IJPSR (2021), Volume 12, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 January 2021; received in revised form, 04 May 2021; accepted, 29 May 2021; published 01 December 2021

CYTOTOXICITY, ANTIMICROBIAL ACTIVITIES AND CHEMICAL PROPERTIES OF TEN PLANTS FROM THE BENIN PHARMACOPOEIA USED FOR ORAL CARE

T. G. P. Guezodje [1, *2], A. J. Agbankpe [3], G. H. Dassou [1], T. V. Dougnon [3], A. Dicko [2] and H. Yedomonhan [1]

Laboratory of Botany and Plant Ecology [1], Faculty of Sciences and Techniques, University of Abomey-Calavi, Benin.
Laboratory of Chemistry and Physics-Multi-scale Approach of Complex Media (LCP-A2MC) [2], University of Lorraine in France.
Research Unit in Applied Microbiology and Pharmacology of Natural Substances [3], Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Abomey-Calavi, Benin.

Keywords:

Oral diseases, Medicinal plants, Toxicity, Antibacterial activity, Benin

Correspondence to Author:
T. G. P. Guezodje

Laboratory of Botany and Plant Ecology, Faculty of Sciences and Techniques, University of Abomey Calavi, Benin.

E-mail: dahguezodje@gmail.com

ABSTRACT: This study aims to valorizeten medicinal plants traditionally used for oral care in Benin by producing scientific data on their toxicities, antimicrobial activities, and chemical properties. The ten medicinal plants (*Zanthoxylum zanthoxyloides*, *Vitellaria paradoxa*, *Prosopis africana*, *Dialium guineense*, *Pseudocedrela kotschyi*, *Parkia biglobosa*, *Azadirachta indica*, *Diospyros mespiliformis*, *Anogeissus leiocarpa* and *Bridelia ferruginea*) were identified following an ethnobotanical survey conducted in Benin. With the exception of the hydroethanolic extract of *Z. zanthoxyloides*, all extracts have an LC_{50} greater than 0.1 mg/mL, indicating that these plants are non-cytotoxic. Of the ten extracts tested, only the hydroethanolic extract of *Z. zanthoxyloides* stem has no activity on the bacterial strains used. The results of the antibacterial test show that the largest diameter of inhibition was obtained in the extract of *A. leiocarpa* on *Escherichia coli* ATCC 25922, a reference strain with 25.67mm. The highest levels of total flavonoids (FVT) and total polypenols (TPP) are recorded respectively in *P. kotschyi* (145.86±0.7 EC/gMS) and *D. guineense* (70.194±0.220 EGA/g Ms). The antiradical powers of the extracts with respect to DPPH and ABTS radicals were determined respectively by the equation lines $y = -0.8784x + 2.0281$ ($R^2 = 0.9925$) and $y = -0.2023x + 0.5099$ ($R^2 = 0.9807$) expressed in Trolox (Trolox Eq mg/gMS). The strongest antiradical activity (ABTS) was recorded in *A. leiocarpa* (14.97±0.83mgET/gMS), *B. ferruginea* (15.57±0.4 mg ET/gMS), *P. africana* (13.25±0.1 mg ET/gMS). These results justify the traditional use of these medicinal plants and deserve to be valued in the fight against oral diseases.

INTRODUCTION: Oral diseases are a global public health problem affecting nearly 3.5 billion people [1].

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DOI: 10.13040/IJPSR.0975-8232.12(12).6642-52

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6642-52

They cause a considerable burden of disease, leading to lifelong pain, discomfort, disfiguring lesions, and even death [1]. Among oral diseases, tooth decay and periodontal disease are the most common chronic conditions in the world [2]. More than 530 million children have deciduous decay, while periodontal disease affects nearly 10% of the world's population [1]. In general, the risk factors for oral diseases take into account, in a non-exhaustive way, poor living conditions, low level of education

(implying a lack of knowledge in terms of dental prevention), and method of life (poor oral hygiene practices, consumption of sugars, tobacco and alcohol) [3]. The main problem associated with the management of oral diseases is the high cost of treatment, especially since they are not included in the Universal Health Coverage. Most low- and middle-income countries have serious challenges in managing oral conditions [1]. Several studies report the use of medicinal plants in the management of oral diseases in Africa [4, 5] but also outside the continent [6]. An ethnobotanical survey carried out in Benin made it possible to identify the medicinal plants used for oral care. The most recorded medicinal plants are: *A. leiocarpa*, *A. indica*, *B. ferruginea*, *D. guineense*, *D. mespiliformis*, *P. biglobosa*, *P. africana*, *P. kotschyi*, *V. paradoxa* and *Z. zanthoxyloides* [7]. These plants are used for their dual interest of vegetable brush and medicinal plant. Unfortunately, there is no relevant scientific work to promote these medicinal plants in the management of oral diseases. Indeed, there are insufficient data on their chemical composition, their toxicity and their activity on microbial strains responsible for oral infections. It is to fill this gap that this study was initiated with the aim of producing scientific data on the toxicity, phytochemistry and antimicrobial activity of these medicinal plants.

MATERIALS AND METHODS:

Material:

Plant Material: The plant material consists of the stems of *Anogeissus leiocarpa* (YH 536/HNB; Combretaceae), *Azadirachta indica* (YH 537/HNB; Meliaceae), *Bridelia ferruginea* (YH 538/HNB; Euphorbiaceae), *Dialium guineense* (YH 539/HNB; Leguminosae), *Diospyros mespiliformis* (YH 540/HNB; Ebenaceae), *Parkia biglobosa* (YH 541/HNB; Legopuminosa bigosae Africana) *Prosopis africana* (YH 542/HNB; Leguminosae Africana), *Pseudocedrela kotschyi* (YH 543/HNB; Meliaceae), *Vitellaria paradoxa* (YH 544/HNB; Spotaceae) and *Zanthoxylum zanthoxyloides* (YH 545/HNB; Rutaceae). The plants used were collected during ethnobotanical surveys. They were carefully washed in distilled water and then dried at laboratory temperature (16-20 °C) for 20 days. These dried plants were then crushed and turned into powder. The powders obtained were passed through a 0.2 mm mesh sieve, then stored in sterile containers at laboratory temperature.

Biological Material:

Microorganisms: Six bacterial strains were used for antimicrobial testing: two reference strains (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923), four clinical and multidrug resistant strains isolated from oral ulcer samples (*K. pneumoniae*, *K. oxytoca*, *Escherichia coli*, *Staphylococcus epidermidis*). The bacterial strains used were obtained from the bacteriology section of the National Laboratory of the Ministry of Health (LNMSP) in the Republic of Benin.

The Eggs of *Artemia salina*: The eggs of *Artemia salina* (ARTEMIO JBL GmbH D-67141 Neuhofem) were used for the larval cytotoxicity test.

Methods:

Obtaining Extracts: The hydro-ethanolic extracts were obtained by maceration. One hundred grams (100 g) of vegetable powder were macerated for 72 h with continuous stirring in 500 ml of the mixture of ethanol and water (50: 50). The macerate was filtered twice through cotton wool and once through Whatman No. 1 filter paper. The filtrate obtained was evaporated in an oven at 50°C [8]. The yield is the ratio between the mass of the dry extract obtained and the mass of the plant material treated [9]. This yield was calculated by the formula:

R= (mass of the extract after evaporation of the solvent) / (mass of the powder of the plant species used for the extraction) × 100

Larval Cytotoxicity: The tests were carried out on larvae obtained by hatching 10 mg of eggs of *Artemia salina* (ARTEMIO JBL GmbH D-67141 Neuhofem) with continuous agitation in 1 L of seawater for 72 h. To 1 ml of each ½ geometric serial dilution of extract prepared from a 40 mg/ml stock solution was added 1 ml of seawater containing 16 larvae. The number of surviving larvae was counted after 24 h of incubation.

The Lethal Concentration 50 (LC_{50}) was determined from the regression line obtained from the curve representative of the number of surviving larvae as a function of the concentration of extracts [10]. Each treatment was repeated once. To interpret

these results, correlation grids associating the degree of toxicity with the LC_{50} were used [11] **Table 1.**

TABLE 1: CORRESPONDENCE BETWEEN LETHAL CONCENTRATION (LC_{50}) AND TOXICITY

Mean Lethal Concentration (LC_{50}) value ranges	Toxicity
LC_{50}> 0,1 mg/ml	- (Non toxic)
0,1 mg/ ml > LC_{50} ≥0,050 mg/ml	+ (Low toxicity)
0,050 mg/ ml > LC_{50}≥0,01 mg/ml	++ (Moderate toxicity)
LC_{50} < 0,01 mg/ml	+ + + (High toxicity)

Antibacterial Tests:

Dissolution of the Extract: The extracts were taken up in sterile distilled water at a rate of 100 mg per 1 ml. Stock solutions of extracts concentrated at 100 mg/ml were thus prepared. They were then sterilized by filtration through a 0.22 µm Millipore membrane using a sterile syringe. The sterility of the stock solutions of extracts was verified by inoculating aliquots of each solution on Mueller Hinton medium, followed by incubation at 37 °C for 24 to 48 h [12].

Preparation of the Bacterial Suspension: A portion of the pure 24-hour colony from Mueller Hinton's medium from each strain was emulsified in 5 ml of physiological water to obtain turbidity of 0.5 on the Mc Farland standard.

Diffusion Test in Agar Medium: Each inoculum was inoculated by swabbing onto Petri dishes containing Mueller Hinton agar [12]. Using the sterile Pasteur pipette tip, wells 6 mm in diameter were made in the agar. 50 µl of each extract were placed in the wells. A well containing sterile distilled water served as a negative control. The Petri dishes were left for 1 hour at room temperature for pre-diffusion of the extracts before being incubated at 37 °C in an oven for 18 h [12]. After the incubation period, the dishes were examined for the measurement of the diameters of zones of inhibition around the wells. The standards used to interpret the inhibition diameters are summarized in **Table 2.**

TABLE 2: CORRESPONDENCE USED FOR READING THE RESULTS OF ANTIBACTERIAL TESTS OF PLANT EXTRACTS [13]

Diamètre de zone d'inhibition (Δ)	Degré de sensibilité du germe	Symbole
Δ < 7 mm	Insensitive	–
7 mm ≤ Δ < 8 mm	Sensible	+
8 mm ≤ Δ < 9 mm	Assez sensible	++
Δ ≥ 9 mm	Very sensitive	+++

Determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Antibiotic Power (AP) of Extracts: Microdilution on 96-well plates was used to determine the MICs of the extracts on the strains. 100 µl of Mueller-Hinton broth (MHB) was deposited in each well of the microplate (1 to 10 wells). 100 µl of the stock solution of each extract was deposited in the first well. After homogenization by aspiration-discharge using a micropipette, 200 µl of extract solution at 50 mg/ml is obtained.

100 µl of this new solution was taken and mixed with the Mueller Hinton broth contained in the second well and this dilution series was carried out from well to well until the 10th well from which the 100 µl was discarded to have concentrations of 50 mg/ml; 25 mg/ml; 12 mg/ml; 6.250 mg/ml; 3.125 mg/ml; 1.562 mg/ml; 0.781 mg/ml; 0.391 mg/ml; 0.195 mg/ml and 0.098 mg/ml.

Finally, 100 µl of the bacterial suspension was added to each well. A positive control consisted of 100 µl of Mueller Hinton broth and 100 µl of the bacterial suspension, while the negative control contained 100 µl of Mueller Hinton broth and 100 µl of the extract stock solution. The microplates were covered with para-film and incubated for 24 h at 37°C. MICs were determined by adding Tetrazolium (developer).

For the determination of MBC, all wells with higher MIC concentrations were then inoculated onto Mueller Hinton agar and placed at 37°C for 24 h. This resulted in the determination of the MBC corresponding to the lowest concentration of extract that showed no bacterial colonies. The antibacterial potency of extracts is judged to be bactericidal or bacteriostatic based on the ratio R= MBC/MIC[11].

Chemical Characterization of Medicinal Plants:

Phytochemical Screening: Qualitative phyto-chemical screening was performed on the powder using the method based on staining and/or precipitation reactions, described by Houghton and Raman [14]. **Table 3** shows the different chemical groups searched for and the principle of the reactions.

TABLE 3: SUMMARY TABLE OF CHEMICAL GROUPS, IDENTIFICATION REACTIONS AND INDICATORS USED

Chemical families	Identification reagents	Indicator (Positive Reaction)
Polyphenols	Ferric chloride $FeCl_3$ (2%)	Appearance of a more or less dark blue-blackish or green coloration
Flavonoids	Hydrochloric Alcohol, Magnesium Copals, Isoamyl Alcohol	Heat release followed by pinkish-orange or purplish coloration
Tannins	Formaldehyde, Concentrated hydrochloric acid	Blue tint
Alkaloids	Dragendorff (Potassium iodobismuthate solution) Burchard (Iodine Iodide Reaction)	Precipitation of reddish-brown coloring
Saponines	Mousse index	Appearance of persistent mossen

Dosages of Phenolic Compounds: The determination of the phenolic compounds: total polyphenols (TPP), total flavonoids (FVT) present in the various plants studied was carried out using a UV-visible spectrophotometer type Cary 50.

Determination of Total Polyphenols (TPP): The total phenol content of the extracts was determined by the Folin-Ciocalteu method [15]. A quantity of 200 µl of the plant extract is introduced into a 2 ml Eppendorf tube; the extract is then diluted with 1800 µl distilled water. Subsequently, 1800 µl of "Folin-Ciocalteu" reagent (1N) and 400 µl of Na_2CO_3 (20%) are added successively. The resulting mixture is incubated at room temperature for 40 minutes in the dark. The absorbance is then measured with a spectrophotometer at 725 nm; a methanol solution is used as a blank. The results obtained are expressed in mg gallic acid equivalent per gram of dry matter (EGA/g Ms). The total phenol contents of the extracts were expressed in mg gallic acid equivalent per gram of dry matter (EGA/g Ms). The contents were determined from the linear regression equation (TPP: y=0.2337x+ 0.1143) of the gallic acid calibration **Fig. 1**.

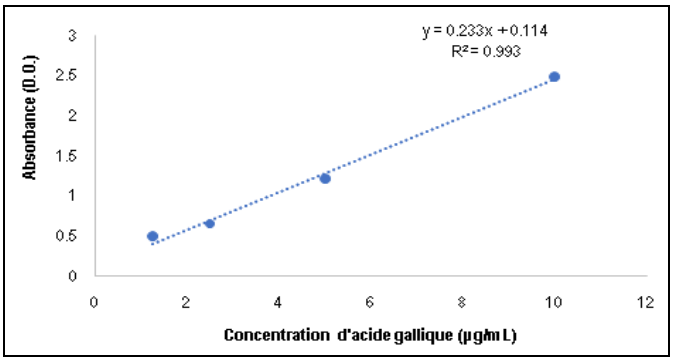


FIG. 1: CALIBRATION OF GALLIC ACID FOR THE DETERMINATION OF TOTAL PHENOL CONTENT

Determination of Total Flavonoids (FVT): The flavonoid content of the extracts was determined using the aluminum trichloride colorimetric method [16]. 250 µl of the studied extract and 1 ml of distilled water were initially deposited in test tubes. At an initial time (t=0) 75 µl of a 5% sodium nitrite solution is added. After 5 minutes, 75 µl of 10% aluminium chloride is added. Six (6) minutes later, 500 µl of a 1N sodium hydroxide (NaOH) solution and 2.5 ml distilled water are added. The absorbance of each mixture obtained is directly measured with the UV-visible spectrophotometer at 510 nm. The results obtained are expressed in mg catechin equivalent per gram dry matter (EC/g DM).

The total flavonoid contents of the extracts were expressed in mg equivalent of catechin per gram of dry matter (EC/g DM). The levels were determined from the linear regression equation (FVT: y= 0.0358x - 0.0112) of the catechin calibration **Fig. 2**.

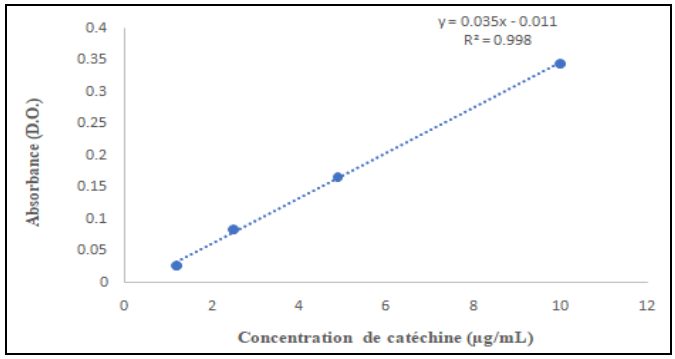


FIG. 2: CATECHIN CALIBRATION CURVE FOR THE DETERMINATION OF TOTAL FLAVONOID CONTENT

Anti-Radical Activity by ABTS Test: This test is based on the ability of an antioxidant to stabilize the cationic radical $ABTS^{\bullet+}$ (ammonium salt of 2,2'- azino bis-(3-ethylbenzothiazoline-6-sulfonic acid)) of greenish-blue coloring. This cationic radical is formed as a result of the oxidation of the initially colourless ABTS with various compounds such as potassium phosphate (KH_2PO_4) and 2,2'-azo-bis (2-amidino-propane) dihydrochloride (AAPH). Thus, the reaction takes place in two steps: In the first step the radical $ABTS^{\bullet+}$ is formed by the removal of an electron e- from a nitrogen

atom of the ABTS. The second takes place in the presence of trolox (or antioxidant donor of $H^{\bullet}$), the nitrogen radical concerned traps a $H^{\bullet}$, leading to ABTSH+, resulting in the discoloration of the solution.

Anti-Radical Activity by the DPPH Test: The method used by Nounagnon *et al.*, [17] was used. Trolox was used as an antioxidant, donor of H^{+}.

Statistical Analyses: For larval toxicity, the lethal concentration causing 50% larval death (LC_{50}) was calculated with a 95% confidence interval using linear regression analysis and the prohibit analysis method. For the spectophotometric tests, Excel software was used to enter the data, which corresponded to the ODs read for each extract. The KC Junior 1-31-5 software (Bio-teck Instrument Inc. USA) was used to read the ODs and determine the UV spectra using a spectrophotometer (µQuant Bio-teck Instrument Inc. USA) coupled to a computer.

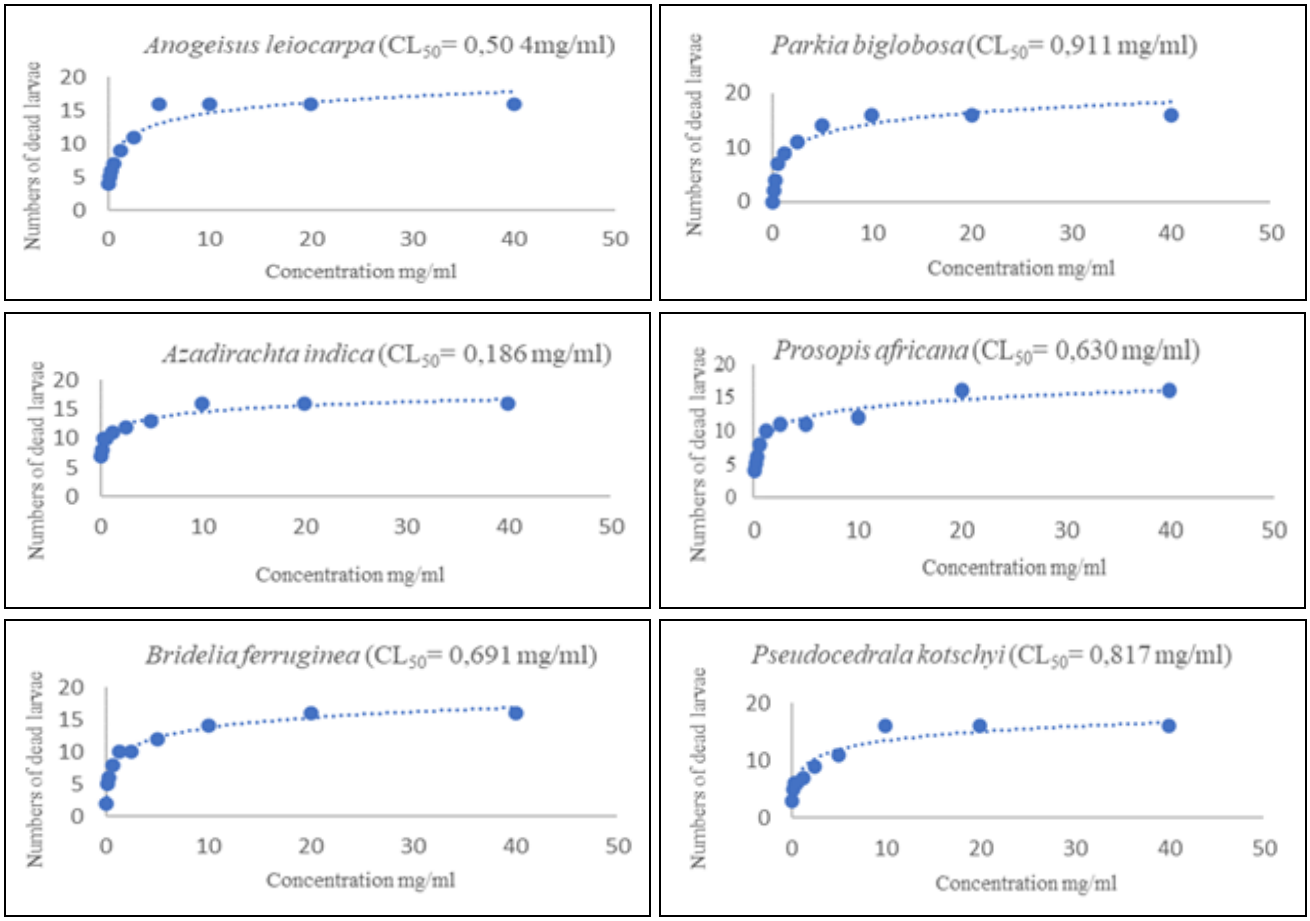
RESULTS:

Extraction Yield: Extraction yields varied from plant to plant **Table 4**. The best yield was obtained in *V. paradoxa* (31.46%) while *D. mespiliformis* had the lowest yield (10.75%).

TABLE 4: EXTRACTION YIELD OF TESTED PLANTS

Plants studied	Performance (%)
Anogeissus leiocarpa	23.02
Azadirachta indica	16.4
Bridelia ferruginea	14.21
Dialium guineense	19.1
Diospyros mespiliformis	10.75
Parkia biglobosa	13.01
Prosopis africana	20.7
Pseudocedrala kotschyi	21.3
Vitellaria paradoxa	31.46
Znathoxylum zanthoxyloides	12.2

Cytotoxicity of Extracts: The number of dead larvae of Artemia salina increases with increasing concentration. The mean lethal concentrations (LC_{50}) obtained for the extracts are shown in **Fig. 3**. With the exception of the hydroethanolic extract of *Z. zanthoxyloides*, all extracts have an LC_{50} greater than 0.1 mg/mL. Based on the toxicity scale established by sparkling [10], the nine (9) other plants are therefore non-cytotoxic. With an LC_{50} = 0.047 mg/ml, the extract of *Z. zanthoxyloides* shows moderate toxicity.



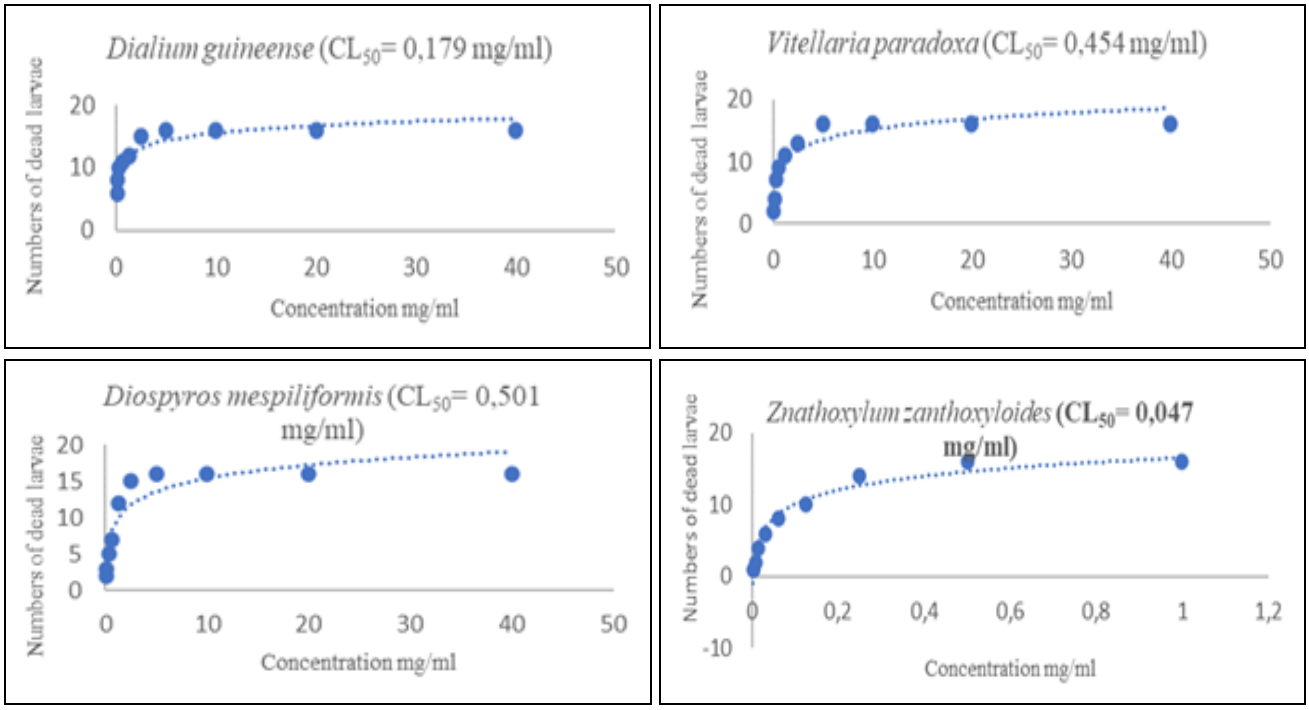


FIG. 3: SENSITIVITY OF *ARTEMIA SALINA* LARVAE TO DIFFERENT EXTRACTS AND CORRESPONDING LC_{50}

Antimicrobial Activity of Extracts:

Results of Diffusion Test in Agar Medium: Of the ten extracts tested, only the hydro-ethanolic extract of the stem of *Z. zanthoxyloides* has no activity on the bacterial strains used in **Table 5**. All other extracts are active on the reference strains. The hydro-ethanolic extracts of the stems of *P. africana* and *A. leiocarpa* are sensitive to all strains except *Klebsiella oxytoca*. The largest diameter inhibition was obtained in the extract of *A. leiocarpa* on *Escherichia coli* ATCC 25922 (25.67mm).

TABLE 5: AVERAGE DIAMETERS OF INHIBITION ZONE OF THE PLANTS EXTRACTS

Plants extracts	Diameter of inhibition zone (mm)					
	Reference strains		Clinical strains			
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 25923	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Escherichia coli*	*Staphylococcus epidermidis*
A. leiocarpa	25.67	17.33	23.67	0	23.67	16.33
A.indica	15.67	15	0	0	0	10.67
B.ferruginea	15	14.33	0	0	0	0
D. guineense	13	13.67	0	0	0	12
D. mespiliformis	15	14.67	0	0	0	11.33
P. biglobosa	11	13	0	0	0	13.33
P. africana	20.33	16.33	20.67	0	21	13.67
P. kotschyi	12.33	14.33	0	0	0	13.33
V. paradoxa	15	14.67	10	0	0	11.67
Z. zanthoxyloides	0	0	0	0	0	0

Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Antibiotic Power: The microplate liquid dilution technique used to determine the MICs and BMCs of the extracts gave the results shown in **Table 6**. The calculation of the MBC/MIC ratio indicates that only the extracts of *A. leiocarpa* and *P. africana* have bactericidal power. These two extracts have bactericidal power on two clinical strains (*Escherichia coli*, *Klebsiella pneumoniae*). The extract of *A. leocarpa* has bactericidal power on the two reference strains (*E. coli* ATCC 25922, *S. aureus* ATCC 25923); on the other hand, *P. africana* has bactericidal power on only one (*E. coli* ATCC 25922).

TABLE 6: MIC, MBC AND ANTIBIOTIC POTENTIAL OF HYDRO-ETHANOL EXTRACTS FROM SELECTED LEAVES ON THE BACTERIAL STRAINS USED

Plants extracts	Parameters (mg/ml)	*Escherichia coli ATCC 25922*	*Escherichia coli*	*Klebsiella pneumoniae*	*Klebsiella oxytoca*	*Staphylococcus aureus ATCC 25923*	*Staphylococcus epidermidis*
A. leiocarpa	CMI	6.25	6.25	6.25	-	6.25	12.50
	CMB	6.25	6.25	6.25	-	12.5	50
	P.a.	1*	1*	1*	-	2*	4
A. indica	CMI	6.25	-	-	-	6.25	12.50
	CMB	25	-	-	-	25	> 50
	P.a.	4	-	-	-	4	-
B. ferruginea	CMI	6.25	-	-	-	6.25	-
	CMB	50	-	-	-	50	-
	P.a	8	-	-	-	8	-
D. guineense	CMI	12.50	-	-	-	6.25	6.25
	CMB	> 50	-	-	-	25	25
	P.a.	-	-	-	-	4	4
D. mespiliformis	CMI	12.50	-	-	-	12.50	12.50
	CMB	50	-	-	-	50	> 50
	P.a.	4	-	-	-	4	-
P. biglobosa	CMI	12.50	-	-	-	12.50	12.50
	CMB	> 50	-	-	-	> 50	> 50
	P.a.	-	-	-	-	-	-
P. africana	CMI	6.25	3.13	6.25	-	6.25	6.25
	CMB	12.50	3.13	12.50	-	25	25
	P.a.	2*	1*	2*	-	4	4
P. kotschyi	CMI	6.25	-	-	-	6.25	12.50
	CMB	50	-	-	-	25	50
	P.a.	8	-	-	-	4	4
V. paradoxa	CMI	6.25	-	-	-	6.25	6.25
	CMB	50	-	-	-	50	50
	P.a.	8	-	-	-	8	8

P.a with * = Bactericidal power; P.a. without * = bacteriostatic power

Chemical Characterization of Medicinal Plants: The chemical characterization of the extracts took into account the qualitative screening, the dosage of phenolic compounds and the determination of the antioxidant power of the extracts. Only the active extracts (9) were taken into account.

Results of the Qualitative Screening: Phyto-chemical analysis of the plants tested revealed the presence of several phytochemical compounds **Table 7**. These are alkaloids, tannins, saponins, flavonoids and polyphenolic compounds. Alkaloids are present only in *P. biglobosa*.

TABLE 7: RESULTS OF PHYTOCHEMICAL SCREENING

Plants: Bark of trunk	Major groups of chemical compounds				
	Alkaloids	**Polyphenols**	**Flavonoids**	**Tannins**	**Saponines**
A. leiocarpa	-	++	++	++	++
A. indica	-	+	++	+	++
B. ferruginea	-	++	++	++	++
D. guineense	-	++	++	++	++
D. mespiliformis	-	++	++	++	++
P. biglobosa	+	++	++	+	++
P. africana	-	++	++	++	+
P. kotschyi	-	++	++	++	+
V. paradoxa	-	+	++	-	++

+ = very positive reaction, + = moderately positive reaction, - = negative test

Content of Phenolic Compounds: Table 8 shows the levels of total polyphenols (TPP) and total flavonoids (FVT) in the various extracts. The levels vary from plant to plant. The highest FVT levels are recorded in *Pseudocedrala kotschyi* with 145.86±0.7 EC/gMS. This is followed by *A. leiocarpa* (82.04±0.4 EC/gMS), *B. ferruginea* (81.34±0.7 EC/gMS), *P. biglobosa* (32.22±0.01 EC/gMS) and *P. africana* (23.49±0.07 EC/gMS). *D. guineense* has the highest PPT content (70.194±

0.220 EGA/g Ms) while the lowest polyphenol content is obtained from the hydro-ethanol extract of *D. mespiliformis* (3.068±0.050 EGA/g Ms).

TABLE 8: DOSAGES OF TOTAL POLYPHENOLS AND FLAVONOIDS

Species	Total flavonoid content (FVT)	Total polyphenol content (TPP)
A. leiocarpa	82.04±0.4	21.51±0.04
A. indica	4.22±0.02	20.43±0.4
B. ferruginea	81.34±0.7	30.98±0.5
D. guineense	3.58±0.13	70.19±0.2
D. mespiliformis	22.02±0.3	3.06±0.05
P. biglobosa	32.22±0.01	9.40±0.6
P. africana	23.49±0.07	10.79±0.5
P. kotschyi	145.86±0.7	64.71±0.2
V. paradoxa	5.7±0.1	19.67±0.2

Antioxidant Power of Extracts: The Antioxidant Power of the extracts was evaluated based on their ability to inhibit free radicals ABTS and DPPH. Trolox and catechin were used as reference molecules. The antiradical powers of the extracts with respect to DPPH and ABTS radicals were determined respectively by the equation lines y = -0.8784x + 2.0281 (R^2 = 0.9925) in Trolox (Trolox Eq mg/gMS) and y = -0.2023x + 0.5099 (R^2 = 0.9807) in catechin (Eq cat mg/MS). **Fig. 4** shows the antiradical capacities of extracts with respect to the DPPH and ABTS radicals. The highest antiradical power with respect to the DPPH radical is obtained with the hydro-ethanolic extract of *P. biglobosa* (22.92±0.15 mg ET/gMS), which records the lowest antiradical power with respect to the ABTS radical (1.61±0, mg ET/gMS).

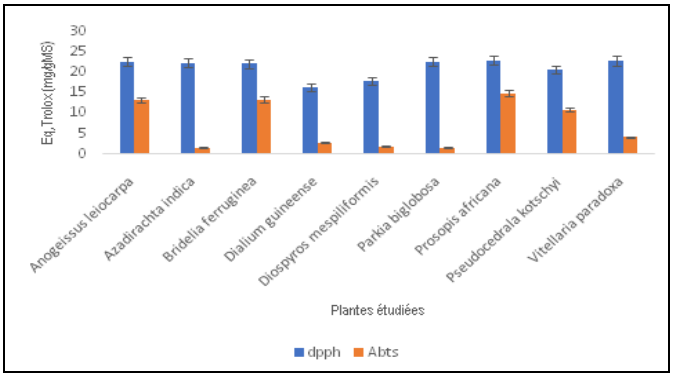


FIG. 4: ANTI-RADICAL CAPACITIES OF EXTRACTS WITH RESPECT TO THE DPPH AND ABTS

The strongest antiradical activity (ABTS) is recorded in the species *A. leiocarpa* (14.97±0.83 mg Cat/gMS), *B. ferruginea* (15.57±0.4 mg Cat/ gMS), *P. africana* (13.25±0.1 mg Cat/gMS) and *P. Kotschyi* (17.35±0.08 mg Cat/gMS). As the ABTS radical of these plants increases, so does their DPPH. These species, therefore, have interesting antioxidant activity. These same species have practically the highest levels of flavonoids and total polyphenols.

DISCUSSION: This study aimed at valorizing 10 medicinal plants traditionally used in Benin for oral care: *Z. zanthoxyloides*, *V. paradoxa*, *P. africana*, *D. guineense*, *P. kotschyi*, *P. biglobosa*, *A. indica*, *D. mespiliformis*, *A. leiocarpa* and *B. ferruginea*. The proposed valorisation strategy involves the production of scientific data on their toxicity, antimicrobial activities and chemical properties.

Extraction results showed variability in extraction yields from one extract to another. The yield expresses the percentage of active ingredients extracted by the solvent. It is an important parameter to discuss the biological activities of an extract. It is known that the affinity of a solvent for phytomolecules and its polarity influence the yield [18]. Thus, the variability of yields could be explained by the variable presence in plants of active molecules with affinity for the water-ethanol mixture.

The *Artemia salina* model was used to assess the cytotoxic effect of the extracts. With the exception of the hydroethanolic extract of *Z. zanthoxyloides*, all extracts have an LC_{50} greater than 0.1 mg/mL. Based on the Toxicity Scale established by sparkling wine [11]; these plants are therefore non-cytotoxic. The cyotoxicity test, according to the *Artemia salina* model is a preliminary screening to determine the degree of cytotoxicity of a product. The work of Parra *et al.*, [19] has shown a good correlation (r= 0.85; p<0.05) between this test and the toxicological effects of a substance on a whole animal. The larval cytotoxicity of *Z. zanthoxyloides* confirms the results of previous work that attributed toxicity to this plant. Indeed, Goodman *et al.*, [20] found that the dichloromethane extract of *Z. zanthoxyloides* was cytotoxic with an LC_{50} of less than 0.1 mg/Ml. The non-cytotoxic character of the extracts guarantees the therapeutic use of medicinal plants without risk to human health. However, the cytotoxicity test is only a preliminary test. Extensive toxicity work, such as acute and chronic toxicity, is necessary to guarantee the non-toxicity of the medicinal plants.

Of the ten extracts tested, only the hydro-ethanolic extract of the *Z. zanthoxyloid* stem has no activity on the bacterial strains used. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (reference strains) and *Staphylococcus epidermidis* (clinical strain) are sensitive to the hydro-ethanolic extract of *D. guineense*. This confirms the work of Osuagwu and Eme [21] who have shown the activity of extracts of this plant on these strains. Olajubu *et al*., [22] also showed the activity of *D. guineense* extracts on *S. aureus* 25923. Of all the strains, the most active extracts are those of *P. africana* and *A. leiocarpa*. The extracts of these two plants inhibited 83.3% of the strains tested, while presenting the largest diameters of inhibition. With regard to *A. leiocarpa*, the results obtained confirm the data previously obtained by Ali *et al*., [23] who showed the inhibition of *S. aureus* and *K. pneumoniae* by the ethyl acetate extract of the stem bark of *A. leiocarpa*. But the diameters of inhibition are much larger than those obtained with the hydro-ethanolic extract in this study, which can be assimilated to the difference in polarity of the extraction solvents.

The inhibition of clinical strains of *E. coli* by aqueous and ethanolic extracts of *A. leiocarpa* roots, as reported by Gbadamosi and Ogunsuyi [24] also supports the results of the present study, making this species a reservoir of bioactive molecules with antimicrobial activities to be used in the fight against microbial infections. With regard to *P. africana*, the results obtained corroborate the work of Kukushe *et al*., [25,] which showed inhibition of *S. aureus, K. pneumoniae* and *E. coli* by aqueous and methanolic extracts of *P. africana*.

It should be noted that the hydro-ethanol extracts of the stems of *D. guineense*, *P. kotschyi*, *P. biglobosa*, *A. indica*, *D. mespiliformis*, *V. paradoxa* and *B. ferruginea* were only active on the reference strain *Escherichia coli* ATCC 25922 and not on the clinical strain *E. coli*. This difference in activity could be explained by the fact that the reference strains are usually already characterized.

Differences in the sensitivity of the strains to the extracts compared to other studies could be related to different parameters: the origin of the strains, the characteristics of the strains and the manipulation techniques. Moreover, the remarkable antibacterial activities recorded to justify the use of medicinal plants for oral care because the extracts have inhibited bacterial strains involved in oral ulcers.

To explain the antimicrobial activity of the extracts, chemical characterization of the extracts was performed. The results of the phytochemical screening show, at the level of the tested plants, the presence of alkaloids, tannins, saponins, flavonoids and polyphenolic compounds. Quantitatively, interesting and variable contents of polyphenol and flavonoid extracts were found. All the extracts showed antioxidant activity with an inhibitory power of DPPH and ABTS radicals.

The variability in the chemical composition of the extracts could be explained by the taxonomic diversity of plant species. In addition, the interesting antibacterial properties of the plants involved in the present study could be explained by their content of polyphenols and flavonoids. Indeed, it is believed that the ability of an herbal remedy to inhibit microbial growth is due to its various components [26]. These are mostly the products of plant metabolism, which from a chemical point of view can belong to the most varied groups of substances: phenolic compounds, tannins, anthocyanins, coumarins, alkaloids and flavonoids [27, 28].

Data on the phytochemistry of extracts support scientific works in the literature that have shown the same chemical components in extracts of *P. africana* [29], *D. guineense* [21], *V. paradoxa* [30], and *A. leiocarpa* [24]. *A. leiocarpa* and *P. africana* presented the best antioxidant activities. These plants can thus effectively contribute to reduce oxidative stress which is associated with several pathologies. Authors believe that the use of natural products rich in antioxidants could play an important role in the prevention of these diseases [31]. An antioxidant activity associated with good antimicrobial activity is therefore an asset.

CONCLUSION: *V. paradoxa*, *P. africana*, *D. guineense*, *P. kotschyi*, *P. biglobosa*, *A. indica*, *D. mespiliformis*, *A. leiocarpa* and *B. ferruginea* have a chemical composition likely to be valued in the management of oral infections, especially since they are non-cytotoxic. The extracts of *A. leiocarpa*

and *P. africana* have been distinguished by having better antioxidant activities, associated with an interesting antibacterial activity. More in-depth work is necessary on these plants for optimal valorization.

ACKNOWLEDGEMENT: We acknowledge the director of the research unit in Applied Microbiology and Pharmacology of Natural Substances for making his technical platform available for this work. We especially thank Mr. Eric Agbodjanto (Msc) for their technical support in the laboratory manipulations.

CONFLICTS OF INTEREST: The authors declare that there is no potential conflict of interest between them.

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How to cite this article:
Guezodje TGP, Agbankpe AJ, Dassou GH, Dougnon TV, Dicko A and Yedomonhan H: Cytotoxicity, antimicrobial activities and chemical properties of ten plants from the Benin Pharmacopoeia used for oral care. Int J Pharm Sci & Res 2021; 12(12): 6642-52. doi: 10.13040/IJPSR.0975-8232.12(12).6642-52.