in vitro phytochemical and biological studies of the hydroethanolic extract of Anchomanes difformis used in phytotherapy in Togo

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Abstract

Background

The proliferation of strains that are multi-resistant to conventional antibiotics has led to a need for research into other active molecules. The aim of this study was to evaluate the antimicrobial activity of *Anchomanes difformis*, a medicinal plant from the West African sub-region used in traditional pharmacopoeia.

Methods

Total hydroethanolic extracts of the different organs (leaves, bulb and rhizomes) were obtained by maceration. Qualitative phytochemistry of the extracts was carried out using staining methods to identify the main chemical groups. Total flavonoids were quantified by spectrophotometric assay. Two methods were used to assess antioxidant activity: the phosphomobyldate reduction method and the FRAP test. *in vitro* antimicrobial tests were carried out using the solid-state diffusion method (Puits et disque).

Results

The highest hydroethanol extraction yield was obtained with the *A. difformis* leaf bark extract (9.47%). The results showed the presence of alkaloids, coumarins and reducing sugars. The highest content of total flavonoids was found in the bulbs, with 33.78 µg Eq routine/mg DM. The plant leaves showed high antioxidant activity in vitro with 37.79 EAA/g DM and 270.50 µmol Eq FeSO4/mg DM. On completion of the antimicrobial tests, there was no visible inhibition zone diameter for all the extracts with the two methods used.

Conclusion

The present study invalidates the local use of this plant in pathologies caused by the microbial strains studied. However, research into other activities associated with this plant could explain its use in traditional medicine.

Introduction

Antibiotic resistance occurs when bacteria develop the ability to evade therapeutic molecules [1,2]. Today, the growing resistance of pathogens to antibiotics is a major global health threat [3,4]. In West Africa, the endemicity of respiratory infections, bacterial meningitis, diarrhoea and other infectious diseases has increased the use of antibiotics both for symptomatic treatment and prophylaxis [5]. This

is eroding the effectiveness of conventional treatments and forcing the urgent discovery of new antimicrobial compounds [6].

In this context, medicinal plants are attracting renewed interest: rich in various secondary metabolites, they represent one of the most promising sources of innovative bioactive molecules [4,7,8]. Numerous studies point out that the plant kingdom produces a wide variety of compounds (alcohols, terpenes, alkaloids, flavonoids, etc.), some of which have already served as models for the development of new drugs [4,7].

At the same time, herbal medicine plays a central role in traditional health care in many countries. According to the World Health Organization, about 80% of the population in some developing countries relies on herbal medicines for their primary care [9,10]. This is especially true in sub-Saharan Africa, where the accessibility and low cost of herbal remedies make them an essential resource. This practical importance goes hand in hand with the need to renew the therapeutic reservoir: the discovery of new molecules of plant origin, with antibacterial, antioxidant or anti-inflammatory activities, has become a major challenge in pharmaceutical research [6,7]. In Togo, traditional knowledge about medicinal plants continues to play an important role in primary health care [11].

In this general framework, *Anchomanes difformis* (Blume) Engl. (family Araceae) appears to be a plant of interest. *Anchomanes difformis*, locally called "Adodo" in Togo, is a medicinal plant used in traditional African medicine, particularly in Nigeria, Togo and Côte d'Ivoire [12,13]. It grows mainly in humid tropical areas, savannahs and along waterways at low or medium altitudes [12,13]. This species is a perennial herbaceous plant, with a horizontal tuber measuring between 40–80 cm long and 8–22 cm in diameter, with annular scars [12,13]. Each season, it produces a single large tripartite leaf, up to 1.5 m wide, with sessile leaflets of variable shape [12,13]. The petiole, greenish purple, reaches 2.5 m in length and bears small green needles [12,13]. The stem is green with a white base [12,13]. The inflorescence emerges from the tuber, with a cylindrical spadix bearing female flowers with purple-pink ovary at the base, followed by creamy-white male flowers [12,13]. It is widely used in traditional local medicine: roots, rhizomes and leaves are used against an impressive variety of ailments (abdominal pain, respiratory disorders, joint problems, infections, digestive disorders, neurological disorders, etc.) [12,13]. The roots of *A. difformis* are used in Benin to treat anal wounds, oral wounds, dysentery, diabetes and its complications and dysentery [14]. Other surveys have shown that all parts of the plant treat asthma [15–17], malaria [18], cough and throat-related problems [13]. Some investigations have reported the use of powdered root mixed with palm oil is used as a remedy for respiratory diseases in children in Zaire [19]. The antimicrobial properties of the plant have been reported [16,20]. It is cited as a diuretic and "all-comer" remedy in the Togolese and Nigerian pharmacopoeia [12,13].

However, despite its widespread traditional use, the available scientific data on this species remain limited. As noted in recent work, very few studies have characterized the chemical composition or assessed the biological activities of *A. difformis* [21–26]. The lack of valid data makes a rigorous review of its active ingredients even more relevant.

In this perspective, the present study aims to fill these gaps with a systematic approach: it aims to analyze the phytochemical composition of the hydroethanolic extract of *Anchomanes difformis* and to evaluate its potential *in vitro* biological activities (in antioxidant and antimicrobial). The aim is to provide a scientific basis for the traditional uses of this plant and to search for new therapeutic agents of plant origin. This approach is in line with the current need to discover, within plant biodiversity, molecules capable of effectively fighting antibiotic-resistant pathogens [4,6].

Materials and methods

Collection of plant material

Leaves (Fig. 1), rhizomes and bulbs (Fig. 2) of *Anchomanes difformis* were collected in Dalavé (Tsévié, Maritime Region of Togo) in October 2021. The material plants have been botanically authenticated at the Herbarium of the Department of Botany, Faculty of Sciences, University of Lome.

Bacterial strains

The microorganisms used for the test were clinical and reference strains of *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, all isolated at the National Institute of Hygiene in Lomé (INH-Lomé).

Extraction

The extraction was carried out in accordance with[27] and taken up by [28]. The samples of each plant were carefully washed under running water and dried in the open air at room temperature for two weeks. After drying, the plant materials were reduced to powder using the grinding machine. The ethanolic extraction was carried out by macerating 100 g of powder in 1,000 ml of 70% ethanol (v/v), under continuous stirring for 48 hours at room temperature. The extract was filtered through WHATMAN N°1 filter paper and evaporated at 45°C using a rotary evaporator until dryness under reduced pressure. The extracts were stored at 4°C in the refrigerator until used.

$$\text{Yield}(\%) = \frac{\textit{Mass of extract}}{\textit{Mass of plant material}} * 100$$

Phytochemical analysis

Phytochemical screening was performed based on staining characteristic tests to highlight major chemical groups. It focused on the hydroethanolic extracts of the plants studied. The chemical groups were identified by reference to the methods described by [29] and taken up by [30–32].

Alkaloids

The dissolved extracts were divided into two tubes at a rate of 2 mL each. In a first tube (test tube), 4 mL of sulphuric acid (H_2SO_4) diluted to 10% and a few drops of Dragendorff's reagent were added. The appearance of an orange-red precipitate indicates the presence of alkaloids in the hydroethanolic extract. The second tube served as a control.

Flavonoids

The dissolved extracts were divided into two tubes at a rate of 2 mL each. In a first tube (test tube), 4 mL of methanol and a few drops of magnesium turns were added; then 1 mL of concentrated HCl was added. The appearance of a red coloration indicates the presence of flavonoids in the hydroethanolic extract. The second tube served as a control.

Saponines

The dissolved extracts were divided into two tubes at a rate of 2 mL each. In a first tube (test tube), a few drops of distilled water were added. The appearance and persistence of foam after stirring indicates the presence of saponosides. The second tube served as a control.

Tannins

The dissolved extracts were divided into two tubes at a rate of 4 mL each. In a first tube (test tube), a few drops of $FeCl_3$ were added. The appearance of a blackish brown colour after homogenization of the mixture indicates the presence of tannins in the hydroethanolic extract. The second tube served as a control.

Phenols

The dissolved extracts were divided into two tubes at a rate of 4 mL each. In a first tube (test tube), a few drops of $FeCl_3$ were added. The appearance of a blackish brown colour after homogenization of the mixture indicates the presence of phenolic compounds in the hydroethanolic extract.

Triterpenes and sterols

The dissolved extracts were divided into two tubes at a rate of 4 mL each. In a first tube (test tube), 1.6 mL of chloroform and 2.6 mL of sulphuric acid (H_2SO_4) were added. The appearance of a reddish-brown ring between two phases, one clear at the bottom and the other green at the top, indicates the presence of triterpenes and sterols in the hydroethanolic extract. The second tube served as a control.

Coumarins

The dissolved extracts were divided into two tubes at a rate of 4 mL each. In a first tube (test tube), 2 mL of distilled water was added, and a small amount of ammonia was added to the mixture. The

appearance of fluorescence after ultraviolet illumination indicates the presence of coumarins in the hydroethanolic extract. The second tube served as a control.

Reducing sugars

The dissolved extracts were divided into two tubes at a rate of 2 mL each. In a first tube (test tube), 2 mL of Fehling's Liquor (1 mL of solution A + 1 mL of solution B) were added and brought to a boil in a boiling sea bath for 2 to 3 min. The appearance of a brick-red precipitate indicates the presence of reducing sugars in the hydroethanolic extract. The second tube served as a control.

Flavonoid content

The flavonoid content is estimated according to the method described by [33] and taken up by [30–32]. It consisted of preparing plant extracts at 1mg/mL in distilled water, aluminum chloride ($AlCl_3$) 2% in distilled water, and rutin at different concentrations of 0; 5 ; 25 ; 50 ; 75 ; 100 ; 150 ; 200 µg/mL in methanol. The operation consisted of mixing 1 mL of the extract solution at 1 mg/ml or 1 mL of each rutin concentration with 1 mL of aluminum chloride ($AlCl_3$) 2% using a vortex. After 10 minutes of incubation, the absorbance was directly measured with a UV-visible spectrophotometer (METASH UV-5200PC UV/VIS Spectrophotometer) at 415 nm against a blank. Rutin was used as a standard. The total flavonoid content of the extracts was inferred from the calibration curve established with Rutin (0–200 µg/mL) and the results were expressed in microgram equivalent of rutin per milligram of dry matter (µg ER/mg DM). Three tests were conducted for each extract. The total flavonoid content (**XFlav**) is obtained using the following formula:

$$\mathbf{XFlav} = \left[\left(\frac{1}{0{,}0078}\mathbf{DO} + \mathbf{0.0428}\right)\right]$$

XFlav

Total flavonoid content (µg ER/mg DM)

OD

Optical Density read at 415 nm

Antioxidant activity

Phosphomobyldate reduction test

Determination of antioxidant activity by the phosphomobyldate reduction assay was performed using the method described by [34] and slightly modified in [31,32]. The phosphomolybdate reagent was prepared (100 mL of reagent) from a mixture of 90 mL of 0.6 M sulphuric acid, 5 mL of 0.1% sodium phosphate and 5 mL of 1% ammonium molybdate. For the test, 1 mL of each extract was added to 9 mL

of the above reagent. The whole thing was brought to a temperature of 95°C in a water bath for 90 minutes. Then the mixture was cooled to room temperature. Absorbances were measured at wavelengths of 820 nm against a blank consisting of reagent and distilled water. Ascorbic acid was used as the standard antioxidant under the same operating conditions and the results were expressed in milligrams of ascorbic acid equivalent per gram of dry matter (mg AAE/g DM). Three tests were performed for each concentration of product tested. The antioxidant activity related to the reducing power of the extracts is expressed in Antioxidant Content (X_{Mob}) using the following formula:

$$XMob = \left[\left(\frac{1}{0{,}0057}DO + 0.0599\right)\right]$$

XMob

Antioxidant Content (mg AAE/g DM)

OD

Optical Density read at 820 nm

Test FRAP (Ferric Reducing antioxidant Power)

The determination of antioxidant activity by the FRAP assay was carried out using the method described by [35] and taken up by [30–32,36]. Thus, 3 mL of the freshly prepared FRAP test reagent in a test tube, 100 µL of the various solutions of iron II sulphate with concentrations between 0 and 2000 µmol. L^{-1} will be added. The mixture was vigorously shaken at the vortex and the optical density was read after 5 min with a spectrophotometer at 593 nm. The absorbance of the TPTZ-Fe^{2+} complex allowed to draw a calibration curve from the concentration range (0–2000 µM) of the iron sulfate solution ($FeSO_4$, $7H_2O$) dissolved in methanol. For the samples of the extracts to be tested, the FRAP reagent (3 mL) and the solution of the extract to be tested (100 µL) with a strength of 1 mg/mL were mixed in the same proportions as for the standard curve plotting. The optical density was read after 5 min at 593 nm. The antioxidant capacity of the extracts will be measured using the calibration curve by the color change related to the formation of the complex (Fe^{2+} TPTZ) and expressed in micromole equivalent of iron sulfate per milligram of dry matter (µmol Eq $FeS0_4$/mg DM). The tests will be repeated 3 times. The antioxidant activity related to the reducing power of the extracts is expressed in Reducing Power ($X_{FRAP)}$ using the following formula:

$$XFRAP = \left[\left(\frac{1}{0{,}0002}DO + 0.0331\right)\right]$$

XFRAP

Reducing power (µmol Eq $FeS0_4$/mg DM)

OD

optical density read at 593 nm

Evaluation of the antimicrobial potency of extracts

The identification of the active extracts was carried out by the solid-state diffusion method [37] and the determination of the minimum inhibitory concentration (MIC) by the liquid dilution method [38].

Preparation of microbial strains

After collection, the strains were transplanted to their appropriate media (Chapman for *S. aureus*, Sabouraud + Chloramphenicol for *C. albicans*, Mac Conkey for *E. coli* and Fresh Blood Agar (FSG) for *S. pneumoniae*) and stored. To obtain the 24-hour colonies that were used to prepare the inoculum, the strains were transplanted on agar medium without inhibitors [39].

Preparation of the inoculum

From an 18–24 h culture on agar medium, a suspension was prepared in saline solution (0.9% NaCl) equivalent to the Mac Farland standard 0.5 (~ 10^8 CFU/ml). Inoculation was sown by flooding with the inoculum suspension diluted to 1/100 (~ 10^6 CFU/ml) in accordance with the necessary safety measures [39].

Preparation and sterilization of the stock solution of extracts

The extracts were dissolved in sterile distilled water to an initial concentration of 200 mg/mL (200 mg of each extract in 1 mL of distilled water). To verify their sterility, an aliquot of each stock solution was taken and inoculated on Muller Hinton agar. After incubation at 37°C for 24 hours, those that gave colonies were filtered with a millipore membrane of 0.45 µm in diameter. After sterilization, each stock solution is diluted 1/4 with distilled water to obtain solutions with a concentration of 50 mg/mL of each extract.

In vitro antimicrobial activity

Antimicrobial testing of extracts on germs tested by the well method

Mueller Hinton (MH) agar plates for bacteria and Sabouraud dextrose + Chloramphenicol for yeasts were inoculated by flooding following the recommendations of the Antibiogram Committee of the French Society of Microbiology [39]. After the dishes have dried, (about 5 min) the agar is perforated into a maximum of six wells (90 mm Petri dishes) with a sterile tip previously cut so that the diameter is about 6 mm. The cavities formed are filled with 50 µL of the 50 mg/mL extract solution (50 µL per well). The

boxes are placed at room temperature 25°C for 15 minutes for a pre-diffusion phase. Incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts. Ciprofloxacin (bacteria), Nystatin (yeast) were used as a positive control and distilled water as a negative control. After 24 hours of incubation, the diameters of the inhibition zones were measured using a graduated ruler. The tests are carried out three times and an average are obtained [37]. MICs and BMCs were determined for extracts with an inhibition diameter greater than or equal to 11 mm.

Antimicrobial testing of extracts on germs tested by the disc method

Mueller Hinton (MH) agar plates for bacteria and Sabouraud dextrose + Chloramphenicol for yeasts is dried (about 5 min) and inoculated by flooding according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [39].

Disc preparation

WATTMAN (No. 1) 6 mm diameter, virgin, autoclaved paper was impregnated with 50 µL of the extracts at the initial concentration of 50 mg/mL. The final load of the disc is 2.5 mg. WATTMAN N°1 papers are dried in an oven (37°C).

Sensitivity test

These discs are placed aseptically on the previously inoculated agar. The boxes are placed at room temperature 25°C for 15 minutes for a pre-diffusion phase. Incubated at 37°C for 24 hours for bacteria and 48 hours for yeasts. Ciprofloxacin (bacteria), Nystatin (yeast) were used as a positive control and distilled water as a negative control. After 24 hours of incubation, the inhibition diameters were measured. The tests are carried out three times and an average are obtained on the three determinations [37]. MICs and BMCs were determined for extracts with an inhibition diameter greater than or equal to 11 mm.

Determination of the minimum inhibitory concentration (MIC)
The minimum inhibitory concentration (MIC) was determined by the microplate microdilution method [38]. Mueller Hinton Broth is used to prepare serial dilutions at half concentrations ranging from 50 to 0.097 mg/mL. The inoculum (10 µL) was added to each cup containing 50 µL of extract. Cupules without inoculum were considered as negative controls. All these cups were incubated at 37°C for 24 h. The first cup in the series showing no sign of culture visible to the naked eye was MIC. An aliquot of the contents of each cupule with no visible growth was taken and then seeded by spreading on Mueller Hinton medium [38]. The dishes are incubated at 37°C for 24 to 48 hours at the end of which the number of colonies is determined. After incubation, the smallest concentration of the extract that does not produce colonies is considered the MBC. To determine whether the antimicrobial effect observed is bactericidal or bacteriostatic, the MBC/CMI ratio is used to assess the activity of the extracts (bactericidal/bacteriostatic).

- For a CMB/MIC ratio greater than 1, the antimicrobial effect is bacteriostatic.[40]
- For a CMB/MIC ratio of 1, the antimicrobial effect is bactericidal [40].

Statistical analysis of the data

The data obtained was subjected to statistical analysis using RStidio 4.1.3 software from 10/03/2022 and Microsoft® Excel® spreadsheet for Microsoft 365 MSO 2021. Quantitative variables (flavonoid content, FRAP test, and phosphomobyldate reduction method) are presented as a mean and standard deviation. One-Factor Analysis of Variance (ANOVA) was used to assess the diameters of the zones of inhibition. The significance level has been set at 5%.

Results

For this study, the best yield is obtained by the extract of the leaves of *A. difformis* (9.47%). The weakest is obtained with the extract of the rhizomes of *A. difformis* (4.10%). All extracts have an acidic pH (pH ≤ 7) and range from 6.28 to 5.40. The solubility is quite good, compared to the solvent used for all the extracts. The colour of the extracts is light yellow with a sticky gum appearance for all three extracts (Table 1).

The extracts were analyzed for alkaloids, tannins, flavonoids, saponosides, reducing sugars, triterpenoids, and sterols. The result is recorded in Table 2. Hydroethanolic extracts of leaves, rhizomes and bulbs *of A. difformis* contain only alkaloids, coumarins and reducing sugars (Table 2).

Quantitative tests show a low presence of flavonoids in all extracts at various doses (Table 3). The highest flavonoid content was measured with the hydroethanolic extract of the bulb of *A. difformis* (33.78 µg ER/mg DM) and the lowest with the hydroethanolic extract of the leaves of *A. difformis* (28.85 µg ER/mg DM).

The evaluation of antioxidant activity revealed that all extracts have antioxidant power. The best antioxidant activity was observed with *A. difformis* leaf extract with 37.79 mg AAE/g DM by the phosphomolybdate reduction assay and 270.50 µmol $FeS0_4$/mg DM by the FRAP assay (Table 4).

Regarding antimicrobial activity, at the initial concentration of 50 mg/ml, the three hydroethanolic extracts tested in this work did not significantly inhibit the growth of microorganisms ($p = 2.e\text{-}16$, cv = 3.862–5.562%) with the two diffusion methods on all strains used (Tables 5 & 6). Therefore, the MIC was not determined (Inhibition Diameter ≤11 mm).

Table 1
Some characteristics of the extracts obtained

Plants	Organs	Aspect	Colour	Solubility	pH	Yield
A. difformis	Leaves	Sg	Ly	Pg	5,40	9,47%
	Bulbs	Sg	Ly	Pg	6,28	4,14%
	Rhizomes	Sg	Ly	Pg	5,65	4,10%
Sg: Sticky Gum; Ly: Light yellow; pH: Hydrogen potential; Pg: Pretty good						

Table 2
Phytochemical study of the studied extracts

Secondary metabolites	Extraits HE of *A. difformis*		
	Leaves	Bulbs	Rhizomes
Alkaloids	+	+	+
Flavonoids	-	-	-
Tannins	-	-	-
Coumarins	+	+	+
Saponosides	-	-	-
Triterpenes	-	-	-
Phenol	-	-	-
Sugar Reducers	+	+	+
Legend: -: Absence; +: Presence; HE: Hydroethanolic			

Table 3
Flavonoid content of the extracts studied

HE Extracts	*A. difformis*		
	Leaves	Bulbs	Rhizomes
Total flavonoid content (µg ER/mg DM)	24,85 ± 0,26	33,78 ± 0,66	29,82 ± 0,88
ER: Equivalent to Rutine; HE: Hydroethanolic; DM: Dry matter			

Table 4
Antioxidant activities *in vitro* Excerpts studied

Hydroethanolic extracts	**Phosphomolybdate Reduction Test (mg AAE/g DM)**	**FRAP Testing (µmol $FeS0_4$/mg DM)**
Feuilles de *A. difformis*	37,79 ± 0,18	270,50 ± 5,00
Bulbs of *A. difformis*	19,59 ± 0,00	202,17 ± 2,89
Rhizomes of *A. difformis*	23,09 ± 0,20	190,50 ± 0,00
AAE: Acide Ascorbique Equivalent ; FRAP: Ferric Reducing antioxidant Power ; DM: Dry matter		

Antimicrobial activities

Table 5
Antimicrobial testing result of extracts on germs tested by The well method

Microbial strains	Extraits HE of *A. difformis*			Water-Ethan	Cyprus/Nyst.	P-value	CV(%)
	Leaves (50 mg/mL)	Bulbs (50 mg/mL)	Rhizomes (50 mg/mL)	NC	PC (50 µg/mL)		
S. aureus ATCC 29213	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6.00 ± 0.00 ^{b}	37.67 ± 0.57 ^{a}	2 $^{e-16}$ ***	3,856
S. aureus 0689	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6.00 ± 0.00 ^{b}	25.67 ± 0.57 ^{a}	2 $^{e-16}$ ***	3,833
E. coli ATCC 25922	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00 ^{b}	44.00 ± 1.00 ^{a}	2 $^{e-16}$ ***	4,225
E. coli 1628	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6.00 ± 0.00 ^{b}	17.00 ± 1.00 ^{a}	2 $^{e-16}$ ***	4,037
S. pneumonia ATCC 49619	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00 ^{b}	42.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	3,922
S. pneumonia 034	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00 ^{b}	33.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	3,362
C. albicans ATCC 10231	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00 ^{b}	32.00 ± 0.57 ^{a}	2 $^{e-16}$ ***	4,495
C. albicans 1134	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	6,00 ± 0,00^{b}	29.33 ± 0.57^{a}	2 $^{e-16}$ ***	5.562

The values are expressed as the Mean ± Standard Error. Values in the same column followed by the same lowercase letter are statistically identical (Duncan, p ≤ 0.05)

HE: Hydroethanolic; NC: Negative control; PC: positive control; CV: Coefficient of Variance

Table 6
Result of antimicrobial testing of extracts on germs tested by the disc method

Microbial strains	Extraits HE of *A. difformis*			Water-Ethan NC	Cyprus/Nyst. PC (50 µg/mL)	P-value	CV(%)
	Leaves (50 mg/mL)	Bulbs (50 mg/mL)	Rhizomes (50 mg/mL)				
S. aureus ATCC 29213	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	38.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	2,91
S. aureus 0689	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	25.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	5,27
E. coli ATCC 25922	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	44.00 ± 1.00 ^{a}	2 $^{e-16}$ ***	5,95
E. coli 1628	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	18.00 ± 1.00 ^{a}	1.8 $^{e-16}$ ***	6,92
S. pneumonia ATCC 49619	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	42.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	5,05
S. pneumonia 034	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	32.67 ± 0.57 ^{a}	2 $^{e-16}$ ***	5,80
C. albicans ATCC 10231	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00^{b}	32.33 ± 0.57 ^{a}	2 $^{e-16}$ ***	11,81
C. albicans 1134	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00 ^{b}	6,00 ± 0,00^{b}	31.00 ± 1.00 ^{a}	2 $^{e-16}$ ***	6.06

The values are expressed as the Mean ± Standard Error. Values in the same column followed by the same lowercase letter are statistically identical (Duncan, p ≤ 0.05)

HE: Hydroethanolic; NC: Negative control; PC: positive control; CV: Coefficient of Variance

Discussion

The study evaluated the antimicrobial activities of *A. difformis* on clinical strains of *E. coli*, *S. aureus*, *S. pneumoniae*, and *C. albicans*. The choice of this plant as study material is guided by their common use in traditional medicine in Africa to cure various diseases.[41]

- Yield

Our results show that for this study, the best yield is obtained by the extract of *A. difformis leaves* (9.47%). The lowest is obtained with the extract of the rhizomes of *A. difformis* (4.10%). These low yields could be explained by the short time (48 hours) and especially the mode and speed of rotation to homogenize the solvent and plant powder mixture[42]. Hydroethanolic extraction is valued because of its ability to extract a wide range of compounds, its relative safety of use, and its low cost compared to other extraction methods.[43]

- Qualitative and quantitative phytochemical study

The qualitative phytochemical study of the three extracts of *A. difformis* revealed the presence of alkaloids, coumarins and reducing sugars. These results do not corroborate those described by [15,44] who, in addition to the alkaloids obtained, also found tannins, flavonoids, saponosides and phenols but not reducing sugars. This could be explained by the extraction method used.

Our results showed a low presence of flavonoids in all extracts at various doses (Table 3). This result confirms the absence of flavonoids in A. *difformis* extracts in qualitative tests (Table 2). The lowest is that of the hydroethanolic extract of the leaves of *A. difformis* (28.85 µg ER/mg DM). Our results corroborate with those of [17,21], who also found that the flavonoid content of *A. difformis* leaves is lower with 4 mg ER/g DM using the same method.

- Antioxidant activity

Regarding antioxidant activity, the FRAP test and the phosphomobyldate reduction test gave a better reducing power of *A. difformis* leaves with 270.5 µmol $FeS0_4$/mg DM and 37.79 mg AAE/g DM respectively. The low presence of flavonoids and other secondary metabolites could explain this low antioxidant activity of the various extracts of *A. difformis* [45].

- Antimicrobial activity

Presumptive testing by the agar diffusion method (Well and Disc) revealed that the three extracts did not significantly inhibit the growth of microorganisms ($p = 2.e^{-16}$, cv = 3.862–5.562%) on all bacterial strains used for the study. Our results are in contradiction with the work of[12] which has shown that the essential oil extract of *A. difformis* has antimicrobial activities on *S. aureus* and *C. albicans*. This could be explained by the difference in the nature of the extracts used in the two studies. The low presence of phytochemicals is thought to be the basis for the antimicrobial inactivities observed with *A. difformis* extracts [31].

Conclusion

The objective of our study was to evaluate the biological and phytochemical activities of *A. difformis* on strains of *E. coli*, *S. aureus*, *S. pneumoniae* and *C. albicans*. Qualitative phytochemicals have shown that the extracts (leaves, rhizome and bulb) of *A. difformis* all contain coumarins, alkaloids and reducing

sugars. Quantitative phytochemistry gave low total flavonoid content for all A. *difformis extracts in vitro*. Our results showed that the three hydroethanolic extracts of *A. difformis* did not significantly inhibit the growth of the microorganisms tested in this study.

Ultimately, we can conclude that the hydroethanolic extracts of the leaves, rhizomes and bulbs of *Anchomanes difformis* would be inactive on all the germs tested (*E. coli*, *S. aureus*, *S. pneumoniae* and *C. albicans*).

This study sheds light on the chemical components and biological activities of the hydroethanolic extract of *Anchomanes difformis* used in herbal medicine in Togo. Our results suggest that this plant could be a valuable source of bioactive compounds with potential applications in the treatment of various conditions other than the strain-related conditions tested in our study.

Abbreviations

ATCC: American Typographic Culture Collection ; MIC: Minimum Inhibitory Concentration ; MBC: Minimum Bactericidal Concentration ; CS NDE: Sisters of Our Lady of the Church Health Center ; INH: National Institute of Hygiene of Lomé ; MH: Mueller-Hinton ; WHO: World Health Organization

Declarations

Ethics Approval and Consent to Participate

Not Applicable

Consent to Publication

Not Applicable

Clinical trial number

Not applicable

Availability of Data and Materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

Tchilabalo Bouyo, Sandrine Tènè Salifou, Jules Koffi Kpatagnon, Passimna Pissang and Komi Koukoura Komi harvested and identified the plant parts used. Tchilabalo Bouyo, Sandrine Tènè Salifou, Kodjovi Sossou, Samadou Tchakondo, Passimna Pissang, Yao Hoekou, Holaly Efui Gbékley and Komi Koukoura Komi participated in the extraction and flavonoid assay. Antioxidant activity tests were carried out by Tchilabalo Bouyo, Bawimodom Bidjada, Sandrine Tènè Salifou, Passimna Pissang, Jules Koffi Kpatagnon, Samadou Tchakondo, Abdoul Kader Ouedraogo, Isidore Kodjovi Anani Gbenonssi, Kodjovi Sossou, Komlan Tchalla, Yao Hoekou and Holaly Efui Gbékley. The results were analyzed statistically by Tchilabalo Bouyo, Holaly Efui Gbékley, Blaise Etienne M'BOUMBA and Passimna Pissang. Finally, Tchilabalo Bouyo, Richard Kouyassa Dessougmba, Passimna Pissang, Komi Koukoura Komi wrote the maniscrit and Komi Koukoura Komi and Tchadjobo Tchacondo approved and supervised the work.

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Figures

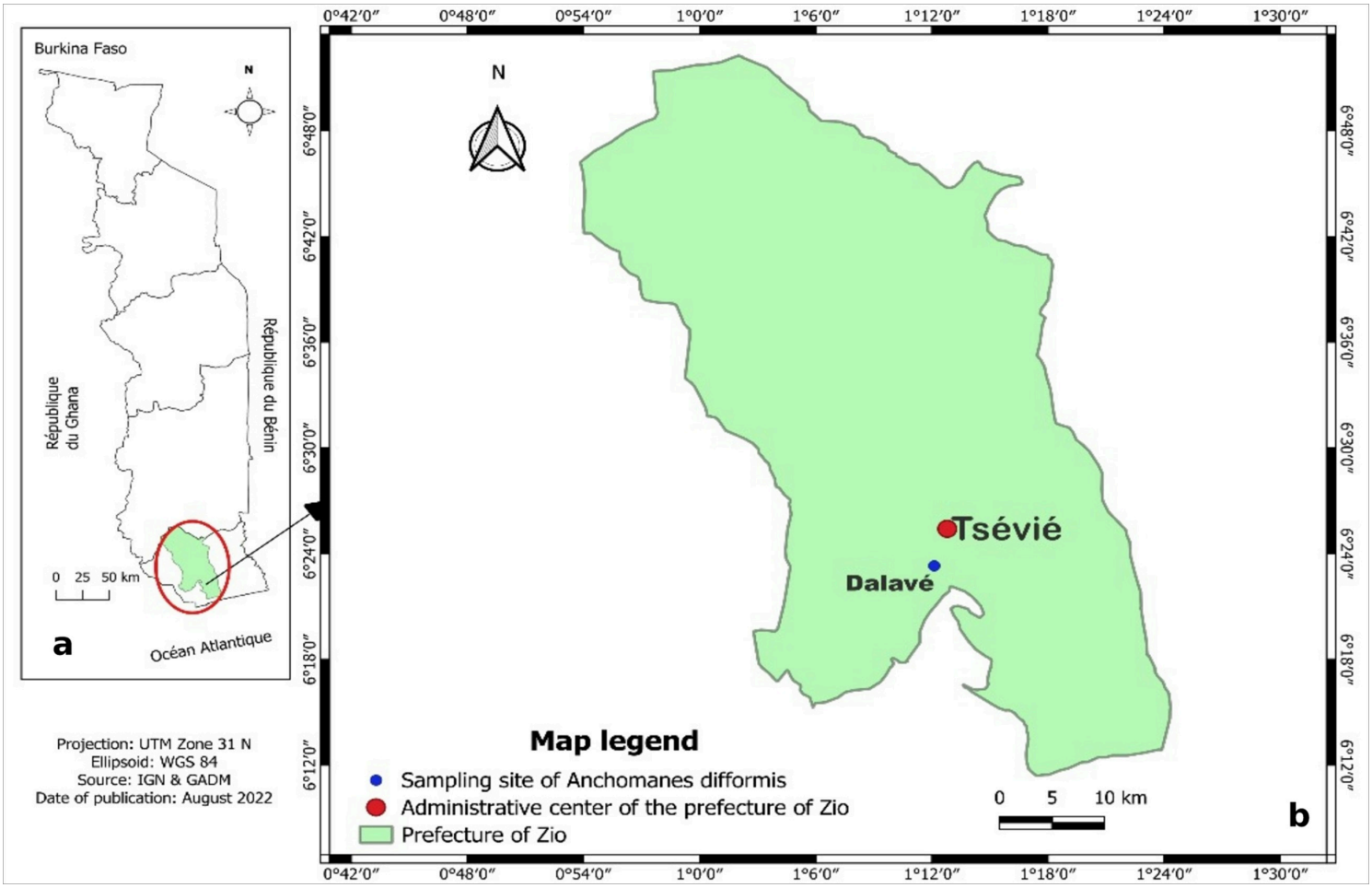

Figure 1

Map of Togo (a) showing the collection area in sky green, and a detailed map of this area (b) highlighting the study areas (Dalavé) in green. The map was generated using QGIS (https://download.qgis.org/downloads/qgis-3.22.9.tar.bz2). The shapefiles of Togo's administrative boundaries were obtained from the IGN database of the National Institute of Geographic and Forest Information (https://www.ign.fr/telechargez-application-cartographique-cartes-ign) and the GADM database of global administrative zones (https://gadm.org, version 4.1, downloaded 2022-07-22), a

publicly available resource providing high-resolution data on administrative boundaries for all countries around the world

Figure 2

Leaves of Anchomanes difformis (Source: Nikon Coolpix P300, 2022)

Figure 3

Bulbs and rhizomes of Anchomanes difformis (Source: Nikon Coolpix P300, 2022)

Figure 4

P extraction process on the métal plate (Source: Nikon Coolpix P300, 2022)

Figure 5

WHATMAN N°1 paper filtration process

(Source: Nikon Coolpix P300, 2022)

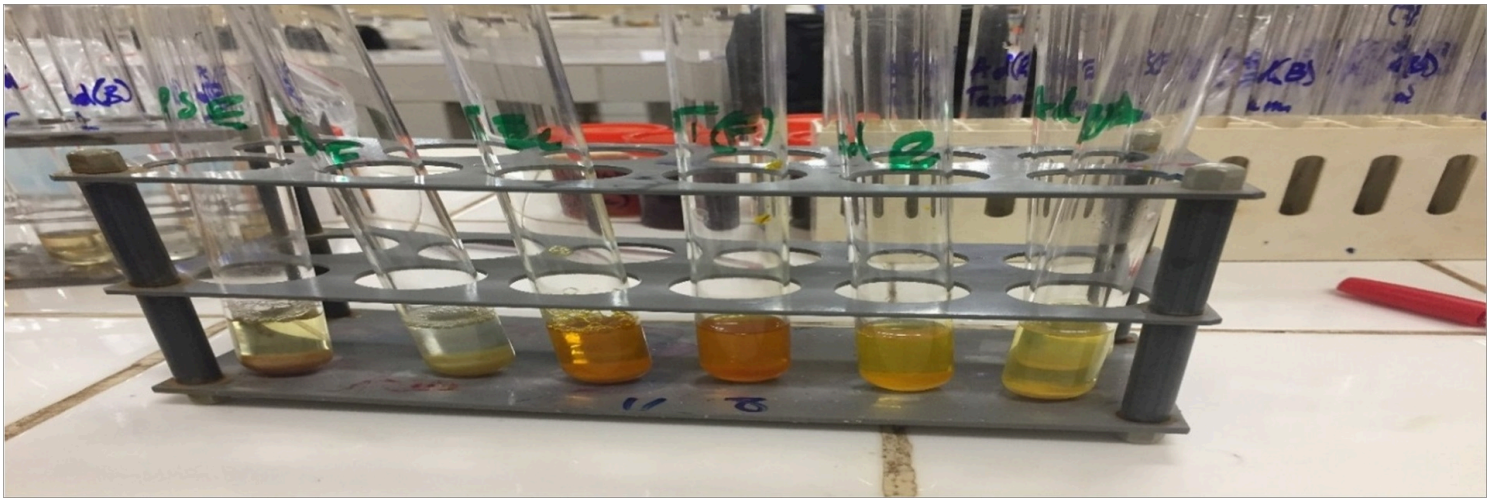

Figure 6

Image showing some qualitative test results of the phytochemical analysis

(Source: Nikon Coolpix P300, 2022)

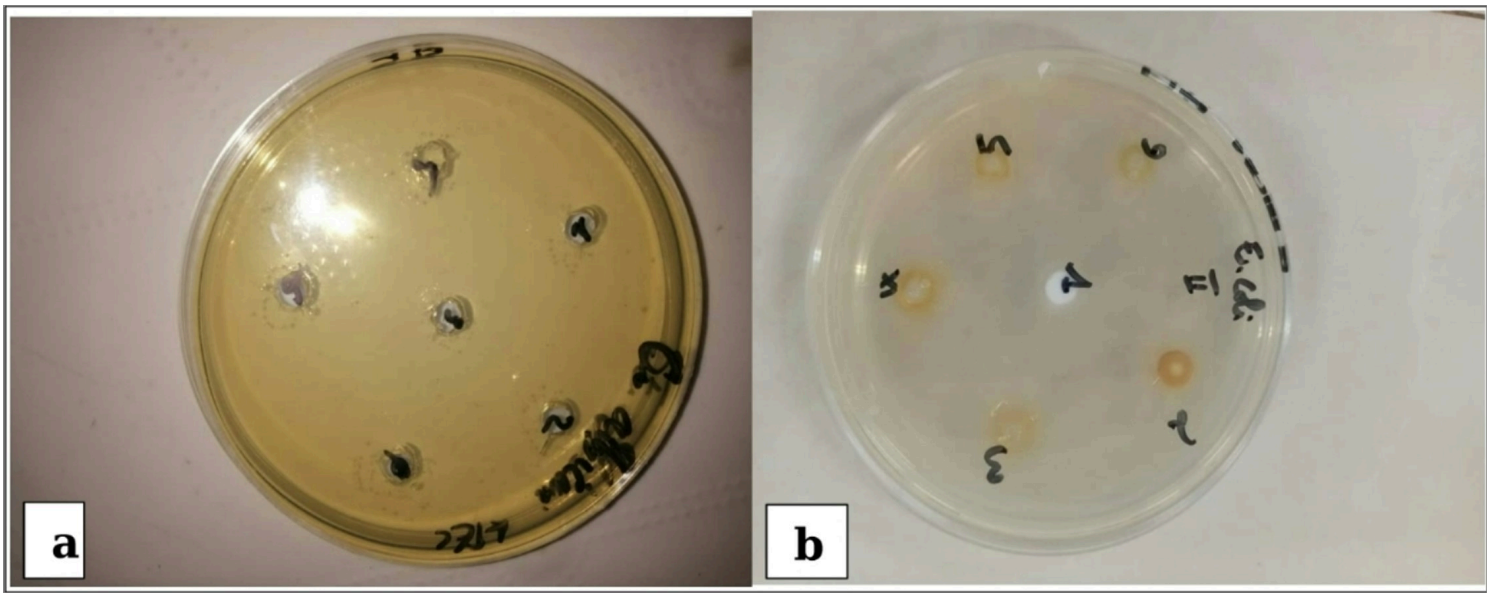

Figure 7

Well Method Presumptive Antimicrobial Testing Process

(Source: Nikon Coolpix P300, 2022).

a: Digging wells on the sterile HD medium

b: the introduction of plant extracts into the wells on the MH medium already sown by flooding

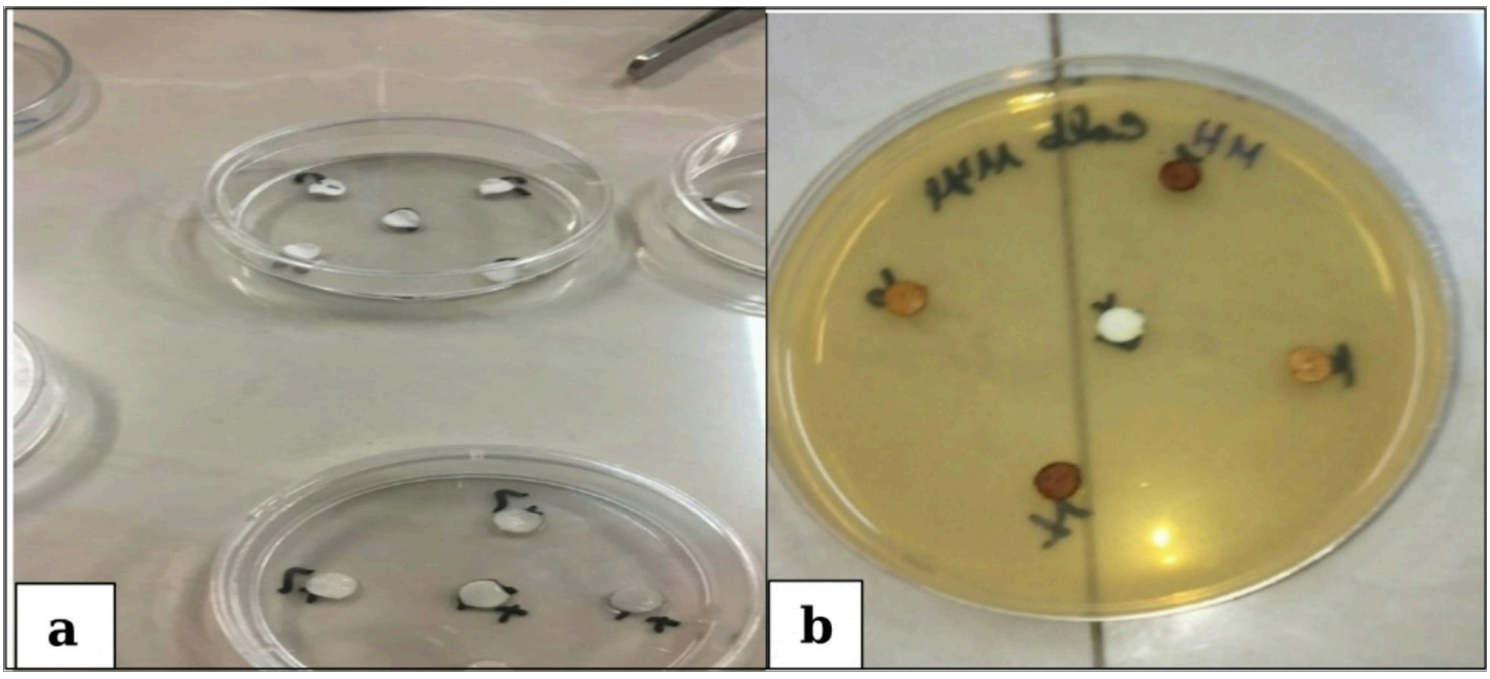

Figure 8

Disc Method Presumptive Antimicrobial Testing Process

(Source: Nikon Coolpix P300, 2022)

a: Design of the discs using WHATMAN N°1 paper

b: Deposits of the extract-impregnated discs on the MH medium already inoculated by flooding with bacteria