

Original Research Article

Antifungal activity of roots barks extract of *Securinega virosa* and *Anogeissus leiocarpa*, two plants used in the traditional treatment of candidiasis in northern Côte d'Ivoire.

ABSTRACT

Aims: To evaluate the anticandidal activity of some solvent extracts of *Securinega virosa* and *Anogeissus leiocarpa* from northern Côte d'Ivoire.

Place and Duration of Study: Biochemical Pharmacodynamics Laboratory, Biosciences Department, University Félix Houphouët-Boigny and Bacteriology-Virology Laboratory University Hospital of Treichville, Côte d'Ivoire between January and December 2013.

Methods: The herbs studied were examined for diameter of inhibition zone using agar well diffusion method; minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) using microdilution method.

Results: All tested plants extracts, except the aqueous extracts, showed varying zones of inhibition against fungi tested. The diameters of inhibition zones for all organic extracts are greater than 10mm for a sample concentration of 500 mg/mL and were significantly higher than for nystatine ($p < 0.05$; $p < 0.01$). The ethanol extract of *A. leiocarpa* revealed the strongest anticandidal activity against all tested strains with MICs ranging from 0.195 to 12.5mg/mL, and MFCs from 0.390 to 50 mg/mL. The phytochemical screening of extracts shows the presence of polyterpenes and sterols, polyphenols, flavonoids, catechin tannins and alkaloids.

Conclusion: *S. virosa* and *A. leiocarpa* possess compounds with good anticandidal properties that may be used for certain infectious diseases caused by certain *Candida* species.

Keywords: *Anogeissus leiocarpa*, *Securinega virosa*, anticandidal activity, northern Côte d'Ivoire.

1 INTRODUCTION

Whatever its origins and its civilization, man has sought in his plant or animal environment remedies to overcome his health problems. Research and knowledge of the properties of drugs have spawned real pharmacopoeia [1]. Medicinal plants and their knowledge are for the African continent a rich heritage hitherto insufficiently unexplored. Adjanohoun identified nearly 50.000 species [2]. In Côte d'Ivoire are nearly 800 species of medicinal plants and more than 1.400 drug recipes that have been identified [3]. The study of biological and chemical properties showed that the Ivorian flora has a real therapeutic and nutritional potential and can be used to treat or prevent many diseases [4]. These plants have a real interest in human health for their antibacterial activities [5, 6, 7], antifungal [8, 9], antiplasmodial [10] and antioxidant [11]. Infectious diseases are a serious public health problem in developing countries where they are the main cause of high mortality rates, and in industrialized countries where resistance to existing antibiotics are growing alarmingly. This creates a growing need to find new antimicrobial compounds and/or inhibitory mechanisms of antibiotic resistance. Therefore, some 250-300.000 plants species inventoried, only 5 to 15% have been investigated for bioactive

molecules, represent a huge pool of potential new bioactive compounds [12]. According to some authors, the naturally occurring compounds have the advantage of a great diversity of chemical structures and they also have a wide range of biological activities [13]. In this context, the present work was focused on two plants whose root barks are used in traditional treatment of microbial infections in northern Côte d'Ivoire: *Securinega virosa* and *Anogeissus leiocarpa*. Various researches have focused on the study of the biological activities of leaves and stem barks of these plants. However, there are very few studies on the evaluation of the antifungal activity of the root bark of plants.

This work intends to evaluate the *in vitro* antifungal properties of different extracts of these two plants of the Ivorian flora on three species of *Candida* namely *Candida albicans*, *Candida tropicalis* and *Candida glabrata*.

2. MATERIAL AND METHODS

2.1 Plant material

The plant material consists of the root bark of *Anogeissus leiocarpa* and *Securinega virosa*. These organs are harvested in January 2013 to Kouto, town located to 725 km north of Abidjan after an ethnobotanical survey of traditional healers in the community. These barks were dried out of the sun for two weeks before being ground into a fine powder by grinding. From the powder obtained after spraying, the different extracts to be tested were prepared.

2.2 Fungal strains

The fungal strains tested were *Candida albicans*, *Candida glabrata* and *Candida tropicalis*, yeasts isolated to pathological products from consulted patients with candidiasis in the Laboratory of Bacteriology-Virology University Hospital Center of Treichville (Abidjan, Côte d'Ivoire). These strains are maintained in a refrigerator at 4 °C for the duration of the experiment.

2.3 Preparation of plant extracts

2.3.1 Preparation of aqueous total extract

The total aqueous extract was obtained by dissolving 50g of the plant powder in 2 L of distilled water. The macerate is mixed for 48 hours using a magnetic stirrer type IKAMAG RCT at a temperature of the laboratory. The homogenate is filtered two times successively on cotton wool and then once on Whatman filter paper N°3. The filtrate obtained is reduced using a rotary evaporator type IKA Labortechnik before being evaporated at 50 °C using an oven type Med Center Venticell. The powder obtained for each plant is the total aqueous extract and were stored at 5 °C.

2.3.2 Preparation of the organic extracts

The extraction method used is that described by Manga *et al* [14]. The plant material is subjected to a first out with solvents of increasing polarity of dichloromethane, ethyl acetate, ethanol and water.

The liquid-liquid chromatography also called partition chromatography is based on the sharing of the solute in the two immiscible liquid phases. This chromatography required the use of three different polarity solvents namely dichloromethane, ethyl acetate, ethanol. To achieve this chromatography, 20g of aqueous extract plant are added to 100 mL of water. This solution was transferred into a separatory funnel of 500 mL in which 100 mL of dichloromethane are then added. The vial was stirred for 2 min and then allowed to sedimentation. The lower aqueous phase is then collected. This

operation is repeated four times and the fractions collected dichloromethane (400 mL) were evaporated to dryness. The aqueous phase is then extracted with 100 mL of ethyl acetate four times. The ethyl acetate fractions (400 mL) were combined and then evaporated to dryness. The aqueous phase is then extracted with 100 mL of ethanol 4 times then ethanol fractions (400 mL) were combined and then evaporated to dryness. The aqueous phase is evaporated to dryness. These different fractions then were kept in refrigerator at 5°C before use.

2.4 Preparation of inoculum

Fungal strains tested are seeded on Petri dishes containing Sabouraud agar and incubated for 48 to 72 hours to obtain young cultures and isolated colonies. From these boxes, using a platinum loop two well-isolated colonies and identical are perfectly picked and put in 10 mL of Sabouraud broth and incubated for 3-5 hours at 37°C for pre-culture. A volume of 1 mL of this broth was taken and added to 10 mL of Sabouraud's broth. This fungal suspension produced is valued approximately at 10^5 cells/mL and was the pure inoculum.

2.5 Preparation of concentration ranges

The range of extracts concentrations was prepared by double dilution method according to a geometric progression of ratio 1/2 with concentrations ranging from 500 mg/mL to 7.81 mg/mL. These tubes are then autoclaved at 121°C for 15 min.

2.6 Antifungal activity

2.6.1 Sensitivity test

Antifungal activity was screened by agar well diffusion method described by Bakkiyaraj and Pandiyaraj [15], Irshad *et al* [16] and Adekunle *et al* [17]. The principle of this method is based on the spread of antimicrobial in solid media into a Petri dish by creating a concentration of gradient after a certain time of contact between the drug and the tested microorganisms. The antimicrobial effect of the drug on the tested microorganisms is assessed by the measurement of a zone of inhibition; and depending on the diameter of inhibition.

Sabouraud medium is poured into sterilized Petri dish to a thickness of 8 mm. After inoculation by flooding adequate dilution (approximately 10^5 cells/mL) of the tested strains made, wells of 6 mm diameter are formed concentrically in the agar. Each well is then filled with 20 µl of a given concentration of the extract. After a pre-diffusion of 45 minutes at room temperature in the hood, the strains are incubated at 37°C for 24 hours after which the diameters of the zones of inhibition are determined. Alongside Nystatin a standard antifungal was used.

2.6.2 Determination of antifungal parameters: Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC)

The incorporation of the different extracts was made in Sabouraud in inclined tubes according to the double dilution method previously described by Yayé *et al* [18] and Ouattara *et al* [19].

A serie of 7 tubes were prepared according to the method of double dilution with concentrations ranging respectively from 50 to 0.390 mg/mL.

The tubes containing were then inclined with a small base at the temperature of the room so that the agar solidifies. After solidification, agar seeding or not containing the extract (control) is performed on

the surface with 1 µl of pure inoculum. The range of both inoculated and control tubes were incubated at 37°C for 24 hours and the MIC is determined. It corresponds to the concentration of the first tube where there is no observable growth visible to the naked eye of the test germ. Then we inoculated the surface of new Sabouraud agar with 0.1 ml of pipe content with concentration greater than or equal to the MIC to determine the MFC. To appreciate the power of each extract, the ratio MFC/MIC was calculated. Indeed, if the ratio MFC/MIC equals to 1 or 2, the effect is fungicidal and if it is between 4 to 16 the effect is fungistatic [20, 21].

2.7 Phytochemical screening

Vegetable extracts obtained were subjected to a phytochemical screening to reveal the major chemical groups it contains. The methods used are those described by Bekro *et al* [22], Bolu *et al* [23], Ouattara *et al* [24] and Shittu *et al* [25].

2.8 Statistical analysis

Analysis and graphical representations of data were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) software. The values expressed are the means of three experiments with the standard error of the mean (mean ± SEM).

3. RESULTS

3.1 Extractions yields

Table 1: Yield and features excerpts

Extract	<i>A. leiocarpa</i>			<i>S. virosa</i>		
	Appearance	Color	Y (%)	Appearance	Color	Y(%)
Ethanol	friable	brown	6,10	friable	brown	2,60
Aqueous	friable	dark Brown	7,80	friable	brown	4,50
Dichloromethane	friable	brown	0,19	pasty	brown	0,91
Ethyl acetate	powdery	brown	0,80	friable	brown	1,11

Y: yield

Table 1 shows the yield and characteristics of each plant extract. All the extracts are friable except the ethyl acetate extract of *A. leiocarpa* which is powdery and dichloromethane extract of *S. virosa* which looks pasty. All extracts are brown.

Aqueous extracts have the highest yields with 4.5% for aqueous extract of *S. virosa* and 7.8% for the aqueous extract of *A. leiocarpa*. The lowest yields are 0.19% and 0.91% respectively for dichloromethane extracts of both plants.

3.2 Phytochemical screening

Table 2: Phytochemical analysis of different solvents extracts of root bark of *Anogeissus leiocarpa*

Chemical groups	Extracts			
	ETHA	EAA	EDMA	ETAA
Sterols (polyterpenes)	++	++	+	+
polyphenols	+++	-	+	+++
flavonoids	+	++	-	+
tannins catechin	++	-	-	++
gallic	-	-	-	-
quinone substances	-	-	-	+
alkaloids	+	+	+	+
saponins	-	-	-	+

ETHA: ethanol extract of *A. leiocarpa*; ETAA: total aqueous extract of *A. leiocarpa*; EDMA : dichloromethane extract of *A. leiocarpa* ; EAA : ethyl acetate extract of *A. leiocarpa*

- : absence;

+: present in low concentration;

++: present in medium concentration;

+++ : present in high concentration.

Table 2 lists the major chemical groups encountered in different extracts of root bark of *A. leiocarpa*. The ethanol extract contains polyterpenes, polyphenols, flavonoids, catechin tannins and alkaloids. In the ethyl acetate extract, we note the presence of polyterpenes, flavonoids and alkaloids. Polyterpenes, polyphenols and alkaloids are also present in dichloromethane extract. As to the total aqueous extract, it contains polyterpenes, polyphenols, flavonoids, catechin tannins, quinone substances, alkaloids and saponins.

Table 3: Phytochemical analysis of different solvents extracts of root bark of *Securinega virosa*

Chemical groups	Extracts			
	ETHS	EAS	EDMS	ETAS
Stérols (polyterpenes)	+	+	+	+
Polyphenols	++	-	-	++
Flavonoids	-	-	-	++
catechin	+	-	-	+
Tanins gallic	-	-	-	-
quinone substances	+	-	-	+
alkaloids	+	+	-	+
saponins	-	-	-	-

ETHS: ethanol extract of *S. virosa*; ETAS: total aqueous extract of *S. virosa*;

EDMS: dichloromethane extract of *S. virosa*; EAS: ethyl acetate extract of *S. virosa*

- : Absence;

+: present in low concentration;

++: present in medium concentration;

+++: present in high concentration.

The results of the phytochemical screening of extracts of roots bark of *S. virosa* are summarized in Table 3. This table shows that ethanol extract contains polyterpenes, polyphenols, catechin, tannins, alkaloids and quinone substances. Ethyl acetate extract contains only polyterpenes and alkaloids while dichloromethane extract contains only polyterpenes. In the total aqueous extract, polyterpenes, polyphenols, flavonoids, catechin tannins, quinone substances and alkaloids have been highlighted.

3.3 Sensitivity tests

The results of susceptibility testing of plant extracts against the strains investigated are shown in figure 1 and figure 2.

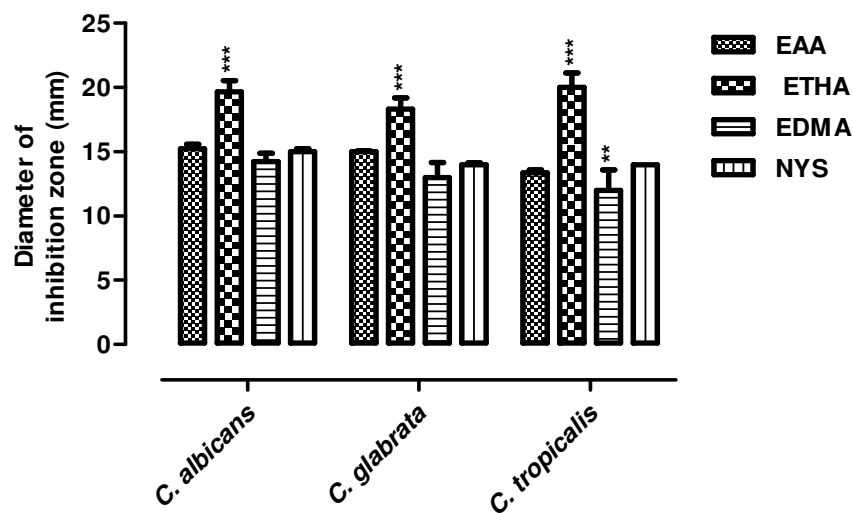


Figure 1: Activity of *A. leiocarpa* extracts on growth of *Candida*

The diameters of the inhibition zones are the arithmetic mean (m) of 3 experiments (n = 3) affected the standard error (m ± SEM).

** There is a significant difference at $p < 0.05$ compared to the average of NYS;

*** There is a significant difference at $p < 0.01$ compared to the average of NYS.

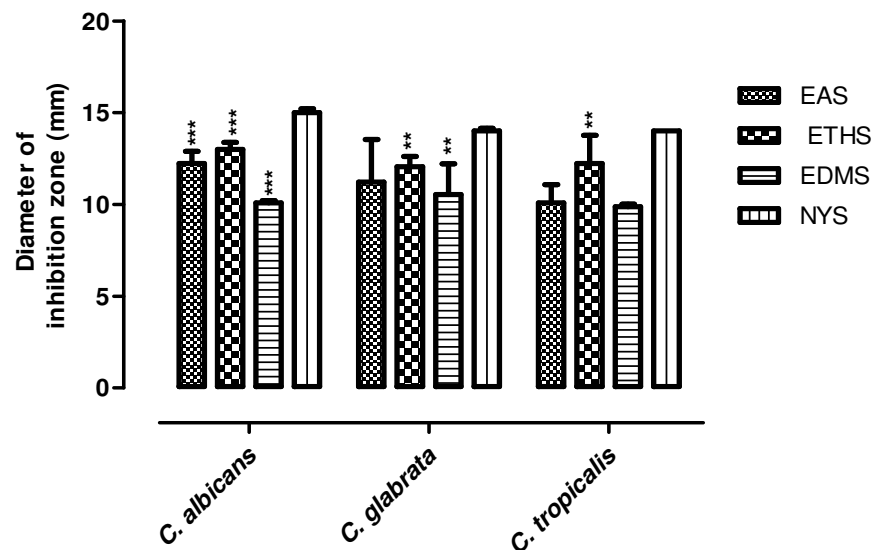


Figure 2: Activity of *S. virosa* extracts on growth of *Candida*

The diameters of the inhibition zones are the arithmetic mean (m) of 3 experiments ($n = 3$) affected the standard error ($m \pm SEM$).

** There is a significant difference at $p < 0.05$ compared to the average of NYS;

*** There is a significant difference at $p < 0.01$ compared to the average of NYS.

Comparing the diameters of inhibition zones, extracts of *A. leiocarpa* (Figure 1) show that the effect of the extracts at the same concentration is more marked with ETHA (ethanol extract of *A. leiocarpa*). It induces inhibition zone diameters of 19.67 ± 0.88 mm; 18.33 ± 0.88 mm and 20.00 ± 1.15 mm respectively on *C. albicans*, *C. glabrata* and *C. tropicalis*. EDMA (dichloromethane extract of *A. leiocarpa*) is the less active extract with diameters of inhibition of 14.23 ± 0.66 mm; 13.00 ± 1.15 mm and 12 ± 1.58 mm respectively on *C. albicans*, *C. glabrata* and *C. tropicalis*. Furthermore the average diameters of inhibition induced by ETHA are significantly greater than those induced by NYS ($p < 0.05$; $p < 0.01$).

As for *S. virosa* (Figure 2), the diameters of inhibition zones induced by its various extracts were lower than those of *A. leiocarpa*. At the same concentration, all extracts of *S. virosa* induce any inhibition zones except ETAS (total aqueous extract of *S. virosa*), which has no effect on all the microorganisms tested. For this plant, ETHS (ethanol extract of *S. virosa*) induces inhibition diameters larger on *C. tropicalis* (14.23 ± 1.53 mm) followed by *C. albicans* (14 ± 0.38 mm) and finally *C. glabrata* (12.05 ± 0.56 mm). These diameters are significantly higher ($p < 0.05$; $p < 0.01$) compared to NYS. Germs were resistant overlook aqueous extracts of both plants.

Tables 4, 5 and 6 show the values of various antifungal parameters of extracts tested. To *A. leiocarpa* MICs determined vary between 0.195 to 12.5 mg/mL and MFCs 0.390 to 50 mg/mL.

Table 4: Values of antifungal parameters and ratios MFC/MIC of ethanol extracts of *A.*

239 *leiocarpa* and *S. virosa*

	ETHA			ETHS		
	MIC (mg/ml)	MFC (mg/ml)	MFC/CMI	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC
<i>C. albicans</i>	0.195	0.390	2	3.125	6.250	2
<i>C. glabrata</i>	0.781	0.781	1	1.562	3.125	2
<i>C. tropicalis</i>	3.125	6.250	2	12.50	25.00	2

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241 **Table 5: Values of antifungal parameters and ratios MFC/MIC of ethyl acetate extracts of *A.***

242 *leiocarpa* and *S. virosa*

	EAA			EAS		
	MIC (mg/ml)	MFC (mg/ml)	MFC/CMI	MIC (mg/ml)	MFC (mg/ml)	MFC/CMI
<i>C. albicans</i>	6.25	6.25	1	12.5	12.5	1
<i>C. glabrata</i>	12.5	50	4	25	50	2
<i>C. tropicalis</i>	6.25	12.5	2	25	25	1

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244 **Table 6: Values of antifungal parameters and ratios MFC/MIC of dichloromethane extracts of *A.***

245 *leiocarpa* et de *S. virosa*

	EDMA			EDMS		
	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC	MIC (mg/ml)	MFC (mg/ml)	MFC/MIC
<i>C. albicans</i>	12.5	50	4	25	50	2
<i>C. glabrata</i>	6.25	31.25	5	6.25	6.25	1
<i>C. tropicalis</i>	12.5	12.5	1	12.5	12.5	1

246 MICs for *S. virosa* range 1.562 to 25 mg/mL and MFCs 3.125 to 50 mg/mL. The fungicidal effect
 247 appreciated by the ratio MCF/CMI shows that ETHA, ETHS, EAS (ethyl acetate extract of *S. virosa*)
 248 and EDMS (dichloromethane extract of *S. virosa*) are fungicidal because this ratio is between 1 to 2
 249 and EAA is fungicidal on *C. albicans* and *C. tropicalis* but fungistatic on *C. glabrata*. EDMA is
 250 fungistatic on *C. albicans* and *C. glabrata*.

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252 Figures 8 and 9 show that *A. leiocarpa* presents the lowest MIC among tested plants. Ethanol extracts
 253 have the lowest MIC.

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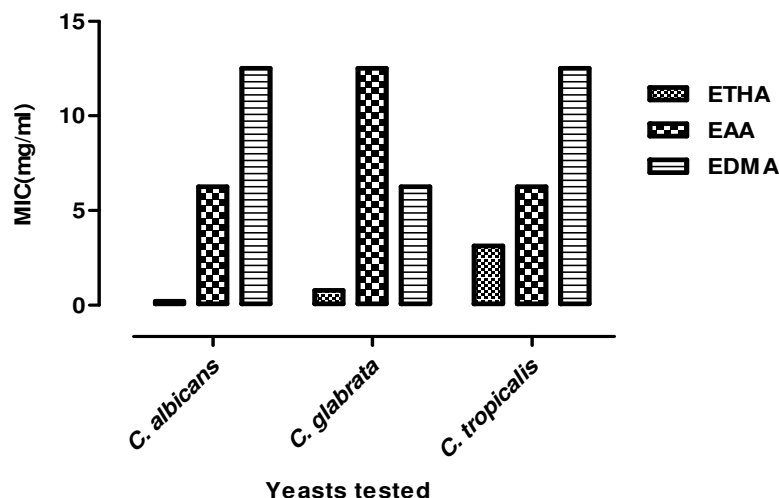
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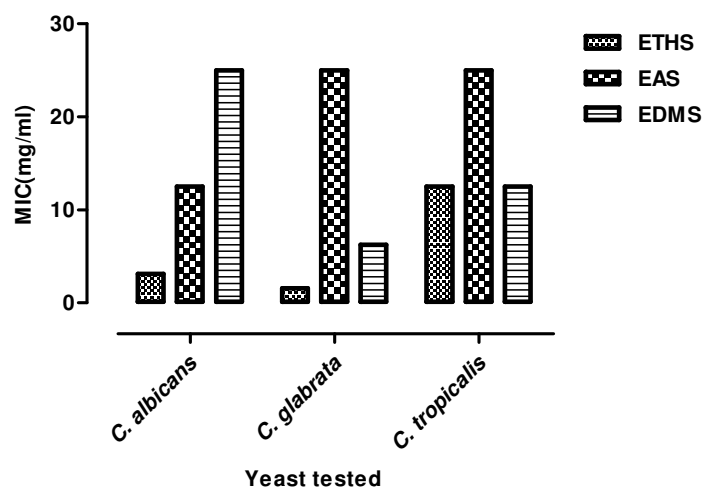


Figure 9: Minimum Inhibitory Concentration extracts of *S. virosa* on fungi tested

4. DISCUSSION

We exhaustively extract a plant powder with solvents of increasing polarity. The polar extracts have the highest yields with 7.80% for aqueous extract of *A. leiocarpa* and 4.50% for aqueous extract of *S. virosa*. The extraction yields reveal that root barks of these plants are richer in polar compounds than unpolar because yields increase with polarity of the solvent used.

In this study the antifungal activity of the extracts of root bark of *A. leiocarpa* and *S. virosa* was evaluated on three species of *Candida* which are: *C. albicans*, *C. tropicalis* and *C. glabrata*. These saprophytes and opportunistic yeast are present in the mucosa where they take advantage of an imbalance of existing flora or immune deficiency to multiply and cause infections. Resistance to azoles, including fluconazole, may explain clinical failures [26]. Determining the diameter of the inhibition zones and determination of antifungal parameters (MIC and MFC) were used to assess their sensitivity. In view of the results, we note that the best extraction yields are obtained with the aqueous extracts, but the best antifungal activity is obtained with ethanol extracts.

About *A. leiocarpa* the diameters of inhibition zones are 18 mm for ETHA the most active extract and 12mm for EDMA at a concentration of 500 mg/mL. Kubmarawa *et al* [27] showed that the ethanol extract of the stem bark of *A. leiocarpa* has significant inhibitory activity against *C. albicans*. Similarly

Adigun *et al* [28] showed that the acid 3,3,4-Tri-o-methylflavellagic glucoside isolated in the stem bark of *A. leiocarpa* has an antimicrobial effect on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. In Togo, studies conducted by Batawila [29] show that the MIC of *A. leiocarpa* for twenty fungal germs vary between 0.25 and 4 mg/mL. Our results show that the MIC range from 0.195 to 12.5 mg/mL and are in agreement with these authors.

As *A. leiocarpa*, *S. virosa* induces diameters of inhibition zone greater than 10 mm and the MIC range from 1.562 to 25mg/mL. These results suggest that the plant has an antifungal activity. This is corroborated by the results of Agassounon *et al* [30] which have shown that this plant has fungicidal properties.

The both plants have antifungal activity but *A. leiocarpa* seems to be more active than *S. virosa*. The roots of *A. leiocarpa* concentrate more active compounds than roots of *S. virosa*.

Therefore, the roots concentrate polar active substances. These results are supported by the work of Moroh [31] which showed that the roots of *Morinda morindoides* concentrate better the antimicrobial active compounds than other organs of the plant. It should be noted that the antimicrobial activity of medicinal plants varies from one geographic region to another. Then, leaf extracts of *A. leiocarpa* from Ghana are deemed to have a better antibacterial activity than extracts of leaves of the same plant from Nigeria [32].

The antifungal activity of both plants would be related to their phytomolecules composition. Indeed phytochemical screening of extracts of *A. leiocarpa* shows the presence of polyterpenes and sterols, polyphenols, flavonoids, catechin tannins and alkaloids. These results are consistent with those of Arbab *et al* [33] showing the same chemical constituents in all organs of the plant.

The phytochemical screening of *S. virosa* extracts shows also polyterpenes and sterols, polyphenols, flavonoids alkaloids and catechin tannins. These compounds have also put in evidence by Ezeonwumelu *et al* [34] in extracts of this plant. These extracts are less rich in bioactive compounds than *A. leiocarapa*. The ethanol extracts were the most active; indicating that they concentrate most of the compounds. Ethanol would be the best extraction solvent of active substances from these plants.

The presence of large chemical groups such as polyterpenes and sterols, polyphenols, flavonoids, catechin tannins and alkaloids in ethanol extracts explains their best activity because these compounds are recognized for their antimicrobial activities [35, 36, 37]. It may also be explained that the activity of antibiotics in plant extracts against fungi or growth may be due to their mechanism of action, chemical structure or spectrum of activity [38].

Flavonoids are phenolic in nature and they act as cytoplasmic poisons, which inhibit the activity of cytoplasmic enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase and ATPase [39, 40]. Tannins coagulate the cell wall proteins resulting in bactericidal activity at higher concentrations, while saponins are surface active agents and they alter the permeability of the cell wall thus facilitating the entry of toxic materials or leakage of vital constituents from the cell [41].

5. CONCLUSION

The extraction of bioactive substances with solvents of increasing polarity showed that the inhibitory activity of the extracts is due to better polar compounds concentrated in ethanol. The extracts are

either fungistatic or fungicidal. These results validate the traditional use of these plants in the treatment of candidiasis.

REFERENCES

1. Videau JY. Accès pour tous aux médicaments de qualité. *Med Trop* 2002; 62: 396-400. French
2. Adjanahoun EJ. Etat de l'ethno-pharmacopée africaine. *Bull Med Trad Pharm* 1990; 4(1): 59-63. French
3. Ake-Assi L. Rapport sur le colloque international sur la médecine traditionnelle et pharmacopée africaine à Abidjan, Côte d'Ivoire. *Bull Med Trad Pharm Afri* 1991; 4(2): 203p. French
4. Dro B, Soro D, Kone MW, Bakayoko A, Kamanzi K. Evaluation de l'abondance de plantes médicinales utilisées en médecine traditionnelle dans le Nord de la Côte d'Ivoire. *J An & Plant Sci* 2013; 17(3): 2631-2646. French
5. Thenmozhi K, Saradha M, Manian S, Paulsamy S. In vitro antimicrobial potential of root extracts of the medicinal plant species, *emilia sonchifolia* (linn.) Dc. *Asian J Pharm Clin Res* 2013; 6(3):149-151.
6. Konan Kouadio F, Guessennd NK, Karamoko O, Bahi C, Adama C, Dosso M. Action antibactérienne de l'extrait éthanolique 70% de *Clerodendrum splendens* (G. Don) (Verbenaceae) sur des souches bactériennes isolées de selles chez des enfants diarrhéiques. *Int. J. Biol. Chem. Sci* 2013; 7(3): 1332-1337. French
7. Sapna P, Vaibhav S, Kakasaheb M. Antimicrobial properties of different *Ocimum* species (Lamiaceae). *J. Adv Pharm Res Biosci* 2013; 1(1): 16-19.
8. Toure A, Bahi C, Ouattara K, Djaman AJ, Coulibaly A. Phytochemical screening and in vitro antifungal activities of extracts of leaves of *Morinda morindoides* (*Morinda*, Rubiaceae). *J of Med Plants Res* 2011; 5(31): 6780-86.
9. Sithapa O, Kra KAM, Kporou KEL, Zihiri GN, Yapi HF, N'Guessan JD, Djaman AJ. Comparative study chromatographic fractions activities from *Terminalia ivorensis* and ketoconazole as standard antifungal on in vitro growth of *Trichophyton mentagrophytes* var *interdigitale*. *J. Drug Delivery Ther* 2013; 3(5): 18-21.
10. Shuaibu MN, Wuyep PA, Yanagi T, Hirayama K, Tanaka T, Kouno I. The use of microfluorometric method for activity guided isolation of antiplasmodial compound from plant extracts. *Parasitol Res* 2008; 102:1119-1127.
11. Bidie AP, N'Guessan BB, Yapo AF, N'Guessan JD, Djaman AJ. Activités antioxydantes de dix plantes médicinales de la pharmacopée ivoirienne. *Sci Nat* 2011; 8(1): 1-11. French
12. Larsonn J, Gottfries J, Bohiin L, Backlund D. Expanding the chemGPS chemical space with natural product. *A. J. Nat. Prod* 2005; 68: 985-991.

- 375 **13.** Rollinger JM, Haupt S, Stuppner H, Langer TJ. Combining ethnopharmacology and virtual
376 screening for lead structure discovery: COX-Inhibitors as application example. Chem. Inf.
377 Comput. Sci 2004; 44: 480-488.
- 378 **14.** Manga A, Gassama A, Sy YG, Bassene E, Lavaud C. Structural determination of new flavones
379 C-glycosides and trans (S, E) - (-) clovamide isolated from *Isocarpha senegalensis* Juss leaves
380 (Icacaceae). J. Soc. Ouest-Afr. Chim 2013; 035: 15-27.
- 381 **15.** Bakkiyaraj S, Pandiyaraj S. Evaluation of potential antimicrobial activity of some medicinal
382 plants against common food-borne pathogenic microorganism. Int J Pharm Bio Sci 2011; 2(2):
383 484-491.
- 384 **16.** Irshad S, Mahmood M, Perveen F. In-Vitro Anti-Bacterial Activities of Three Medicinal Plants
385 Using Agar Well Diffusion Method, Res. J. Bio 2012; 2(1): 1-8.
- 386 **17.** Adekunle O. Phytochemical Screening and Antimicrobial Efficacy of Aqueous and Methanolic
387 Extract of *Mangifera indica* (Mango Stem Bark). World J Life Sci. and Medical Research 2012;
388 2(2): 81-85.
- 389 **18.** Yaye YG, Kra AKM, Ackah JAAB, Djaman AJ. Évaluation de l'activité antifongique et essai de
390 purification des extraits de *Terminalia mantaly* (H. Perrier), une Combretaceae sur la croissance
391 in vitro de *Candida albicans*. Bull Soc R Sci Liège 2011; 80: 953-964. French
- 392 **19.** Ouattara S, Kporou KE, Kra KAM, Yapi HF, Zirihi GN, N'Guessan JD, Bidié ADP, Djaman AJ.
393 Optimization of the in vitro antifungal activity of hydroalcoholic extract of *Terminalia ivorensis*. A.
394 Chev. Nat. Prod. Plant Resour 2013; 3(4): 29-33.
- 395 **20.** Berche P, Gaillard JL, Simonet M. Les bactéries des infections humaines. Editeur: Flammarion,
396 Médecine-Sciences 1991; 660p. French
- 397 **21.** Loubaki BC, Ouattara AS, Ouattara AS, Ouedraogo-Traore R, Traore AS.. Activités
398 antimicrobiennes des extraits aqueux totaux de *Detarium microcarpum* (Cesalpiniaceae) sur huit
399 espèces bactériennes impliquées dans certaines maladies infectieuses au Burkina Faso.
400 Rev.CAMES-Série A 1999; 1: 6673 p. French
- 401 **22.** Bekro Y, Bekro J, Boua BB, Tra BF, Ehile E. Etude ethnobotanique et screening phytochimique
402 de *Caesalpinia benthamiana* (Baill.) Herend et Zarrucchi (Caesalpiniaceae). Sciences et Nature
403 2007; 4(2): 217-225. French
- 404 **23.** Bolou GEK, Bagré I, Ouattara K, Djaman AJ. Evaluation of the antibacterial activity of 14
405 medicinal plants in Côte d'Ivoire. Trop J Pharm Res 2011; 10(3): 335-340.
- 406 **24.** Ouattara K, Traore Z, Doumbia I, Coulibaly AF, Coulibaly A. In vitro antibacterial activity of
407 ethanol extract of *Abrus precatorius* Linn (Fabaceae) on bacteria responsible for nosocomial
408 infections. Int. Res. J Pharm. App Sci 2013; 3(1):12-27.
- 409 **25.** Shittu OB, Olabode OO, Omemu AM, Oluwalana SA, Adeniran S, Akpan I.. Phytochemical and
410 antimicrobial screening of *Spondias mombin*, *Senna occidentalis* and *Musa sapientum* against
411 *Vibrio cholerae* O1. Int. J. Curr. Microbiol. App. Sci 2014; 3(5): 948-961.

- 412 **26.** Vanessa C. Les candidoses vaginales récidivantes à candida albicans : Diagnostic,
413 physiopathologie, traitements. Université Henri Poincare-Nancy 1, France. Thèse de Pharmacie,
414 2001; 131 p. French
- 415 **27.** Kubmarawa D. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants
416 from Nigeria. Afr J Biotechnol 2007; 6(14):1690-1696.
- 417 **28.** Adigun JO, Amupitan JO, Kelly DR. Isolation and investigation of antimicrobial effect of 3,4,3 '-
418 tri-Omethylflavellagic acid and its glucoside from *Anogeissus leiocarpus*. B Chem Soc Ethiopia
419 2000; 14(2): 169-174.
- 420 **29.** Batawila K. Antifungal activities of five Combretaceae used in Togolese traditional medicine.
421 Fitoterapia 2005; 76(2): 264-8.
- 422 **30.** Agassounon DTM, De Souza C, Anani KT, Koumaglo K, Toukourou F, Gbeassor M, Evaluation
423 des activites cytotoxique, antivirale, antibacterienne et antifongique de six plantes medicinales.
424 Pharm. Méd.Trad. AF JOOI 2001; 11: 93-105. French
- 425 **31.** Moroh AJL. Résistance bactérienne et phytomolécules antimicrobiennes issues de *Morinda*
426 *morindoides*. Université de Bretagne Occidentale, France, Université Félix Houphouet Boigny,
427 Côte d'Ivoire. Thèse de doctorat 2013; 205 p. French
- 428 **32.** Mann A, Yahaya Y, Bansa A, Ajayi GO. Phytochemical and Antibacterial screening of
429 *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds. African
430 J. of Microbiol Res 2008, 2: 060-062.
- 431 **33.** Arbab AH. Review on *Anogeissus leiocarpus* a potent african traditional drug. International
432 Journal Of Research In Pharmacy And Chemistry 2014; 4(3): 496-500.
- 433 **34.** Ezeonwumelu COJ, Matuki KE, Ajayi MA, Okoruwa GA, Tanayen KJ, Adiukwu PC, Goji TDA,
434 Dare S, Okonkwo OC, Byarugaba F. Phytochemical screening, acute toxicity and analgesic
435 properties of aqueous extract of *Flueggea virosa*'s root in rats. Ibbosina J Med BS 2013: 15-21.
- 436 **35.** Mada SB, Garba A, Muhammad A, Mohammed A, Adekunle DO. Phytochemical screening and
437 antimicrobial efficacy of aqueous and methanolic extract of *Mangifera indica* (mango stem bark).
438 World J Life Sci. and Medical Research 2012; 2(2):81-85.
- 439 **36.** Mbengui DR, Guessennd KN, M'Boh GM, Golly KJ, Okou OC, N'Guessan JD, Dosso M,
440 Djaman AJ. Phytochemical screening and study of comparative antibacterial activity of aqueous
441 and alcoholic extracts of the leaves and barks of *Terminalia catappa* on multiresistant strains. J.
442 Appl. Biosci 2013; 66: 5040-5048.
- 443 **37.** Gbadamosi ET, Ogunsuyi A. An appraisal of the potency of roots of *Anogeissus leiocarpus*
444 (DC.) Guill. & Perr. and *Terminalia glaucescens* Benth. in the management of *E. coli* related
445 infections J. Appl. Biosci. 2014; 78: 6646-6653
- 446 **38.** Calderon CB, Sabundayo BP. Antimicrobial Classifications: Drugs for Bugs. In Schwalbe R,
447 Steele-Moore L, Goodwin AC. Antimicrobial Susceptibility Testing Protocols. CRC Press. Taylor
448 & Frances group. 2007.

- 449 **39.** Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of
450 flavonoids as anti-inflammatory agents. *Inflamm Allergy Drug Targets* 2009; 8(3): 229-35.
- 451 **40.** Arullappan S, Rajamanickam P, Thevar N, Kodimani CC. In vitro screening of cytotoxic,
452 antimicrobial and antioxidant activities of clinacanthus nutans (Acanthaceae) leaf extracts. *Trop*
453 *J Pharm Res*, September 2014; 13(9): 1455-1461.
- 454 **41.** Iwu MM, Igboko OA, Okeniyi CD, Tempesta MS. Inhibition of the enzyme activity of
455 aldose reductase of some flavonoids by some flavonoids. *J Pharm Pharmacol.* 1990; 42: 290-
456 292.