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PHYTOCHEMICAL AND PHARMACOGNOSTIC CHARACTERIZATION OF TWO PLANTS USED IN WEST AFRICA FOR THE ALTERNATIVE MANAGEMENT OF STOMA TO LOGICAL INFECTIONS

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Keywords:

Oral infections, *Cocos nucifera*, *Anacardium occidentale*, Phytochemical analysis, Toxicity, antiradical activity

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ABSTRACT: This work aims to contribute to the phyto-therapeutic treatment of oral diseases that do not have a recognized management program, such as malaria, HIV-AIDS and tuberculosis. The aim is to qualitatively and quantitatively determine the chemical composition of *Anacardium occidentale* and *Cocos nucifera*, as well as the toxicity and sensitivity to their extracts of the microbial strains responsible for oral infections. Medicinal plant sellers in 03 markets of Porto-Novo use plant species to treat oral infections. *C. nucifera* (A) and *A. occidentale* (B) were the most frequently cited, used in decoction form. Qualitative phytochemical analysis using the standard method showed that C. nucifera roots contain tannins, steroids, mucilages, leuco-anthocyanins and alkaloids, while *A. occidentale* roots contain tannins, flavonoids, anthocyanins, leuco- anthocyanins, steroids, reducing compounds, coumarins and mucilages. The contents of total phenols (18.79±0.46 mgEAG/g(A)) and (20.05±1.01 mgEAG/g (B)), flavonoids (39.04±18.8 mgEQ/g) for extract B, condensed tannins (8.36±0.22 mgEC/g (A)) and (5.77±0.27mgEC/g(B)), justify the biological potential of these plants. Evaluation of the free radical scavenging activity of the decocts of both plants revealed moderate inhibitory concentrations (IC50=7.31mg/mL (A); IC50=15.54 mg/mL (B)) compared with ascorbic acid (IC50 = 3.18 mg/mL). The general toxicity test carried out on *Artemia salina* Leach larvae, showed that these extracts were a priori harmless on human cells with a lethal half concentration equal to 1.50 mg/mL for extract A and 0.46 mg/mL for extract B. Both extracts showed good activity against *Candida albicans* ATCC 10231 and Streptococcus mutans strains, with inhibition diameters ranging from 7.2±0.4 mm for the *C. nucifera* aqueous extract to 10.7±0.1 mm for the *A. occidentale* extract and thus constitute sources of natural anti-infective agents against oral diseases.

INTRODUCTION: Oral diseases and infections are responsible for a significant burden of disease in many countries and make their effects felt throughout life, causing discomfort, pain, disfiguring lesions and even death (WHO; 2018) and this applies to both children and adults. Some of these infections can be avoided with good dental hygiene, bearing in mind that most of them last only a few days, especially those common in children. Other dental conditions, however, may be at a more advanced stage and take longer to

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resolve. Oral infections are one of the ills that undermine human flourishing and affluence in Africa, especially in Benin. In the WHO African Region, poor oral health causes pain to millions of people, increases the financial burden on society, and significantly affects the quality of life and well-being of the individuals concerned. Oral diseases are among the most common preventable non-communicable diseases (NCDs) in the world. They are all multifactorial in origin and share modifiable risk factors with the main NCDs (WHO, 2014; WHO-Africa, 2016).

Dental caries is one of the chronic diseases worldwide that can affect an individual throughout his or her lifetime (Selwitz *et al.*, 2007) [51]. In Europe, progress has been made in the field of oral health. Conversely, many problems persist in communities in developing countries, particularly among disadvantaged groups [6, 33]. In Africa, the treatment of caries and periodontal disease, in addition to the oral manifestations of HIV, are overlooked by health systems, with training programs for dental health care personnel (WHO, 2000). Oral diseases constitute a crucial public health problem, due to their high prevalence and the considerable impact they have on general health and quality of life [33].

Like industrialized countries, the African region has a dysfunctional oral health information system [46]. Although antifungal drugs are available today, the treatment of oral infections remains difficult, partly because of the limited number of effective principles and their very high cost, and partly because of the emergence of strains resistant to certain conventional antimycotics. In addition to bacteria, yeasts are frequently present in the mouth as commensals. This is the case of Candida albicans, which in certain circumstances is associated with oral-pharyngeal lesions. Oral candidiasis can take many forms, but thrush remains the best-known [32].

In view of the difficulties encountered by synthetic antibiotics against microorganisms resistant to their effects, it is more urgent to propose an effective, less costly and more credible alternative solution with medicinal plants used in traditional Beninese medicine, which could solve the problem of oral infection in the medium and long term.

In Africa, the therapeutic power of plants was known empirically by ancestors and relatives [39], but the chemical composition of the medicines used daily by these numerous populations for their health care was ignored. Indeed, for most of these plants, the chemical compounds responsible for the reported biological activities as well as their toxicity remain unknown [31]. In Congo, among the plants cited in an ethnobotanical survey, *Anarcadium occidentale* is identified as a plant whose leaves and roots are used to treat tooth decay [35].

In north- central Nigeria, the leaves or bark of *Anarcadium occidentale* are used as a decoction in water, to treat typhoid fever, ulcers and fungal diseases [38]. Similarly, *Cocos nucifera* L. is used in Côte d'Ivoire to treat tooth decay [7]. In Benin, as in other developing countries, several scientific studies [2, 10, 27] have focused on ethnobotanical inventory to contribute to the knowledge of medicinal plants. The ethnobotanical study of plants for oral use conducted in the communes of Dassa-Zoumè and Savè (Djakpa, 2015) identified 18 species, including *Cocos nucifera* and *Anacardium occidentale*, which represents the second most common plant (5.36%) after *Jatropha curcas*. In Porto-Novo, women selling medicinal plants in the markets gave priority to two species, *Anacardium occidentale* and *Cocos nucifera*, to treat mouth infections and tooth diseases. The general aim of this work is to evaluate the chemical and pharmocognosic potentials of these two plant species in order to broaden the range of remedies for oral and dental ailments in Africa.

MATERIALS AND METHODS:

Material: Roots of *Anacardium occidentale* and *Cocos nucifera* were harvested in the southeastern region of Benin, in the commune of Akpro-Misserete, brought to the laboratory and laid out in a room at constant temperature until completely dry for 14 days. They were then ground to a fine powder using a grinder, which formed the starting matrix for the analyses. **Fig. 1** and **2** show the two species studied. The biological material consisted of *Artemia salina* (Leach) shrimp larvae for the toxicity test, and strains of *Candida albicans* ATCC 10231 and *Streptococcus mutans* isolated from decayed teeth.

FIG. 1: AERIAL PART OF *C. NUCIFERA*

FIG. 2: AERIAL PART OF *A. OCCIDENTALE*

Methods:

Ethnobotanical Survey: The survey was conducted using the interview method (Moyabi *et al.,* 2020; [37] Deguenon *et al.,* 2023) [11], with the aid of a questionnaire and an interview guide with 6 randomly selected sellers of traditional medicinal plants in three different markets in the city of Porto-Novo (OUANDO, AGBOKOU medicinal plant market, and the large AWANGBO market). The questionnaire is based on the FARMEL form, widely used in the collection of plant kingdom information [8]. The citation frequency (CF) of each species was determined using the formula below [11, 17, 19]:

CF = Number of citations for the plant / Total citations for all plants × 100

Phytochemical Analysis: Secondary metabolites were identified using the standard method of tube reactions, differential staining and precipitation characteristic of each plant chemical compound [18, 41, 4, 11]. Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars, Fehling's test for glycosides, Liebermann-Burchard's test for triterpenoids, Liebermann-Burchard's test for steroids, frothy test for saponins, Shinoda's and sodium hydroxide tests for flavonoids, ferric chloride test for tannins, Guignard's test for free cyanogenetics derived and Borntrager's test for free anthraquinones.

Preparation of Crude Extracts: Total chemical principles were extracted from species using the decoction method as described in the literature [18]. 50 g of powder were dissolved in 500 mL of distilled water. The mixture is brought to a moderate boil for 30 min. After cooling, the mixture is filtered (3 times in succession) on absorbent cotton and the filtrate is transferred to a 1000 mL flask, then evaporated at 40°C using a rotavapor. The dry residue obtained is the decoctate. Extraction is repeated twice on the same quantity (50g). Finally, the various dry residues obtained are weighed and the yield calculated.

Quantitative Determination of Some Major Metabolites

Determination of Total Phenols: 125µL of 1mg/ml sample is taken and dissolved in 625µL of Folin-Ciocalteu reagent. After incubation for 5 min, 500µL of sodium carbonate Na_2CO_3 at 75mg/mL is added. The mixture is vortexed and incubated for 2 h in the dark. Absorbance readings are taken with a Genova brand spectrophotometer at 760 nm[11, 34]. Polyphenol concentrations are deduced using equation (α) established from gallic acid calibration ranges (0- 10mg/ml) and are expressed as mg gallic acid equivalent per gram of dry extract [4, 26, 29, 34].

$$T\ (mgeqAG / g) = C.\ Vr / Vp.\ Cp \quad (\alpha)$$

T = Content of compounds; C = Concentration obtained from calibration curve Vr = Reaction volume; Vp = Volume of extract taken of extract taken; Cp = Concentration of extract solution taken.

Determination of Flavonoids: Remove 500µL of AlCl3 solution (2%) and add 500µL of sample. Add 3mL of methanol and incubate for 10 min. Optical densities were read using a spectrophotometer at 415nm against a blank consisting of 500µL AlCl3 and 3.5mL methanol. Quercetin was used as a control, prepared at a concentration of 10 mg/mL methanol [34]. Flavonoid contents are calculated from equation (β) derived from the regression line of the standard (quercetin) and are expressed as mg quercetin equivalent per gram of dry extract (Kim et al, 2003; [34].

T (mgeqQ / g) = C. Vr / Vp. Cp....... (β)

T = Content of compounds; C = Concentration obtained from calibration curve Vr = Reaction volume; Vp = Volume of extract taken of extract taken; Cp = Concentration of extract solution taken.

Determination of Condensed Tannins: 500µL of extract is taken and 1.5mL of vanillin solution (4%) prepared in methanol, 1.5mL of concentrated hydrochloric acid and 2mL of methanol are added. The vanillin solution is prepared by dissolving 4g vanillin in 100mL methanol, and the catechin solution is prepared from 20mg catechin in 4mL methanol [21]. The mixture is incubated for 15 min and the absorbance is read at 500 nm. The calibration line is established with catechin (0-500µg/mL). Tannin contents are calculated by applying equation (γ) derived from the regression line for the standard (catechin) and are expressed in µg catechin equivalent (CAT) per milligram of dry extract [34].

T (geqCAT / mg) = C. Vr / Vp. Cp....... (γ)

T = Content of compounds; C = Concentration obtained from calibration curve; Vr = Reaction volume; Vp = Volume of extract taken; Cp = Concentration of extract solution taken.

Assessment of Anti-free Radical Activity: This activity was assessed by direct reduction of the DPPH radical. (2,2-diphenyl-1- picrylhydrazyl) radical. This involves measuring the ability of extracts to donate an H° radical (Gandonou *et al*., 2018). DPPH exhibits a violet coloration at 517 nm in solution, which changes to yellow when reduced by a free radical scavenger (antioxidant).

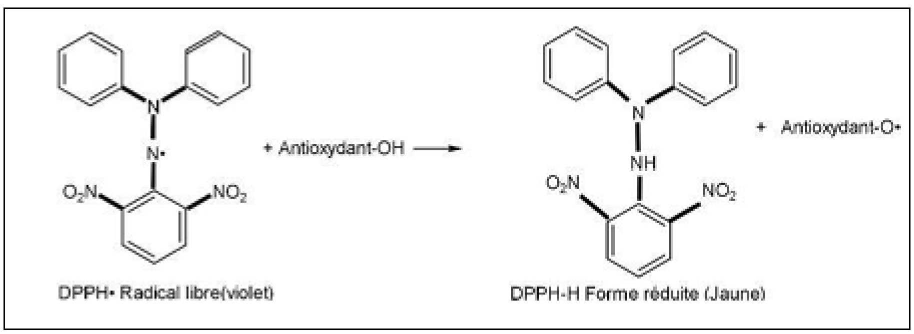

FIG. 3: REDUCTION REACTION OF DPPH RADICAL

A stock solution is prepared at 1mg/mL for the extract and 0.4mg/mL for the DPPH radical using analytical-grade methanol. 1.5mL of the extract solution is then mixed with 3mL of the DPPH° solution. After 15 min in the dark, the optical density is read at 517 nm. The regression curve is constructed with a reference antioxidant (vitamin C, Sigma Aldrich) in the range 0-10mg/mL. The percentage reduction of DPPH is calculated according to the relationship.

(% DPPH) = 100 × (Abs of control - Abs of sample /Abs of control)..... (δ).

Where Abs blank is the absorbance of the control (reaction mixture excluding test compounds) and Abs sample is the absorbance of the test compounds. IC_{50} values, corresponding to the concentration of plant drug extract that caused 50% DPPH radical neutralization, are calculated from the graph of percent recovery versus DPPH concentration. *In-vitro* toxicity test Toxicity on shrimp larvae correlates with cytotoxicity on human cells 9PS, 9KB, A-549, HT-29 [9, 45]. The test is performed according to the standard method used by Sakirigui *et al* (2012a); [48] Sakirigui *et al* (2012b); [49]. *Artemia salina* L. eggs are incubated in seawater until they hatch for 48 h. A series of 10 diluted solutions of extracts at variable and progressive concentrations at half (1/2) the stock solution at 100mg/mL, is brought into contact with a colony of 16 larvae each. All solutions, together with control solutions containing no active substance, are left under agitation for 24 hours. Surviving larvae in each solution are counted under an electron microscope to assess the toxicity of the solution. In the event of death in the control medium, the data are corrected using Abbott's formula:

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

Dose-response data were log-transformed and the LC50 determined. To compare cytotoxic activity from LC_{50} values we exploited the correspondence **Table 1** established by Mousseux (1995) [36] and widely used in the literature [18, 41].

TABLE 1: RELATIONSHIP BETWEEN LC50 AND TOXICITY

LC_{50}	Degree of toxicity
$LC_{50} \geq 100$ µg/mL	-(non-toxic)
100 µg/mL> $LC_{50} \geq$ 50µg/mL	+(low)
50 µg/mL> $LC_{50} \geq$10µg/mL	++(moderate)
LC_{50}< 10 µg/mL	+++(strong)

***In-vitro* Antimicrobial Test:** Strains of *Candida albicans* ATCC 10231 and *Streptococcus mutans* (isolate) were tested for their sensitivity to extracts using the well diffusion technique employed by Dougnon *et al*, 2013) [17].

To this, 106 UFC inoculum from the strains was swabbed into petri dishes containing Mueller Hinton Agar. Sterile pasteur pipettes were used to make 6 mm diameter wells. Then, using a sterile cone adapted to a micropipette, 50 µl of each extract at 20mg/mL was deposited in the previously dug wells. A well containing sterile distilled water served as a negative control. Petri dishes were left for 1 hour at room temperature to pre-diffuse the substances, before being incubated at 37°C in the oven for 18 hours. After incubation, the plates were removed to read the diameters of the zones of inhibition around the wells. The degree of sensitivity was assessed using the scale **Table 2** of Tsirinirindravo and Andrianarisoa (2009).

TABLE 2: STRAIN SENSITIVITY AS A FUNCTION OF INHIBITION DIAMETER

Inhibition diameter D	Degree of sensitivity	Symbol
<7mm	Insensitive	-
7mm≤Δ < 8mm	Fairly sensitive	+
8mm≤Δ < 9mm	Sensitive	++
Δ≥ 9mm	higher sensitive	+++

RESULTS AND DISCUSSION:

Selected Plants: The ethnobotanical survey enabled us to select two species most frequently cited in the treatment of oral infections in Benin, belonging to two botanical families. The first is *Anacardium occidentale* (Anacardiaceae) called Akadjou-tin (in Fon and Goun), with a frequency of quotation (FC) equal to 64.29. The second species is *Cocos nucifera* (FC=43.51) from the Arecaceae family, called Agon-tin (in Fon and Goun) and Yovoninti, Yevunetsi, Netsi (in Ewe and Mina).

Chemical Groups Identified: The results of the phytochemical screening are shown in **Table 3** below:

TABLE 3: SECONDARY METABOLITES IDENTIFIED

Chemical groups	Species		
	Cocos nucifera	*Anacardium occidentale*	Totals*
Catechictannins	++	++	02
Gallictannins	-	-	00
Flavonoids	-	++	01
Leuco-Anthocyanins	++	++	02
Anthocyanins	-	+	01
Alkaloids	+	-	01
Reducing compounds	-	+	01
Mucilage	++	++	02
Saponoside	-	-	00
Cyanogenic derivatives	-	-	00
Triterpenes	-	+	01
Steroids	++	++	02
Coumarins	-	++	01
Quinone derivatives	-	-	00
Free anthratracenics	-	-	00
C-Heterosides	-	-	00
O-Heterosides	-	-	00
Cardiotonic derivatives	-	-	00

+: positive reaction ++: strongly positive reaction -: negative reaction * Number of species containing a given chemical group.

The results show that the plants studied are rich in secondary metabolites. They contain a total of eight (08) different chemical groups. They do not contain the toxic chemical groups cardiotonic heterosides and cyanogenic derivatives, making them a priori safe for oral use. In addition, both plants contain catechic tannins, leuco-anthocyane, mucilages and steroids. We can therefore deduce that these 4 chemical groups are the most common in plants used as oral anti-infectives. However, they do not include compounds such as saponosides, quinone derivatives, triterpenes, free anthracenics, c-heterosides and o-heterosides. It should also be noted that certain chemical groups with proven antimicrobial properties have been singularly identified with these plants [15, 20]. These are essentially alkaloids for *Cocos nucifera*, and flavonoids for *Anacardium occidentale*. The literature indicates that the antimicrobial activity of several plant species is inherent to certain main chemical groups, namely flavonoids, tannins, anthocyanins and alkaloids [25, 31]. Steroids are also powerful antioxidants and antimicrobials [28]. Previous work has shown the presence of flavonoids and coumarins in aqueous and methanolic extracts of *Anarcadium occidentale*. Also we detected triterpenes in the plant, which is in agreement with the results of Togola et al., (2020) [54], when they observed terpenoids in extracts of the plant's root bark. The same authors noted the presence of alkaloids in aqueous and methanolic extracts of *Anarcadium occidentale* leaves and root bark, while tannins were absent in leaf extracts. Our results are contrary to those reported in the literature with regard to alkaloids and tannins. The chemical composition of this species is similar to that obtained by other researchers [1, 13, 53].

The presence of tannins and steroids is in line with previous work by Kadja [24] and colleagues, who in 2020 in Côte d'Ivoire proved through phytochemical analysis that *Cocos nucifera* hulls contain coumarins, flavonoids, steroids, terpenes and tannins. Ultimately, the pharmacological properties of these major chemical groups, namely tannins, alkaloids and flavonoids, may well explain the properties of these plants in combating oral infections.

Extraction Yield: The yield of the extracts are in the table below:

TABLE 4: YIELD OF DIFFERENT EXTRACTS

Plants	Organ	Yield (%)
Anacardium occidentale	Root'sBark	12.86±0.17
Cocos nucifera	Root	24.71±0.08

Water was used to extract the majority of chemical compounds from both plants. The quantities extracted were significant, and the mass yield was twice as high for *Cocos nucifera* roots as for *A. occidentale* bark. This result shows that the plant contains compounds with an affinity for water, i.e. polar and thermoresistant molecules. Anti-radical activity The optical density values obtained were used to calculate percentages of radical inhibition and to plot curves **Fig. 4, 5** and **6** of almost complete reduction of DPPH to its molecular form. From these curves we determined the IC_{50} value for the extract. The smaller the IC_{50} value, the greater the antioxidant activity of the extract.

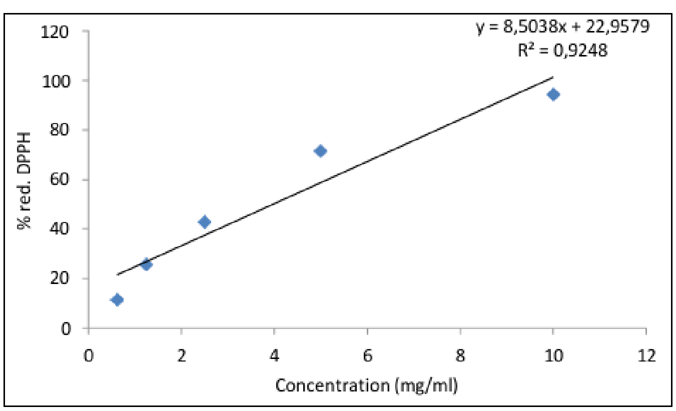

FIG. 4: CALIBRATION CURVE FOR VITAMIN C DPPH-H

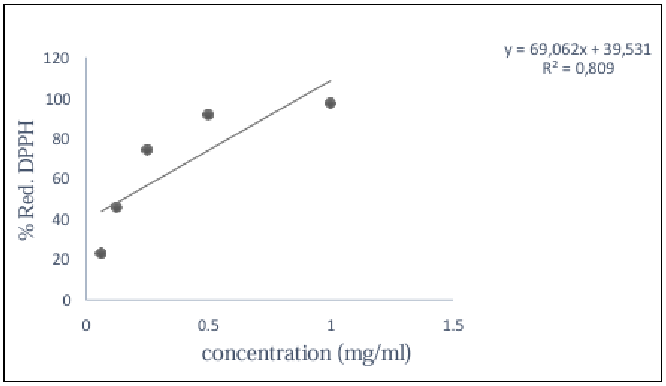

FIG. 5: DPPH-H CALIBRATION CURVE FOR *COCOS NUCIFERA* EXTRACT

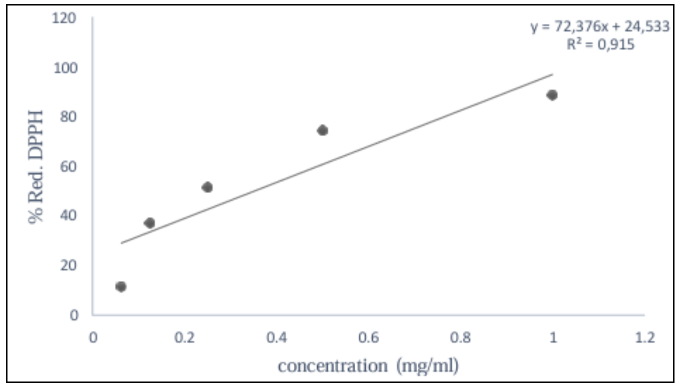

FIG. 6: DPPH-H CALIBRATION CURVE FOR *A. OCCIDENTALE* EXTRACT

The results show that the percentage reduction is proportional to the concentration of extract and vitamin C used as reference. More precisely, an increase in the concentration of the sample causes an increase in the percentage of free radical reduction, and consequently high antioxidant activity is exhibited. The antioxidant activity of the extract is expressed in IC_{50} values shown in **Table 5.**

TABLE 5: CALCULATED IC50 VALUES

Extracts / reference	IC_{50} (mg/mL)
Vitamine C	3.18±0.02
Cocos nucifera	7.31±0.06
A. occidentale	15.54±0.13

In order to better appreciate the difference between the values found, we have constructed with the Excel 2013 system, a histogram represented in **Fig. 7** below. The plant extracts studied show antioxidant power, but lower than the reference represented here by vitamin C. We also note that the *C. nucifera* extract has a stronger antioxidant power than that of *A. occidentale*. These results provide evidence that these plants, through the organs studied, would therefore be useful as free radical scavengers and thus help in the treatment of numerous diseases caused by reactive oxygen species. These include aging, inflammation, cancer, diabetes and microbial infections. Anti-radical activity is due to the presence of major chemical groups including tannins and flavonoids [28]. This result reinforces that of the phytochemical screening which highlighted these polyphenolic compounds for one or other of the species. The leaf and stem bark extracts of *Anacardium occidentale* demonstrated very interesting antioxidant activity with IC_{50} = 5.24±0.34 µg/ml for the ethyl acetate extract [54].

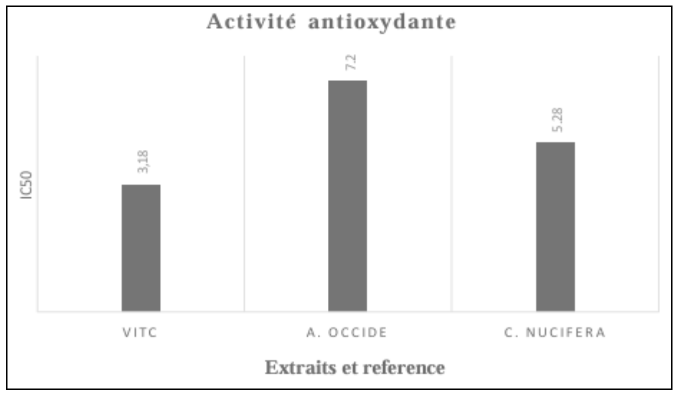

FIG. 7: ANTIOXIDANT ACTIVITY EXPRESSED AS IC_{50} FOR DIFFERENT EXTRACTS

The antioxidant activity of the methanolic and ethyl acetate extracts of *A. occidentale* leaves was almost identical. The aqueous extract showed the lowest total antioxidant capacity value of 7.83±1.26 mg EAA/g) for stem bark, against 18.12±1.23 mg EAA/g for leaves. These activities are higher than in our work. Moreover, other previous work has reported that the antioxidant activity of young *A. occidentale* leaves is higher than that of stem bark [52]. This difference in results can be justified by the fact that Togola *et al* [54]. Worked on organic extracts of leaves and stems, whereas our work focused on the aqueous root extract of the same plant. Several research studies have indicated that antioxidant capacity depends on the type of extract tested for a given species [5, 47, 50]. According to Kadja et al. (2020) [24], the aqueous extract of *C. nucifera* showed very high antioxidant activity in the DPPH radical test (EC_{50} = 2.56). This activity is twice as high as the value found in our results, which is inherent to the plant part used.

Polyphenolic Metabolite Content: The concentrations of total phenols, tannins and flavonoids are calculated from the calibration range calibration range established with gallic acid, catechin and quercetin respectively. **Fig. 8, 9** and **10** show the different calibration lines.

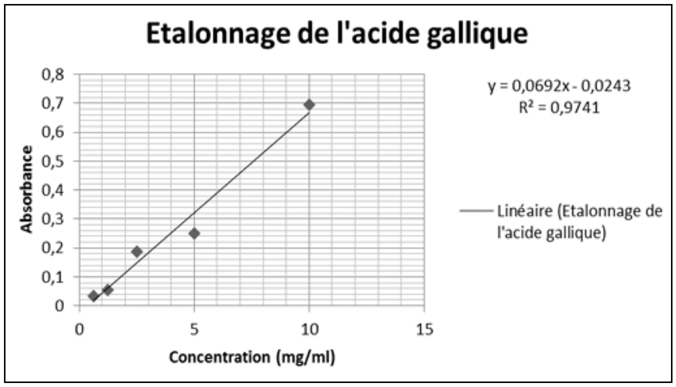

FIG. 8: GALLIC ACID CALIBRATION CURVE

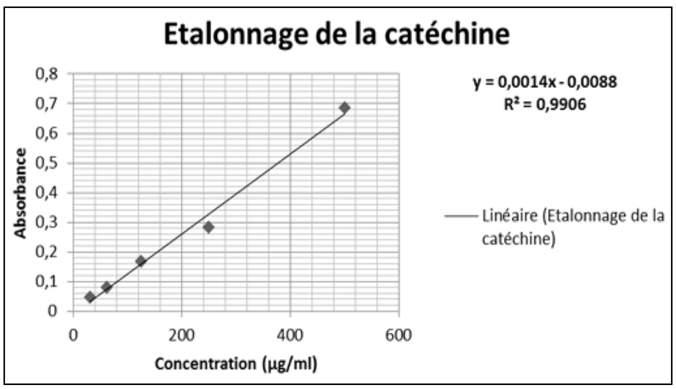

FIG. 9: CATECHIN CALIBRATION CURVE

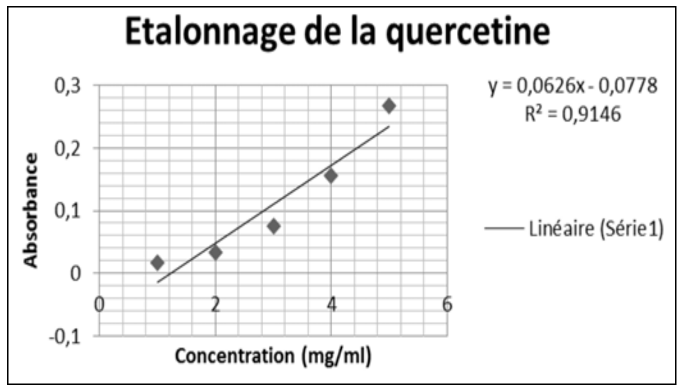

FIG. 8: QUERCETIN CALIBRATION CURVE

The calculated contents of total phenols, flavonoids and condensed tannins are summarized in the table below:

TABLE 6: CONTENT OF SOME METABOLITES IN EXTRACTS

Chemical compounds		Quantity of metabolites	Curve equation	R^2
Total phenols	A	18.79 ± 0.46mg EAG/g ES		
			Y =0.0692x-0.0243	0.9741
	B	20.05 ± 1.01mg EAG/gES		
Total flavonoids	A	Nondeterminé		
	B	39.04 ± 1.08 mgEQ/gES	Y =0.0626x-0.0778	0.9146
Condensed tannins	A	8.36 ± 0.22 mgEC/gES		
	B	5.77 ± 0.27mgEC/gES	Y =0.0014x-0.0088	0.9906

EAG: equivalent of gallic acid, EQ: equivalent of quercetin, EC: equivalent of catechin, ES: dry extract. A: *Coco nucifera*. B: *Anacardium occidentale*.

The histograms below allow us to better interpret the levels of these groups of compounds.

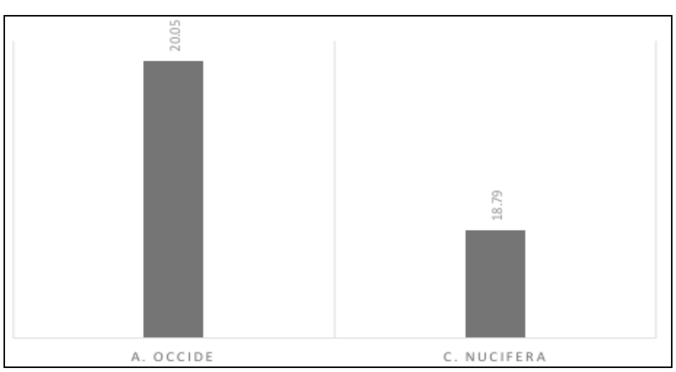

FIG. 11: TOTAL PHENOL CONTENT

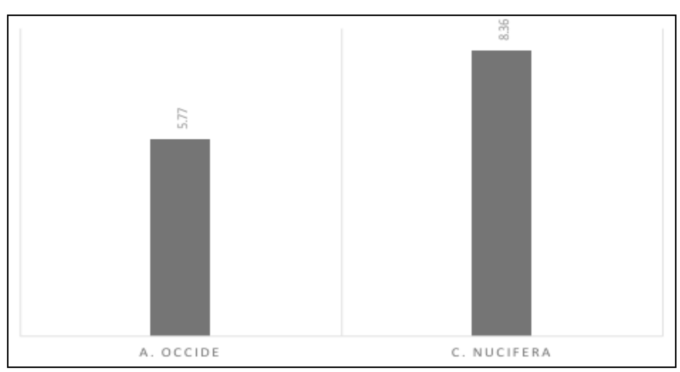

FIG. 12: TOTAL TANNIN CONTENT

A. occidentale extract is richer in polyphenols than *C. nucifera* extract. On the other hand, *C. nucifera* extract is richer in tannins. It is also worth noting that the *A. occidentale* extract has a high total flavonoid content, compared with a total absence in the *C. nucifera* extract revealed by phytochemical screening. These results are in line with those of the phytochemical screening, which had previously shown the qualitative presence of these groups in the samples analyzed. It should be added that the levels obtained for these metabolites support the antioxidant potential of these extracts. Cytotoxic activity of extracts **Fig. 13** and **14** show the variation in larval cell sensitivity as a function of extract concentration.

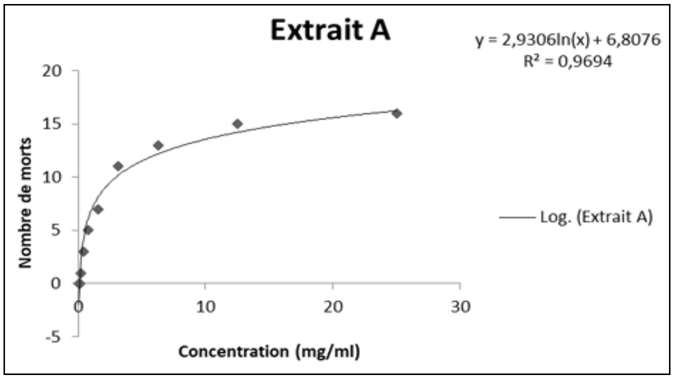

FIG. 13: SENSITIVITY CURVE OF *ARTEMIA SALINA* LARVAE TO THE *COCOS NUCIFERA* AQUEOUS ABSTRACT

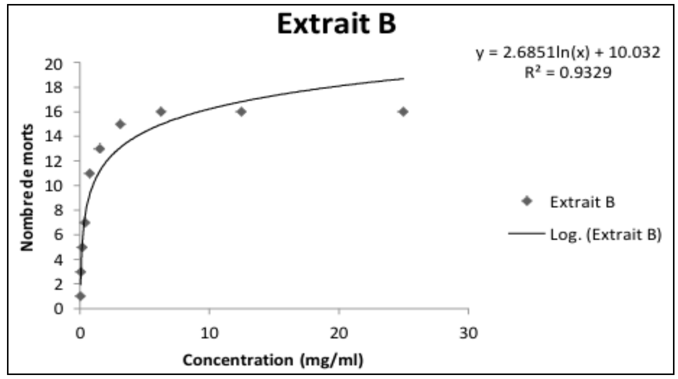

FIG. 14: SENSITIVITY CURVE OF *ARTEMIA SALINA* LARVAE TO THE *A. OCCIDENTALE* AQUEOUS ABSTRACT

All graphs show a good correlation between progressive extract concentrations and larval mortality. Larvae are sensitive to the extracts tested, following a dose-dependent relationship. The LC_{50} of the various extracts tested were determined using the expression: $LC_{50} = e[(8-\beta)/\boldsymbol{\alpha}]$ with β the y-intercept and α the directing coefficient of the equation ($y=\alpha \ln x+\beta$) of the logarithmic regression curve. The various values found are summarized in **Table 9** below:

TABLE 7: SUMMARY OF CALCULATED LC_{50} VALUES

Aqueous extracts	α	ℑ	LC_{50} (mg/mL)
Extract A	2.93	6.807	1.50
Extract B	2.685	10.03	0.46

A: *Coco nucifera*. B: *Anacardium occidentale*.

According to the correspondence table drawn up by Mousseux (1995) [36], we can say that the extracts show no toxicity in the range of concentrations analyzed, since the LC_{50} values obtained are between 0.46 and 1.50 mg/mL for *A. occidentale* and *C. nucifera* decocts respectively, which are well above the set limit. Occidentale and *C. nucifera* decocts respectively, well above the set limit. Considering the correlation between cytotoxicity on shrimp larvae and on cells, we can say a priori that the extracts tested are free of cytotoxic activity. This result justifies the results of the phytochemical screening, which showed the absence of cardiotonic heterosides, cyanogenic derivatives and quinonic derivatives, which are generally toxic compounds [18].

Antifungal and Antibacterial Activity of Extracts: The diameters of the inhibition zones measured are shown in **Table 8.**

TABLE 8: STRAIN INHIBITION DIAMETERS BY EXTRACTS (MM)

Extract (decocted)	Diameter of inhibition zones (mm)	
	Streptococcus mutans	***Candida albicans* ATCC10231**
A. occidentale	8.6±0.2	10.7±0.1
C. nucifera	7.2±0.4	7.84±0.6

The table shows that both extracts have zones of inhibition with diameters ranging from 7.2 mm to 10.7 mm. Based on the scale used, we conclude that *Candida albicans* is very sensitive to the aqueous extract of *A. occidentale*, and fairly sensitive to that of *C. nucifera*. Similarly, *Streptococcus mutans* is sensitive to *A. occidentale* extract and fairly sensitive to *C. nucifera* extract. This result justifies the use of plants in the treatment of oral infections. Both plants can therefore be used to treat thrush and tooth decay. Indeed, the flavonoids and coumarins identified in the plants have anti-inflammatory, antiseptic [23], antifungal and antibacterial properties [30], which would justify their use in the treatment of candidiasis in children for thrush.

Terpenoids also have excellent antibacterial activity against a variety of bacteria, including *Candida albicans*, the causative agent of candidiasis [16]. Aqueous extracts of *C. nucifera* prepared at variable temperatures revealed bactericidal power against strains of *Streptococcus mutans* responsible for dental caries [24]. The results of this earlier work are similar to those obtained in our research. Both extracts are therefore potential candidates to enrich the therapeutic arsenal against oral infections such as thrush and dental caries.

CONCLUSION: *Cocos nucifera* and *Anacardium occidentale* are the plants frequently used by Africans in general, and Beninese in particular, to treat oral infections. These plants are rich in chemical groups that justify their antimicrobial potential. Shrimp larvae are tolerant to root shavings from the two plants studied, and consequently these species do not present cellular toxicity, given the absence of toxic metabolites, also revealed by phytochemistry.

These extracts have been shown to have antioxidant, antifungal and antibacterial properties, making them useful free-radical scavengers in the treatment of many diseases, including oral infections (dental caries, bad breath and thrush). Our research brings added value to the valorization of plant resources in traditional Beninese medicine, against the spread of oral infections. It would be essential to extend this work by formulating and studying the quality control of an organic toothpaste using these two antioxidant and anticariogenic extracts.

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