-ethanolic extract, SI = LC_{50} / MIC: Selectivity index. The indices 1, 2... 13 were the numbers of tested plants in Table 1.

There is a net correlation between toxicity against shrimp larvae and cytotoxicity on 9KB and 9PS (nasopharygien human carcinoma) cells, on the one hand [10] and cytotoxicity on A-549 carcinoma lung cells and HT -29 colon carcinoma cells, on other hand [11]. To assess the degree of toxicity from the LC_{50} values, we used the correlation of **Table 4** [12].

TABLE 4: CORRESPONDENCE BETWEEN LC_{50} AND TOXICITY

LC_{50}	Toxicity
$LC_{50} \geq 0.10$ mg/mL	No toxic
0.10 mg/mL > $LC_{50} \geq 0.05$ mg/mL	Low
0.05 mg/mL > $LC_{50} \geq 0.01$ mg/mL	Moderate
$LC_{50} < 0.01$ mg/mL	Higth

According to **Table 4,** LC_{50} values proved that 22 extracts were not toxic; 6 extracts showed low toxicity; 10 extracts were moderately toxic while only one extract (DCM2) had a high toxicity. The aqueous extracts are non-toxic. The A13 extract was the least toxic extract with LC_{50} value of 1.35 mg / mL and the second one was DCM_{11} extract (LC_{50} = 1.26 mg/mL). A13 exhibits on both strains a very nice selectivity index of 432 on *Escherichia coli* and 864 on *Staphylococcus aureus*.

For all the tested extracts, MICs values ranged from 0.625 mg/mL to 5 mg/mL for *E. coli* 25922 and 0.078 to 1.25 mg/mL for *S aureus* 25923. Moreover, the CMBs range from 0 to 2.5 mg / mL for *E. coli* and 0.3125 mg / mL to 2.5 mg / mL for *S. aureus*. Similarly, we note that only the hydroethanolic extract of *Acanthospermum hispidum* (HE7) showed an antibiotic power (ap ≤ 4) on *E coli* and four extracts (HE7, HE3, HE10 and HE9) showed on *S. aureus*. *E coli* (Gram-) were the most resistant organism and *S. aureus* (Gram+) was the most sensitive to these plant extracts. The *Acanthospermum hispidum* dominated by the presence of gallic tannins and accessorily triterpenes, steroids and mucilage, showed bactericidal activity against *E. coli* ATCC 25922. This activity seemed to be due to its composition rich in gallic tannins and a synergistic activity with flavonoids, triterpenes, steroids and mucilage [13]. It was the same with regard to its bactericidal property against *S. aureus* ATCC 25923, with MBC four (4) times less than that obtained on *E. coli* ATCC 25922. As extracts HE3, HE9, HE10, they did not present bactericidal activity against *E. coli* ATCC 25922.

However, they had a bactericidal effect on *S. aureus* ATCC 25923 with MBC values of 0.3125 mg/mL, 0.625 mg/mL and 1.25 mg/mL, respectively. These MBC values were identical to the corresponding CMI and their antibiotic power identical and equal to unity. This shows that the hydroethanolic extracts of *Ocimum gratissimum*, *Caesalpinia bonduc* and *Calotropis procera* were bioactive against strains of *S. aureus* ATCC 25923, a gram +ve.

The extract HE10 has a MBC twice higher than HE3 extract. Its bactericidal activity against *S. aureus* ATCC 25923 seemed to be due to the presence in this extract of saponins, triterpenes, steroids, flavonoids and reducing compounds that strengthen the activity of the compounds having in common with the HE3 extract as gallic tannins, anthocyanins and mucilage. In the same way, the extract HE8 has bactericidal activity against *S. aureus* ATCC 25923 but its antibiotic power is negligible.

The HE8 extract contained the same compounds as the extract HE10 with the only difference that HE10 contained more anthocyanins and reducing compounds. These two compounds gave a better efficiency compared to extract HE8 that has a low antibiotic power (Pa = 8). Among the four extracts (HE3, HE7, HE9, and HE10) that have bactericidal activity on *S. aureus* ATCC 25923, HE7 was the most active one on *E. coli* ATCC 25922 with a fair selectivity (SI = 1.25 > 1).

This extract deserves to be investigated because of its antibiotic power (Pa = 2) on both tested strains. Our results were in harmony with those of Kpadonou-Kpoviessi *et al* [13] who report antimicrobial activities and toxicity of ethanol extracts and fractions of Ocimum gratissimum and those of Kraus *et al* [14] who showed bactericidal and fungicidal effects of methanol extracts of the aerial part of *Acanthospermum hispidum* on pathogenic germs as well as nonpathogenic namely *Bacillus subtilis*, *Pseudomonas stutzen* and *Cladospodum cucumerinum*.

Also in 2003, Fleisher *et al* [15] showed that polar fractions of ethanol extracts of leaves and flowers of the plant had a good antibiotic activity on various pathogens, those nonpolar one were found less active. As for the aqueous extract, no activity was shown. In 2007, the antibiotic properties of six isolated sesquiterpenes of *Acanthospermum hispidum* DC (Asteraceae) were tested by Cartagena *et al* [16]. In total, this dual bactericidal activity showed on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 confirmed its antibiotic power against the two germs.

Therefore, it is active on both of the positive and negative gram organisms whose bacterial membranes do not have the same chemical compositions. Gram-negative organisms contain in their walls, in addition to peptidoglycan, lipopolysaccharides and phospholipids which reduce the penetration of the extract into the cytoplasm. Thus gram-negative strains are less sensitive to the plant extracts than gram -ve [9, 17, 18, 19]. This could explain the lack of MBC in some extracts tested.

CONCLUSION: This study helps to show that all plant extracts were less toxic against *Artemia salina* LEACH except dichloromethane extract from *Momordica charantia L*. All extracts inhibited the growth of tested bacteria. But only hydroethanolic extracts of four plants showed bactericidal effects. These included the extract of *Acanthospermum hispidum* (HE7) that presented bactericidal activity in both *E. coli* and *S. aureus* and extracts of *Ocimum gratissimum* (HE3), *Caesalpinia bonduc* (HE9) and *Calotropis procera* (HE10) which killed only *S. aureus*.

It should be pointed out however that A13 exhibits a very nice profile with a selectivity index culminating at 432 on *Escherichia coli* and 864 on *Staphylococcus aureus.*

The results reinforce those of the phytochemical analysis in the justification of their antimicrobial activity attributed to these plants. These bioactive extracts are therefore good candidates for the enrichment of the therapeutic arsenal against infectious diseases. Our work could also help for further work on the activity study of these plants in other strains (*Mycobacterium tuberculosis*, *Mycobacterium ulcerans* and *Neisseria gonnhoreae*) involved in opportunistic sexually infections transmitted HIV - AIDS and on the possible isolation of active compounds.

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REFERENCES:

1. Nikiema P and Wendpagnagdé R : Propriétés pharmacochimiques de *Calotropis procera* Ait. Récolté au Mali : Etude préclinique des effets anti-inflammatoires et antimicrobiens des extraits des écorces de racines. Thèse de Doctorat 2005; Université de Bamako.
2. Etou-Ossibi A-W, Nzonzi J, Mombouli JV, Nsondé-Ntandou GE, Ouanba JM, Abena AA : screening chimique et effets de l'extrait aqueux du *Lippia mutiflora* Moldenke sur le cœur isolé du crapaud. J Phytothérapie 2005; 5:193-199.
3. Houngnihin RA, Gbaguidi F, Agbani OP, Glo V, Dossou P, Dissou F, Medegan-Fagla VK, Trougnin V : Monographie de cent (100) plantes utilisées en Médecine traditionnelle pour la prévention et le traitement des affections opportunistes du VIH-SIDA, 1ère Edition 2010 Bénin; 104p.
4. Moussaid M, Elamrani A A, Bourhim N and Benaissa M: Bulbes d'*androcymbium gramineum* (*cav.*) *J.f.macbr.* (colchicaceae): composition chimique, étude pharmacologique et toxicologique chez les souris. *Bulletin de la Société Royale des Sciences de Liège* 2012 ; 81 : 90 – 100.
5. Dougnon TV, Klotoé JR, Sègbo J, Atègbo J, Edorh AP, Gbaguidi F, Hounkpatin AS, Dandjesso C, Fah L, Fanou B, Dramane K, Loko F: Evaluation of the phytochemical and hemostatic potential of *Jatropha multifida* sap. African Journal of Pharmacy and Pharmacology 2012; 6 (26):1943-1948
6. Fatondji HR, Gbaguidi F, Kpoviéssi 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

7. Sakirigui A, Kpoviessi DSS, Gbaguidi F, Kossouoh 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
8. Sakirigui A, Kpoviessi salomé DSS, Gbaguidi F, Kossouoh C, Bero J, Quetin-Leclercq J, Moudachirou M, Poupaert Jacques, Accrombessi CG: Selective trypanocide activity of some substituted thiosemicarbazones of citral from benin *cymbopogon citratus* essential oil and their toxicity against *Artemia salina* Leach. ijrras 2012; 12 (3):454-462
9. Kpadonou-Kpoviessi BGH, Yayi LE, Kpoviessi DSS, Gbaguidi F, Yèhouenou B, Quetin-Leclercq J, Figueredo G, Moudachirou M and Accrombessi GC : Chemical variation of essential oil constituents of *ocimum gratissimum* L. from Benin., and Impact on antimicrobial Properties and toxicity against *Artemia Salina* LEACH. Chem. Biodiversity 2012; 9: 139-150.
10. Pelka M, Distler W, Petschelt A and Dent J: A new screening test toxicity testing of dental materials 2000; 28: 341-345.
11. Carballo JL, Hernandez-Inda ZL, Pérez P and Garcia-Gràvalos MD: A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. BMC Biotechnology 2002; 2:17.
12. Mousseux M : Test de toxicité sur larves d'*Artemia salina* : entretien d'un élevage de balanes. 1995; Nouméa, Centre Universitaire de Nouvelle Calédonie. 20 p.
13. Kpadonou-Kpoviessi BGH, Kpoviessi DSS, Yayi-Ladekan E, Gbaguidi F, Yehouenou B, Mansourou M and Accrombessi GC: Phytochemical screening, antimicrobial activities and toxicity against *Artemia salina* Leach of extracts and fractions of *Ocimum gratissimum Linn* from Benin. J Chem Pharm Res 2013; 5(10):369-376
14. Kraus W, K6ll-Weber M, Maile R, Wunder T, and Vogler B: Biologically active constituents of tropical and subtropical plants. Pure & Appl Chem 1994; 66: 2347-2352.
15. Fleischer TC, Ameade EPK and Sawer IK: Antimicrobial activity of the leaves and flowering tops of Acanthospermum hispidum. Fitoterapia 2003; 74 (1-2):130-132.
16. Cartagena E, Bardon A, Catalan CAN, de Hernandez ZNJ, Hernandez LR and Joseph-Nathan P: Germacranolides and a new type of guaianolide from *Acanthospermum hispidum.*. Journal of Natural Products 2000; 63: 1323-1328.
17. Yèhouenou B, Noudogbèssi JP, Sessou P, Avlessi F and Sohounhloué D : Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de *Xylopia aethiopica* (DUNAL) A. Rich. contre les pathogènes des denrées alimentaires. J Soc Ouest-Afr Chim 2010 ; 029 : 19-27.
18. Yèhouenou B, Wotto V, Bankolé L, Sessou P, Noudogbèssi JP and Sohounhloué D: Chemical Study and antimicrobial activities of volatile extracts from fresh leaves of *Crassocephalum rubens* (Juss & Jack) S., Moore against food-borne pathogens. Scientific Study & Research 2010; 11 (3): 343-351.
19. Bajpai KV, Dung NT, Kwon JO and Kang SC: Analysis and the potential application of essential oil and leaf extracts of *Silene Americana* L. to control food Spoilage and food-borne pathogens. J Food Technol 2008; 227: 1613-1620.

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