Antifungal Activity of Roots Barks Extract of Securinega virosa (Roxb. ex Willd.) Baill and Anogeissus leiocarpa (DC.) Guill. & Perr, Two Plants Used in the Traditional Treatment of Candidiasis in Northern Côte d'Ivoire

Kouangbé Mani Adrien¹,²*, Bahi Calixte¹, Tia Honoré², Boga Gogo Lucien¹, Edoh Vincent³, Djaman Allico Joseph¹,³ and N’Guessan Jean David¹

¹Biosciences Department, Biochemical Pharmacodynamics Laboratory, University of Félix Houphouët-Boigny, Côte d’Ivoire.
²Department of Bacteriology-Virology, Central Laboratory of University Hospital of Treichville, Abidjan, Côte d’Ivoire.
³Department of Clinical and Basic Biochemistry, Pasteur Institute of Côte d’Ivoire, Côte d’Ivoire.

Authors’ contributions

This work was done as a team with all authors. Author KMA designed the study, performed the different tests and wrote this article and performed statistical analysis. Authors BC and BGL wrote the first draft of the manuscript. Authors TH and EV wrote the protocol, managed the analyses of the study analysis and managed literature. Authors DAJ and NJD provided the data included to the manuscript and managed the literature search. All authors read and approved the final version of the manuscript.

Article Information

DOI: 10.9734/IJBcRR/2015/17481
Editor(s):
(1) Tomio Yabe, Department of Applied Life Science, Gifu University, Japan.
Reviewers:
(1) Anonymous, Mahidol University, Thailand.
(2) Patricia Garcia Palencia, Department of Microbiology and Infection Control, Autonomous University of Nuevo León, Mexico.
(3) Dorota Wojnicz, Department of Biology and Medical Parasitology, Wroclaw Medical University, Poland.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?tid=1244&id=3&aid=9700

Received 16th March 2015
Accepted 29th April 2015
Published 11th June 2015

*Corresponding author: E-mail: kmania1@yahoo.fr
ABSTRACT

Aims: To evaluate the anticandidal activity of some solvent extracts of Securinega virosa and Anogeissus leiocarpa from northern Côte d'Ivoire.
Study Design: In vitro assay of antifungal activity.
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 10 mm 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 Anogeissus leiocarpa revealed the strongest anticandidal activity against all tested strains with MICs ranging from 0.195 to 12.5 mg/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 possesse compounds with good anticandidal properties. This results support their traditional use in treatment of 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 [5-7], antifungal [8,9], antiplasmodial [10] and antioxidant [11] activities. 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 Securinega virosa and Anogeissus leiocarpa whose root barks are used in traditional treatment of microbial infections in northern Côte d'Ivoire. 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 both plants of the Ivorian flora on three species of Candida namely Candida albicans, Candida tropicalis and Candida glabrata.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted of the root bark of A. leiocarpa and S. virosa. These organs were harvested in January 2013 to Kouto, town located to 725 km north of Abidjan after an ethnobotanical survey of traditional healers in the community. Plants used in this study were identified by Professor Aké-Assi Laurent of the National Floristic Center, University of Félix Houphouet Boigny Cocody Abidjan.
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 C. albicans, C. glabrata and C. 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 were 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 50 g of the plant powder in 2 l of distilled water. The macerate was mixed for 48 h using a magnetic stirrer type IKAMAG RCT at a temperature of the laboratory. The homogenate was filtered two times successively on cotton wool and then once on Whatman filter paper N°3. The filtrate obtained was reduced using a rotary evaporator type IKA Labortechnick before being evaporated at 50°C using an oven type Med Center Venticell. The powder obtained for each plant was the total aqueous extract and were stored at 5°C.

2.3.2 Preparation of the organic extracts

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

The liquid-liquid chromatography also called partition chromatography was 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 and ethanol. To achieve this chromatography, 20 g of aqueous extract plant were added to 100 ml of water. This solution was transferred into a separatory funnel of 500 ml in which 100 ml of dichloromethane were then added. The vial was stirred for 2 min and then allowed to sedimentation. The lower aqueous phase was then collected. This operation was repeated four times and the fractions collected dichloromethane (400 ml) were evaporated to dryness. The aqueous phase was 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 were seeded on Petri dishes containing Sabouraud agar and incubated for 48 to 72 h to obtain young cultures and isolated colonies. From these boxes, using a platinum loop two well-isolated colonies and identical were perfectly picked and put in 10 ml of Sabouraud broth and incubated for 3-5 h 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 was valued approximately at 10⁶ 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 were 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 was 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 was assessed by the measurement of a zone of inhibition; and depending on the diameter of inhibition.

Sabouraud medium was poured into sterilized Petri dish to a thickness of 8 mm. After inoculation by flooding adequate dilution (approximately 10⁶ cells/ml) of the tested strains made, wells of 6 mm diameter were formed concentrically in the agar. Each well was then filled with 20 μl of a given concentration of the
extract. After a pre-diffusion of 45 min at room temperature in the hood, the strains were incubated at 37°C for 24 h after which the diameters of the zones of inhibition were 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. and Ouattara et al.

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 solidified. 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 37ºC for 24 h and the MIC was determined. It corresponded to the concentration of the first tube where there was no observable growth visible to the naked eye of the tested 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 was between 4 to 16 the effect was fungistatic.

### 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., Yusuf et al., Mehta et al. and Shittu et al.

### 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 were the means of three experiments with the standard error of the mean (mean ± SEM).

### 3. RESULTS

#### 3.1 Extractions Yields

Table 1 shows the yield and characteristics of each plant extract. All the extracts were friable except the ethyl acetate extract of A. leiocarpa which was powdery and dichloromethane extract of S. virosa which looked pasty. All extracts were brown.

Aqueous extracts haved 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 were 0.19% and 0.91% respectively for dichloromethane extracts of both plants.

#### 3.2 Phytochemical Screening

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

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

#### 3.3 Sensitivity Tests

The results of susceptibility testing of plant extracts against the strains investigated were shown in Figs. 1 and 2.
Table 1. Yield and features excerpts

| Extract           | A. leiocarpa | S. virosa |
|-------------------|--------------|-----------|
| 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 2. Phytochemical analysis of different solvents extracts of root bark of *A. leiocarpa*

| Chemical groups              | ETHA | EAA | EDMA | ETAA |
|------------------------------|------|-----|------|------|
| Sterols (polyterpenes)       | +    | +   | +    | +    |
| Polyphenols                  | +    | -   | +    | +    |
| Flavonoids                   | +    | +   | -    | +    |
| Tannins Catechin             | +    | -   | -    | +    |
| Gallic                       | -    | -   | -    | -    |
| Quinone substances           | -    | -   | -    | +    |
| Alkaloids                    | +    | +   | +    | +    |
| Saponins                     | -    | -   | -    | +    |

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

**Fig. 1. Activity of *A. leiocarpa* extracts on growth of *Candida***

The diameters of the inhibition zones are the arithmetic mean (mm) of 3 experiments (n = 3) affected the standard error (mm ± 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* (Fig. 1) showed that the effect of the extracts at the same concentration was more marked with ETHA (ethanol extract of *A. leiocarpa*). It induced inhibition zone diameters of 19.67±0.88 mm; 18.33±0. and 20.00±1.15 mm respectively on *C. albicans*, *C. glabrata* and *C. tropicalis*. EDMA (dichloromethane extract of *A. leiocarpa*) was 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 ETAH was significantly greater than those induced by NYS (p < 0.05; p < 0.01).

As for S. virosa (Fig. 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 induced any inhibition zones except ETAS (total aqueous extract of S. virosa), which had no effect on all the microorganisms tested. For this plant, ETHS (ethanol extract of S. virosa) induced 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 were 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 varied between 0.195 to 12.5 mg/ml and MFCs 0.390 to 50 mg/ml.

**Table 3. Phytochemical analysis of different solvents extracts of root bark of S. virosa**

| Chemical groups                  | Extracts       |
|---------------------------------|----------------|
|                                 | ETHS | EAS | EDMS | ETAS |
| Stérols (polyterpenes)          | +    | +   | +    | +    |
| Polyphenols                     | +    | -   | -    | -    |
| Flavonoids                      | -    | -   | -    | -    |
| Tanins Catechin                 | +    | -   | -    | +    |
| 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; +: presence

**Fig. 2. Activity of S. virosa on growth of Candida**

The diameters of the inhibition zones are the arithmetic mean (mm) of 3 experiments (n = 3) affected the standard error (mm ± 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.
MICs for *S. virosa* ranged 1.562 to 25 mg/ml and MFCs 3.125 to 50 mg/ml. The fungicidal effect appreciated by the ratio MCF/CMI showed that ETHA, ETHS, EAS (ethyl acetate extract of *S. virosa*) and EDMS (dichloromethane extract of *S. virosa*) were fungicidal because this ratio was between 1 to 2 and EAA was fungicidal on *C. albicans* and *C. tropicalis* but fungistatic on *C. glabrata*. EDMA was fungistatic on *C. albicans* and *C. glabrata*.

Figs. 3 and 4 show that *A. leiocarpa* presents the lowest MIC among tested plants. Ethanol extracts have the lowest MIC.

### Table 4. Values of antifungal parameters and ratios MFC/MIC of ethanol extracts of *A. leiocarpa* and *S. virosa*

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

### Table 5. Values of antifungal parameters and ratios MFC/MIC of ethyl acetate extracts of *A. leiocarpa* and *S. virosa*

|          | EAA           | EAS           |
|----------|---------------|---------------|
|          | MIC (mg/ml)   | MFC (mg/ml)   | MFC/CMI | MIC (mg/ml) | MFC (mg/ml) | MFC/CMI |
| *C. albicans* | 6.25          | 6.25          | 1       | 12.5        | 12.5        | 1       |
| *C. glabrata* | 12.5          | 50            | 4       | 25          | 50          | 2       |
| *C. tropicalis* | 6.25          | 12.5          | 2       | 25          | 25          | 1       |

### Table 6. Values of antifungal parameters and ratios MFC/MIC of dichloromethane extracts of *A. leiocarpa* and *S. virosa*

|          | EDMA          | EDMS          |
|----------|---------------|---------------|
|          | MIC (mg/ml)   | MFC (mg/ml)   | MFC/MIC | MIC (mg/ml) | MFC (mg/ml) | MFC/MIC |
| *C. albicans* | 12.5          | 50            | 4       | 25          | 50          | 2       |
| *C. glabrata* | 6.25          | 31.25         | 5       | 6.25        | 6.25        | 1       |
| *C. tropicalis* | 12.5          | 12.5          | 1       | 12.5        | 12.5        | 1       |

**Fig. 3. MIC of *A. leiocarpa* extracts of fungi tested**
4. DISCUSSION

We performed the extraction of the 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 12 mm 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 Batawlì [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 25 mg/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, catechin tannins and alkaloids in ethanol extracts. 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. leiocarpa*. 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 [35-37]. 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.

ACKNOWLEDGMENTS

The authors thank Blégui Douh Blaise, Anoh Bodouin, Yoboué Sylvestre, Dakrom Richard, Ahoussi Corneille and Mrs Honorine Coulibaly for their technical assistance and as well as the entire staff of the department of Bacteriology-Virology of the Central laboratory of University Hospital of Treichville, Abidjan Côte d’Ivoire.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Videau JY. Access for all to quality medicines. Med Trop. 2002;62:396-400. French
2. Adjanahoun EJ. State of the African ethnomedicines. Bull Med Trad Pharm. 1999;4(1):59-63. French
3. Ake-Assi L. Report on the International Symposium on African traditional medicine and pharmacopoeia in Abidjan, Côte d’Ivoire. Bull Med Trad Pharm Afr. 1991; 4(2):203. French
4. Dro B, Soro D, Kone MW, Bakayoko A, Kamanzi K. Evaluation of the abundance of medicinal plants used in traditional medicine in Northern 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. Antibacterial action of the ethanol extract 70% of *Clerodendrum splendens* (G. Don) (Verbenacae) on bacterial strains isolated from diarrheal stools in children. 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 ivorenensis* 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. Antioxidant activities of ten medicinal plants of the Ivorian Pharmacopoeia. Sci Nat. 2011; 8(1):1-11. French

12. Larsson J, Gottfries J, Bohiin L, Backlun D. Epanding the chemGPS chemical space with natural product. A. J. Nat. Prod. 2005; 68:985-991.

13. Rollinger JM, Haupt S, Stupnner H, Langer TJ. Combining ethnopharmacology and virtual screening for lead structure discovery: COX-Inhibitors as application example. Chem. Inf. Comput. Sci. 2004; 44:480-488.

14. Manga A, Gassama A, Sy YG, Bassene E, Lavaud C. Structural determination of news flavones C-glycosides and trans (S, E) - (-) clovamide isolated licencia senegalensis Juss leaves (Icacinaceae). J. Soc. Ouest-Afr. Chim. 2013;035:15-27.

15. Bakkiyaraj S, Pandiyaraj S. Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. Int J Pharm Bio Sci. 2011;2(2):484-491.

16. Irshad S, Mahmood M, Perveen F. In vitro anti-bacterial activities of three medicinal plants using agar well diffusion method. Res. J. Bio. 2012;2(1):1-8.

17. Adekunle O. Phytochemical Screening and Antimicrobial Efficacy of Aqueous and Methanolic Extract of Mangifera indica (Mango Stem Bark). World J Life Sci. and Medical Research. 2012;2(2):81-85.

18. Yaye YG, Kra AKM, Ackah JAAB, Djaman AJ. Evaluation of the antifungal activity assay and purification of extracts from Terminalia Mantaly (H. Perrier) a Combretaceae on the growth in vitro of Candida albicans. Bull Soc R Sci Liège. 2011;80:953-964. French

19. Ouattara S, Kporou KE, Kra KAM, Yapi HF, Zirhi GN, N’Guessan JD, Bidie ADP, Djaman AJ. Optimization of the in vitro antifungal activity of hydroalcoholic extract of Terminalia ivorensis. A. Chev. Nat. Prod. Plant Resour. 2013;3(4):29-33.

20. Berche P, Gaillard JL, Simonet M. The bacteria of human infections. Editor: Flammarion, Medecine-Sciences. 1991; 660. French

21. Loubaki BC, Ouattara AS, Ouattara AS, Ouedraogo-Traore R, Traore AS. Antimicrobial activities of total aqueous extracts of Detarium microcarpum (Cesalpinaceae) on eight bacterial species involved in infectious diseases in Burkina Faso. Rev. CAMES-Serie A. 1999;1:6673. French

22. Bekro Y, Bekro J, Boua BB, Tra BF, Ehile E. Ethnobotanical study and phytochemical screening of Caesalpinia benthamiana (Baill.) Herend and Zarrucchi (Caesalpiniacea). Sci Nat. 2007;4(2):217-225. French

23. Mehta K, Patel BN, Jain BK, Phytochemical analysis of leaf extract of Phyllantus fraternus. Res. J. Recent. Sci 2013;2:12-15.

24. Yusuf AZ, Zakir A, Shemau ZI, Abdullahi M, Halima SA. Phytochemical analysis of methanol leaves extract of Paulinia pinnata linn J. Pharmacognosy Phytother 2014;6(2):10-16.

25. Shittu OB, Olabode OO, Omemu AM, Oluwalana SA, Adeniran S, Akpan I.. Phytochemical and antimicrobial screening of Spondias mombin, Senna occidentalis and Musa sapientum against Vibrio cholerae O1. Int. J. Curr. Microbiol. App. Sci. 2014;3(5):948-961.

26. Vanessa C. Vaginal candidiasis recurrent to Candida albicans: Diagnosis, pathophysiology, treatment. University Henri Poincaré -Nancy 1, France. Thesis Pharmacy. 2001;131. French

27. Kubmarawa D. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. Afr J Biotechnol. 2007;6(14):1690-1696.

28. Adiug JO, Ampiton J, Kelly DR. Isolation and investigation of antimicrobial effect of 3,4,3’-tri-Omethylflavellagic acid and its glucoside from Anogeissus lecogrus. B Chem Soc Ethiopia. 2000; 14(2):169-174.

29. Balawila K. Antifungal activities of five Combretaceae used in Togolese traditional medicine. Filoterapia. 2005;76(2):284-8.

30. Agassounou DTM, De Souza C, Anani KT, Kouglo K, Toukourou F, Gbeassor M, Evaluation of the cytotoxic, antiviral, antibacterial and antifungal activities of six
medicinal plants. Pharm. Méd. Trad. AF JOO1. 2001;11:93-105. French
31. Moroh AJL. Bacterial resistance and antimicrobial phytomolecules from *Morinda morindoides*. University of Bretagne Occidentale, France, University Félix Houphouet Boigny, Côte d’Ivoire. Thesis 2013;205. French
32. Mann A, Yahaya Y, Banso A, Ajayi GO. Phytochemical and Antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds. African J. of Microbiol Res. 2008; 2:060-062.
33. Arbab AH. Review on *Anogeissus leiocarpus* a potent african traditional drug. Int. J. Res. Pharm. Chem. 2014;4(3):496-500.
34. Ezeonwumelu COJ, Matuki KE, Ajayi MA, Okoruwa GA, Tanayen KJ, Adiukwu PC, Goji TDA, Dare S, Okonkwo OC, Byarugaba F. Phytochemical screening, acute toxicity and analgesic properties of aqueous extract of *Flueggea virosa*'s root in rats. Ibnosina J Med BS. 2013;15-21.
35. Mada SB, Garba A, Muhammad A, Mohammed A, Adekunle DO. Phytochemical screening and antimicrobial efficacy of aqueous and methanolic extract of *Mangifera indica* (mango stem bark). World J Life Sci. and Medical Research. 2012;2(2):81-85.
36. Mbengui DR, Guessend KN, M'Boh GM, Golly KJ, Okou OC, N'Guessan JD, Dosso M, Djaman AJ. Phytochemical screening and study of comparative antibacterial activity of aqueous and alcoholic extracts of the leaves and barks of *Terminalia catappa* on multiresistant strains. J. Appl. Biosci. 2013;66:5040-5048.
37. Gbadamosi ET, Ogunsuyi A. An appraisal of the potency of roots of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Benth. in the management of *E. coli* related infections J. Appl. Biosci. 2014;78:6646-6653.
38. Calderon CB, Sabundayo BP. Antimicrobial Classifications: Drugs for Bugs. In Schwalbe R, Steele-Moore L, Goodwin AC. Antimicrobial Susceptibility Testing Protocols. CRC Press. Taylor & Frances Group; 2007.
39. Rathee P, Chaudhary H, Rathee S, Rathee D, Kumar V, Kohli K. Mechanism of action of flavonoids as anti-inflammatory agents. Inflamm Allergy Drug Targets. 2009;8(3): 229-35.
40. Arullappan S, Rajamanickam P, Thevar N, Kodimani CC. In vitro screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (Acanthaceae) leaf extracts. Trop J Pharm Res. September, 2014;13(9):1455-1461.
41. Iwu MM, Igboko OA, Okenyi CD, Tempesta MS. Inhibition of the enzyme activity of aldosoereductase of some flavonoids by some flavonoids. J Pharm Pharmacol. 1990;42:290-292.