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In vitro phytochemical and biological evaluation of hydroethanolic extract of *Anchomanes difformis* In Togo

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The increasing proliferation of strains that are multidrug-resistant to conventional antibiotics has created a pressing need to explore alternative bioactive compounds. This study aimed to evaluate the antimicrobial activity of different organs of *Anchomanes difformis*, a medicinal plant widely used in the traditional pharmacopoeia of West Africa, against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Candida albicans*. Total hydroethanolic extracts from the leaves, bulbs, and rhizomes were obtained by maceration. The phytochemical composition of the extracts was assessed qualitatively using standard staining techniques to identify major secondary metabolite groups. Total flavonoid content was quantified spectrophotometrically. Antioxidant activity was evaluated using two complementary methods: the phosphomolybdate reduction assay and the FRAP (Ferric Reducing Antioxidant Power) test. In vitro antimicrobial activity was assessed by solid diffusion methods (well and disk diffusion assays). The highest hydroethanolic extraction yield was recorded for the leaf bark extract of *A. difformis* (9.47%). Phytochemical screening revealed the presence of alkaloids, coumarins, and reducing sugars. The bulb extract exhibited the highest total flavonoid content (33.78 µg R Eq /mg DE). The leaf extract demonstrated strong in vitro antioxidant potential, with 37.79 AA Eq /g DE and 270.50 µmol $FeSO_4$ Eq /mg DE. However, none of the extracts produced visible inhibition zones in the antimicrobial assays performed by either diffusion method. The findings indicate that hydroethanolic extracts from the organs of *Anchomanes difformis* did not exhibit antibacterial or antifungal activity against the tested microorganisms. Nonetheless, further investigations into other biological activities of this plant could help elucidate the basis of its traditional medicinal use.

Keywords *Anchomanes difformis*, Phytochemistry, Activity, Antioxidant, Antimicrobial

Abbreviations

ATCC American Typographic Culture Collection

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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

Antibiotic resistance occurs when bacteria develop the ability to withstand the action of therapeutic agents[1,2]. Today, the increasing resistance of pathogenic microorganisms to antibiotics represents a major global public health threat[3,4]. In West Africa, the endemic nature of respiratory infections, bacterial meningitis, diarrheal diseases, and other infectious conditions has led to widespread antibiotic use, both for symptomatic treatment and prophylaxis[5]. This trend is gradually undermining the effectiveness of conventional therapies and underscores the urgent need to discover new antimicrobial compounds[6].

In this context, medicinal plants, rich in diverse secondary metabolites, have gained renewed scientific interest[4,7,8]. Numerous studies have shown that plants produce a wide range of compounds such as alcohols, terpenes, alkaloids, and flavonoids some of which have already served as structural models for the development of modern drugs[4,7].

Furthermore, herbal medicine continues to play a central role in traditional health care systems across many regions. According to the World Health Organization, approximately 80% of the population in some developing countries relies primarily on herbal remedies for their health care needs[9,10]. This is particularly true in sub-Saharan Africa, where the accessibility and affordability of plant-based medicines make them indispensable therapeutic resources. In this setting, revitalizing the natural therapeutic reservoir through the discovery of new plant-derived compounds with antibacterial, antioxidant, or anti-inflammatory properties has become a key objective of pharmaceutical research[6,7]. In Togo, traditional knowledge of medicinal plants continues to play a significant role in primary health care[11].

Within this general framework, *Anchomanes difformis* (Blume) Engl. (family Araceae) emerges as a plant of considerable interest. Locally known as "Adodo" in Togo, *A. difformis* is a medicinal species widely used in traditional African medicine, particularly in Nigeria, Togo, and Côte d'Ivoire[12,13]. It typically grows in humid tropical regions, savannas, and along watercourses at low to medium altitudes[12,13]. This perennial herbaceous plant is characterized by a horizontal tuber measuring 40–80 cm in length and 8–22 cm in diameter, marked by distinct annular scars[12,13]. Each growing season, the plant produces a single large tripartite leaf, up to 1.5 m wide, with sessile leaflets of variable shapes[12,13]. The greenish-purple petiole reaches 2.5 m in length and bears small green needles[12,13]. The stem is green with a whitish base[12,13]. The inflorescence arises directly from the tuber and consists of a cylindrical spadix bearing female flowers with purple-pink ovaries at the base, followed by creamy-white male flowers[12,13].

A. difformis holds a prominent place in local traditional medicine: its roots, rhizomes, and leaves are used to treat a wide range of ailments, including abdominal pain, respiratory disorders, joint pain, infections, digestive disturbances, and neurological conditions[12,13]. In Benin, the roots are used to treat anal and oral wounds, dysentery, diabetes and its complications[14]. Ethnobotanical surveys have further revealed that all parts of the plant are employed against asthma[15–17], malaria[18], cough, and throat-related ailments[13]. In Zaire, powdered roots mixed with palm oil are traditionally administered to children for respiratory diseases[19]. The antimicrobial properties of *A. difformis* have been scientifically documented[16,20]. Moreover, the species is recognized as a diuretic and a general-purpose ("all-comer") remedy in both the Togolese and Nigerian pharmacopoeia[12,13].

However, despite its widespread use in traditional medicine, the available scientific data on this species remain scarce. Recent studies have highlighted that only a few investigations have characterized the chemical composition or evaluated the biological activities of *A. difformis*[21–26]. The scarcity of reliable data makes a rigorous investigation of its active constituents even more relevant.

In this context, the present study seeks to address these gaps through a systematic approach. Specifically, it aims to analyze the phytochemical composition of the hydroethanolic extract of *Anchomanes difformis* and to assess its potential in vitro biological activities, including antioxidant and antimicrobial properties. The goal is to provide a scientific basis for the traditional uses of this plant and to identify new plant-derived therapeutic agents. This approach aligns with the current global effort to discover bioactive molecules within plant biodiversity that can effectively combat 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, Togo (Fig.3)) in October 2021. The plant materials were botanically authenticated at the Herbarium of the Department of Botany, Faculty of Science, University of Lomé.

Bacterial strains

The microorganisms used in this study included both 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 procedure was performed according to the method described by[27] and later adopted by[28]. Each plant part was thoroughly washed with running water and air-dried at room temperature for two weeks. After drying, the plant materials were ground into a fine powder using an electric grinder. Hydroethanolic extraction

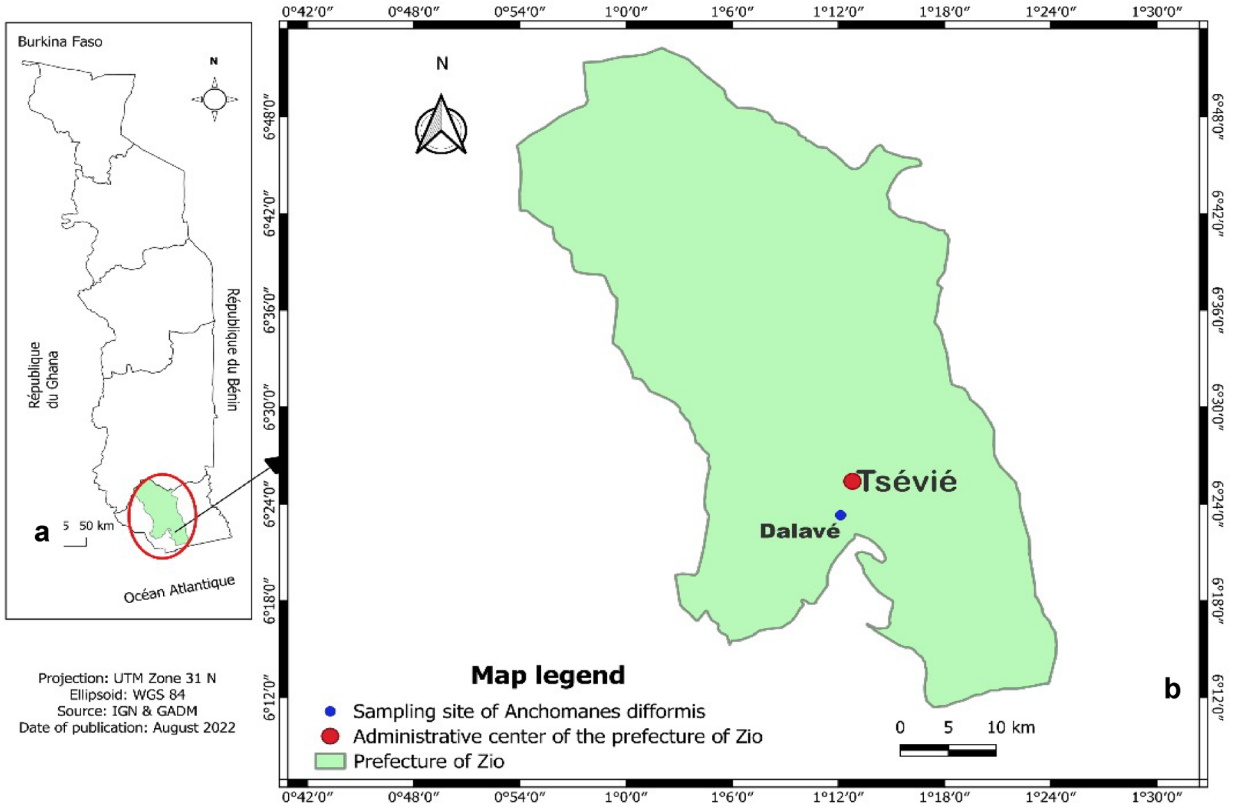

Fig. 3. Map of Togo (**a**) showing the collection area in sky green, and a detailed map of this area (**b**) highlighting the study areas (Dalavé) in green. The map was generated using QGIS (https://download.qgis.org/downloads/qgis-3.22.9.tar.bz2). The shapefiles of Togo's administrative boundaries were obtained from the IGN database of the National Institute of Geographic and Forest Information (https://www.ign.fr/telecharger-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.

Fig. 1. Leaves of *Anchomanes difformis*. (*Source* Nikon Coolpix P300, 2022)

was performed by maceration of 100 g of powdered material in 1000 mL of 70% ethanol (v/v) under continuous stirring for 48 h at room temperature. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated to dryness under reduced pressure at 45 °C using a rotary evaporator. The dried extracts were stored at 4 °C until further use.

Fig. 2. Bulbs and rhizomes of *Anchomanes difformis*. (*Source* Nikon Coolpix P300, 2022)

$$\textbf{Yield}\,(\%) = \frac{Mass\ of\ extract}{Mass\ of\ plant\ material} \times 100$$

Phytochemical analysis

Phytochemical screening was carried out using qualitative staining tests to identify the major classes of chemical compounds. The analysis focused on the hydroethanolic extracts of the studied plants. The identification of the chemical groups was performed according to the methods described by[29] and subsequently adopted by[30–32].

Alkaloids

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

Flavonoids

The dissolved extracts were divided into two test tubes, each containing 2 mL of solution. In the first tube, 4 mL of methanol and a few magnesium turnings were added, followed by 1 mL of concentrated hydrochloric acid (HCl). The appearance of a red coloration indicated the presence of flavonoids in the hydroethanolic extract. The second tube served as a control.

Saponins

The dissolved extracts were divided into two test tubes, each containing 2 mL of solution. In the first tube, a few drops of distilled water were added. The formation and persistence of foam after shaking indicated the presence of saponins. The second tube served as a control.

Tannins

The dissolved extracts were divided into two test tubes, each containing 4 mL of solution. In the first tube, a few drops of ferric chloride ($FeCl_3$) solution were added. The development of a dark brown or blackish coloration after mixing indicated the presence of tannins in the hydroethanolic extract. The second tube served as a control.

Phenols

The dissolved extracts were divided into two test tubes, each containing 4 mL of solution. In the first tube, a few drops of ferric chloride ($FeCl_3$) solution were added. The appearance of a dark brown coloration after homogenization indicated the presence of phenolic compounds in the hydroethanolic extract. The second tube served as a control.

Triterpenes and sterols

The dissolved extracts were divided into two test tubes, each containing 4 mL of solution. In the first tube, 1.6 mL of chloroform and 2.6 mL of sulfuric acid (H_2SO_4) were added. The formation of a reddish-brown ring at the interface between a clear lower phase and a greenish upper phase indicated 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 test tubes, each containing 4 mL of solution. In the first tube, 2 mL of distilled water and a few drops of ammonia were added. The appearance of fluorescence under ultraviolet light indicated the presence of coumarins in the hydroethanolic extract. The second tube served as a control.

Reducing sugars

The dissolved extracts were divided into two test tubes, each containing 2 mL of solution. In the first tube, 2 mL of Fehling's reagent (1 mL of solution A + 1 mL of solution B) were added, and the mixture was heated in a boiling water bath for 2–3 min. The formation of a brick-red precipitate indicated the presence of reducing sugars in the hydroethanolic extract. The second tube served as a control.

Flavonoid content

Flavonoids are major secondary metabolites that play a key role in the antioxidant and antimicrobial properties of plants[33]. Their quantification is essential for the continuation of our study. The flavonoid content was determined according to the method described by[34] and later modified by[30–32]. Briefly, plant extracts were prepared at a concentration of 1 mg/mL in distilled water. Aluminum chloride ($AlCl_3$) 2% was also prepared in distilled water, and rutin solutions were prepared in methanol at concentrations of 0, 5, 25, 50, 75, 100, 150, and 200 µg/mL.

The procedure consisted of mixing 1 mL of the plant extract (1 mg/mL) or 1 mL of each rutin standard solution with 1 mL of 2% aluminum chloride solution using a vortex mixer. After 10 min of incubation, absorbance was measured at 415 nm against a blank using a UV–visible spectrophotometer (METASH UV-5200PC UV/VIS Spectrophotometer). Rutin was used as the standard.

The total flavonoid content of the extracts was calculated from the calibration curve obtained with rutin (0–200 µg/mL), and results were expressed as micrograms of rutin equivalent per milligram of dry extract (µg R Eq /mg DE). Each extract was analyzed in triplicate. The total flavonoid content (XFlav) was calculated using the following formula:

$$\mathbf{XFlav} = \left[\left(\frac{1}{0.0078}\mathbf{DO} + 0.0428\right)\right]$$

XFlav: Total flavonoid content (µg R Eq /mg DM), **OD**: Optical Density read at 415 nm.

Antioxidant activity

Phosphomolybdate reduction test

The antioxidant activity was determined using the phosphomolybdate reduction assay as described by[35], with slight modifications according to[31,32]. The phosphomolybdate reagent (100 mL) was prepared by mixing 90 mL of 0.6 M sulfuric acid, 5 mL of 0.1% sodium phosphate, and 5 mL of 1% ammonium molybdate. For the assay, 1 mL of each extract was combined with 9 mL of the prepared reagent. The mixture was then incubated in a water bath at 95 °C for 90 min, followed by cooling to room temperature.

Absorbance was measured at 820 nm against a blank consisting of the reagent and distilled water. Ascorbic acid served as the reference antioxidant under the same experimental conditions, and results were expressed as milligrams of ascorbic acid equivalent per gram of dry extract (mg AA Eq /g DE).

Each concentration of the tested extract was analyzed in triplicate. The antioxidant activity, corresponding to the reducing power of the extracts, was expressed as the Antioxidant Content (XMob) according to the following formula:

$$\mathbf{XMob} = \left[\left(\frac{1}{0.0057}\mathbf{DO} + 0.0599\right)\right]$$

XMob: Antioxidant Content (mg AA Eq /g DE). **OD**: Optical Density read at 820 nm.

Test FRAP (Ferric reducing antioxidant Power)

The antioxidant activity was determined using the FRAP (Ferric Reducing Antioxidant Power) assay according to the method described by[36] and subsequently adopted by[30–32,37]. Thus, 3 mL of freshly prepared FRAP reagent were placed in a test tube, and 100 µL of various ferrous sulfate ($FeSO_4$) solutions with concentrations ranging from 0 to 2000 µmol L^{-1} were added. The mixture was vortexed vigorously, and the optical density was measured after 5 min using a spectrophotometer at 593 nm. The absorbance of the Fe^{2+}–TPTZ complex was used to construct a calibration curve within the concentration range of 0–2000 µM, using $FeSO_4 \cdot 7\ H_2O$ dissolved in methanol.

For the extract samples, 3 mL of FRAP reagent and 100 µL of the extract solution (1 mg/mL) were mixed in the same proportions as for the standard curve. The optical density was measured after 5 min at 593 nm.

The antioxidant capacity of the extracts was determined from the calibration curve, based on the color change corresponding to the formation of the Fe^{2+}–TPTZ complex, and expressed as micromoles of ferrous sulfate equivalent per milligram of dry extract (µmol $FeSO_4$ Eq /mg DE).

All tests were performed in triplicate. The antioxidant activity, reflecting the reducing power of the extracts, was expressed as the Reducing Power (XFRAP) according to the following formula:

$$\mathbf{XFRAP} = \left[\left(\frac{1}{0.0002}\mathbf{DO} + 0.0331\right)\right]$$

XFRAP: Reducing power (µmol $FeS0_4$ Eq /mg DE). **OD**: Optical Density read at 593 nm.

Evaluation of the antimicrobial potency of extracts

The identification of active extracts was performed using the solid-state diffusion method[38], followed by the determination of the minimum inhibitory concentration (MIC) through the liquid dilution method[39].

Preparation of microbial strains

After collection, the microbial strains were subcultured onto their respective selective media Chapman agar for *Staphylococcus aureus*, Sabouraud agar supplemented with chloramphenicol for *Candida albicans*, MacConkey agar for *Escherichia coli*, and Fresh Blood Agar (FSG) for *Streptococcus pneumoniae* and subsequently preserved.

To obtain 24-hour-old colonies used for inoculum preparation, the strains were streaked onto non-inhibitory agar media[40].

Preparation of the inoculum

From an 18–24-hour culture grown hyon agar medium, a bacterial suspension was prepared in saline solution (0.9% NaCl) to match the 0.5 McFarland standard (approximately 10^8 CFU/mL). Inoculation was carried out by flooding the medium with the inoculum suspension diluted 1/100 (approximately 10^6 CFU/mL), following the required safety procedures[40].

Preparation and sterilization of extract stock solutions

Each extract was dissolved in sterile distilled water to obtain an initial concentration of 200 mg/mL (200 mg of extract per 1 mL of distilled water). To verify sterility, an aliquot of each stock solution was inoculated onto Mueller–Hinton agar. After incubation at 37 °C for 24 h, any solution that produced bacterial growth was filtered through a 0.45 µm Millipore membrane. Following sterilization, each stock solution was diluted 1/4 with distilled water to obtain a final concentration of 50 mg/mL for each extract.

In vitro antimicrobial activity

Antimicrobial testing of extracts on germs tested by the well method

Mueller Hinton (MH) agar plates were used for bacteria, and Sabouraud dextrose agar supplemented with chloramphenicol was used for yeasts. The plates were inoculated by flooding, following the recommendations of the Antibiogram Committee of the French Society of Microbiology[40].

After drying for approximately 5 min, the agar was perforated with up to six wells (in 90 mm Petri dishes) using a sterile tip previously cut to obtain a diameter of about 6 mm. Each well was filled with 50 µL of the extract solution (50 mg/mL). The plates were then left at room temperature (25 °C) for 15 min to allow pre-diffusion, followed by incubation at 37 °C for 24 h for bacteria and 48 h for yeasts.

Ciprofloxacin (for bacteria) and Nystatin (for yeasts) were used as positive controls, while distilled water served as the negative control. After incubation, inhibition zone diameters were measured using a graduated ruler. All tests were performed in triplicate, and the mean values were recorded[38].

Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were determined for extracts showing inhibition zones greater than or equal to 11 mm (Figs. 4 and 5).

Antimicrobial testing of extracts against microorganisms using the disc diffusion method

Mueller–Hinton (MH) agar plates were used for bacteria, and Sabouraud dextrose agar supplemented with chloramphenicol was used for yeasts. The plates were dried for approximately 5 min and then inoculated by flooding, following the recommendations of the Antibiogram Committee of the French Society for Microbiology[40].

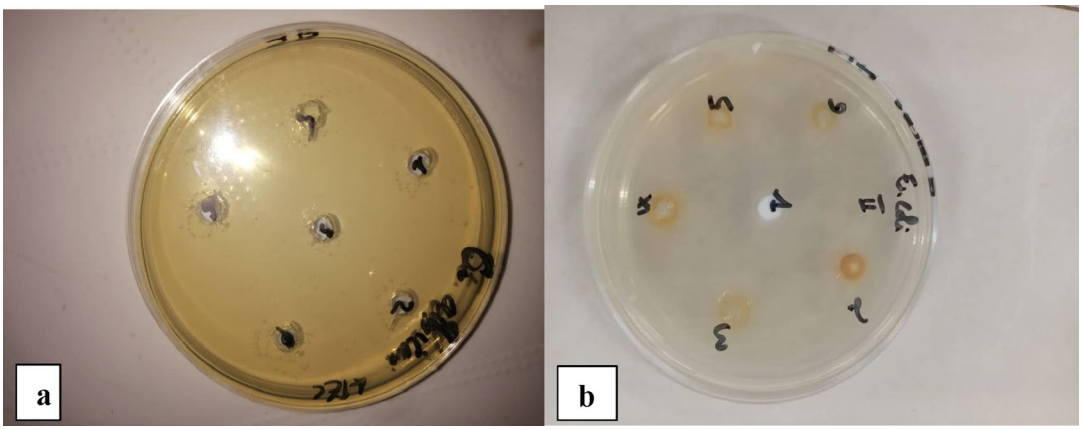

Fig. 4. Well method presumptive antimicrobial testing process. (**a**) Digging wells on the sterile MH medium, (**b**) the introduction of plant extracts into the wells on the MH medium already sown by flooding.(*Source* Nikon Coolpix P300, 2022).

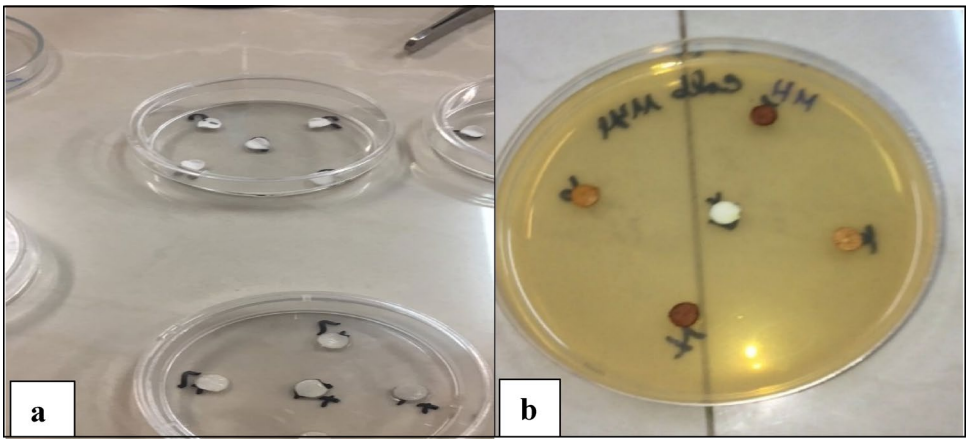

Fig. 5. Disc method presumptive antimicrobial testing process. (**a**) Design of the discs using Whatman No.1 paper. (**b**) Deposits of the extract-impregnated discs on the MH medium already inoculated by flooding with bacteria.(*Source* Nikon Coolpix P300, 2022)

Disc preparation Whatman No.1 filter paper (6 mm diameter, virgin, autoclaved) was impregnated with 50 µL of the extracts at an initial concentration of 50 mg/mL, resulting in a final disc load of 2.5 mg. The Whatman No.1 discs were then dried in an oven at 37 °C.

Sensitivity test The prepared discs were aseptically placed on agar plates previously inoculated with the test microorganisms. The plates were kept at room temperature (25 °C) for 15 min to allow a pre-diffusion phase, then incubated at 37 °C for 24 h for bacteria and 48 h for yeasts. Ciprofloxacin (for bacteria) and Nystatin (for yeasts) were used as positive controls, while distilled water served as the negative control. After 24 h of incubation, the diameters of the inhibition zones were measured. All tests were performed in triplicate, and the mean values were calculated from the three determinations[38]. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for extracts showing inhibition zones ≥ 11 mm.

Determination of the minimum inhibitory concentration (MIC)
The minimum inhibitory concentration (MIC) was determined using the microdilution method in microplates[39]. Serial twofold dilutions of the extract were prepared in Mueller–Hinton Broth, ranging from 50 to 0.097 mg/mL. An inoculum of 10 µL was added to each well containing 50 µL of the extract. Wells without inoculum served as negative controls. The plates were incubated at 37 °C for 24 h.

The MIC was defined as the lowest concentration of the extract that showed no visible growth. From each well showing no visible growth, an aliquot was taken and spread on Mueller–Hinton agar[39]. The plates were then incubated at 37 °C for 24 to 48 h, after which the number of colonies was counted. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the extract that produced no visible colony growth.
To determine whether the antimicrobial effect was bactericidal or bacteriostatic, the MBC/MIC ratio was calculated:

- When the MBC/MIC ratio is greater than 1, the antimicrobial effect is considered bacteriostatic[41].
- When the MBC/MIC ratio equals 1, the antimicrobial effect is considered bactericidal[41].

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 phosphomolybdate 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 highest yield was obtained from the leaf extract of *A. difformis* (9.47%), while the lowest yield was recorded for the rhizome extract (4.10%). All extracts exhibited an acidic pH (pH < 7), ranging from 6.28 to 5.40. Their solubility was satisfactory relative to the solvent used for all extracts. This solubility assessment was conducted to ensure complete dissolution of the extracts for subsequent analyses (phytochemical and others). All extracts were light yellow and had a sticky, gum-like texture (Table 1).

Plants	Organs	Aspect	Colour	Solubility	pH	Yield (%)
A. differmis	Leaves	Sg	Ly	Pg	5.40	9.47
	Bulbs	Sg	Ly	Pg	6.28	4.14
	Rhizomes	Sg	Ly	Pg	5.65	4.10

Table 1. Some characteristics of the extracts obtained. Sg, Sticky Gum; Ly, Light yellow; pH, Hydrogen potential; Pg, Pretty good.

	Extraits HE of *A. differmis*		
Secondary metabolites	Leaves	Bulbs	Rhizomes
Alkaloids	+	+	+
Flavonoids	–	–	–
Tannins	–	–	–
Coumarins	+	+	+
Saponosides	–	–	–
Triterpenes	–	–	–
Phenol	–	–	–
Reducing sugars	+	+	+

Table 2. Phytochemical analysis of the extracts. –, Absence; +, Presence; HE, Hydroethanolic.

	A. differmis		
HE Extracts	Leaves	Bulbs	Rhizomes
Total flavonoid content (µg R Eq /mg DE)	24.85 ± 0.26	33.78 ± 0.66	29.82 ± 0.88

Table 3. Flavonoid content of the extracts studied. Eq R, Equivalent to Rutine; HE, Hydroethanolic; DE, Dry Extract.

Hydroethanolic extracts	Phosphomolybdate reduction test (mg AA Eq /g DE)	FRAP testing (µmol $FeS0_4$ Eq /mg DE)
Leaves of *A. differmis*	37.79 ± 0.18	270.50 ± 5.00
Bulbs of *A. differmis*	19.59 ± 0.00	202.17 ± 2.89
Rhizomes of *A. differmis*	23.09 ± 0.20	190.50 ± 0.00

Table 4. Antioxidant activities in vitro excerpts studied. AA Eq, Acide Ascorbique Equivalent ; FRAP, Ferric Reducing antioxidant Power; DE, Dry Extract.

The extracts were analyzed for the presence of alkaloids, tannins, flavonoids, saponosides, reducing sugars, triterpenoids, and sterols, and the results are presented in Table 2. The hydroethanolic extracts of the leaves, rhizomes, and bulbs of *A. differmis* contained only alkaloids, coumarins, and reducing sugars (Table 2).

Quantitative analyses revealed a low flavonoid content across all extracts at varying concentrations (Table 3). The highest flavonoid content was found in the hydroethanolic extract of the bulb of *A. differmis* (33.78 µg Eq R/mg DE), whereas the lowest was recorded for the leaf extract (28.85 µg Eq R/mg DE).

The evaluation of antioxidant activity demonstrated that all extracts possessed antioxidant potential. The strongest antioxidant activity was observed in the leaf extract of *A. differmis*, with 37.79 mg AA Eq /g DE in the phosphomolybdate reduction assay and 270.50 µmol $FeSO_4$ Eq /mg DE in the FRAP assay (Table 4).

Regarding antimicrobial activity, at the initial concentration of 50 mg/mL, the three hydroethanolic extracts tested did not significantly inhibit microbial growth ($p = 2 \times 10^{-16}$, CV = 3.86 to 5.56%) using either of the diffusion methods across all tested strains (Tables 5 and 6). Consequently, the minimum inhibitory concentration (MIC) was not determined (inhibition diameter < 11 mm).

Antimicrobial activities

Discussion

This study assessed the antimicrobial activity of *A. differmis* against clinical strains of *E. coli*, *S. aureus*, *S. pneumoniae*, and *C. albicans*. The selection of this plant was based on its widespread use in traditional African medicine for treating various ailments[42].

Microbial strains	Extraits HE of *A. difformis*			Water-Ethan NC	Cipro/Nyst. PC (50 µg/mL)	*p*-value	CV(%)
	Leaves (50 mg/mL)	Bulbs (50 mg/mL)	Rhizomes (50 mg/mL)				
S. aureus ATCC 29,213	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 $^{e-16\ ***}$	3.86
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 $^{e-16\ ***}$	3.83
E. coli ATCC 25,922	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 $^{e-16\ ***}$	4.22
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 $^{e-16\ ***}$	4.04
S. pneumonia ATCC 49,619	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 $^{e-16\ ***}$	3.92
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 $^{e-16\ ***}$	3.36
C. albicans ATCC 10,231	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 $^{e-16\ ***}$	4.49
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 $^{e-16\ ***}$	5.56

Table 5. Results of antimicrobial testing of extracts against tested microorganisms using the well diffusion method. 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.

Microbial strains	Extraits HE of *A. difformis*			Water-Ethan NC	Cipro/Nyst. PC (50 µg/mL)	*p*-value	CV(%)
	Leaves (50 mg/mL)	Bulbs (50 mg/mL)	Rhizomes (50 mg/mL)				
S. aureus ATCC 29,213	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 25,922	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 49,619	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 10,231	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

Table 6. Results of antimicrobial testing of extracts on the tested microorganisms using the disc diffusion method. 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.

Yield

Our results indicated that the highest extract yield was obtained from *A. difformis* leaves (9.47%), whereas the lowest yield came from the rhizomes (4.10%). The relatively low yields may be attributed to the short extraction duration (48 h) and the speed and method of rotation used to homogenize the solvent plant powder mixture[43]. Hydroethanolic extraction is advantageous due to its ability to recover a broad spectrum of phytochemicals, its relative safety, and its lower cost compared to other extraction techniques[44].

Qualitative and quantitative phytochemical study.

The qualitative phytochemical analysis of the three *A. difformis* extracts revealed the presence of alkaloids, coumarins, and reducing sugars. These findings differ from those reported by[15,45], who detected alkaloids as well as tannins, flavonoids, saponins, and phenols, but did not observe reducing sugars. This discrepancy may be attributed to differences in the extraction methods employed.

Quantitative analysis indicated a low flavonoid content in all extracts at the tested concentrations (Table 3), which aligns with the absence of flavonoids observed in the qualitative tests (Table 2). The hydroethanolic leaf extract of *A. difformis* showed the lowest flavonoid content (28.85 µg R Eq /mg DE). These results are consistent with those of[17,21], who also reported a low flavonoid content in *A. difformis* leaves (4 mg R Eq /g DE) using the same method.

Antioxidant activity

Regarding antioxidant activity, the FRAP assay and the phosphomolybdate reduction test demonstrated a notable reducing power of *A. difformis* leaves, with values of 270.50 µmol $FeSO_4$ Eq /mg DE and 37.79 mg AA Eq /g DE, respectively. The relatively low content of flavonoids and other secondary metabolites may explain the modest antioxidant activity observed in the various *A. difformis* extracts[46]. Indeed, several studies have highlighted flavonoids as key metabolites involved in cellular oxidative processes, functioning either by neutralizing free radicals or by protecting cells against oxidative stress[33,47–50].

Antimicrobial activity

Presumptive testing using the agar diffusion methods (well and disc) revealed that the three extracts did not significantly inhibit the growth of any of the microorganisms tested ($p = 2\ e^{-16}$, cv = 3.86% to 5.56%) across all bacterial strains examined. Our findings contradict those reported by[12], who demonstrated that the essential oil extract of *Anchomanes difformis* exhibited antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. This discrepancy may be attributed to differences in the nature and composition of the extracts used in the two studies. The low content of phytochemicals such as flavonoids could explain the lack of antimicrobial activity observed with the hydroethanolic extracts of *A. difformis*[31]. Secondary metabolites, particularly flavonoids, are known to play a key role in microbial inhibition mechanisms, either by disrupting bacterial cell membranes, inhibiting biofilm formation, efflux pumps, and bacterial enzymes, or through synergistic interactions with other compounds[51–54].

Limitations and prospects

This study provides valuable insights into the phytochemical composition, antioxidant, and antimicrobial properties of different organs of *Anchomanes difformis*, using two in vitro prescreening methods. However, some limitations should be noted, including the use of a single extraction method, the absence of quantitative analysis of secondary metabolites, and the focus on a limited range of biological activities.

Future research should consider exploring various solvents or advanced extraction techniques, such as supercritical fluid extraction, to enhance the yield and efficiency of bioactive compounds. Moreover, further investigations into the pharmacological properties particularly the anti-inflammatory and antimicrobial activities against emerging bacterial strains as well as in vivo antimicrobial studies, will be crucial for assessing the bioavailability and therapeutic efficacy of the active compounds.

Such efforts will contribute to a more comprehensive understanding and optimal utilization of the therapeutic potential of this plant.

Conclusion

Our study evaluated the biological, antioxidant, and phytochemical properties of *Anchomanes difformis* against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Candida albicans*. The results revealed that the three hydroethanolic extracts of *A. difformis* did not significantly inhibit the growth of the tested microorganisms. Therefore, it can be concluded that the hydroethanolic extracts from the leaves, rhizomes, and bulbs of *Anchomanes difformis* showed no notable antimicrobial activity against any of the strains examined in this study.

Data availability

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

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Author 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 manuscript and Komi Koukoura Komi and Tchadjobo Tchacondo approved and supervised the work.

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Competing interests

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