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Phytochemical Study, Antioxidant and Antibacterial Activity *Dichaetanthera Altissima* Cogn. Root Extracts (Melastomaceae)

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ABSTRACT: *Dichaetanthera altissima* is a species endemic to Madagascar which is among the family Melastomataceae. It was harvested in the North-West part of Madagascar and more particularly in the DIANA Region, Ambanja district, Maherivaratra rural commune, Fokontany Maherivaratra in June 2019. Traditionally, a decocted fresh root of this plant is recommended for the treatment of diarrhea and stomach aches. The phytochemical screening made it possible to detect the presence of low alkaloid concentration, digitalis glycosides, glycosides, polysaccharides, quinones, steroids, reducing sugar, triterpenes and leucoanthocyanins. Dichloromethane and ethyl acetate extracts of the root of this plant have antimicrobial activity against respectively 5 strains such as *Enterobacter cloacae*, *Salmonella enteridis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Candida albicans* and only one such as *Streptococcus pneumoniae*. According to the preliminary test on TLC in the presence of the DPPH radical, the antioxidant activity of this plant is localized in the hydroalcoholic extract and in ethyl acetate. The IC₅₀ calculation of the residual DPPH radical after the test by the kinetic method gave (0.363 ± 0.009) µg/mL for the ethyl acetate extract and (1.113 ± 0.008) µg/mL for the hydroethanolic extract.

KEYWORDS: Antimicrobial, Antioxidant, phytochemical screening, *Dichaetanthera altissima*

I. INTRODUCTION

Phytochemical screening makes it possible to detect different chemical families in plants. While the reducing power of the DPPH radical can qualify a substance to have antioxidant activity and the antibiogram proves that it can be active against a microorganism. Herein, we apply them on the species *Dichaetanthera altissima* known for its healing capacity for the traditional treatment of diarrhea and stomach aches. These methods make it possible to know whether this plant has an active substance against pathogenic microorganisms or an antioxidant or both at the same time. After extraction by increasing polarity, several specific coloring reactions were carried out and depending on the concentration of the substance a precipitate can form in the mixtures. The colors are characteristic of each chemical family of natural substances. The extracts obtained were selected by a preliminary antimicrobial test before carrying out an antibiogram. Only those which present antimicrobial activity will be retested for the reducing power of the DPPH radical by the kinetic method. In this article, the second part concerns the bibliographical synthesis relating to the methods adopted. The third part, drying and maceration by increasing polarity will be presented. After the preliminary antimicrobial test and the antibiogram, the bioactive extracts will be subjected to antioxidant testing in the presence of the DPPH radical by the kinetic method. The fourth part is devoted to the results followed by the conclusion.

II. RELATED WORK

For thousands of years, humanity has used various resources found in its environment to treat and cure all kinds of illnesses. And in recent years, antibiotic resistance in pathogenic microorganisms has become an increasingly important public health problem worldwide. However, this resistance to antibiotics reminds us that it is important to find new molecules with antibacterial activity according to Lee et al. [1]. Currently, in [2], due to poverty the World Health Organization (WHO) estimates that approximately 81% of humanity resorts to traditional herbal preparations as primary health care. Faced with massive exploitation, several researchers are working on the endemic medicinal plant

of Madagascar, in order on the one hand, to deepen scientific knowledge and on the other hand, to save some threatened species, as announced by Rabarison et al. [3]. On this subject, we chose the plant scientifically named: “*Dichaetanthera altissima* (Melastomataceae)”. It is one of the endemic plants of Madagascar and has therapeutic benefits with curative properties for stomach aches. Its extracts have been studied for biological testing, including antimicrobial and antioxidant properties. For the *Dichaetanthera* genus, maceration at room temperature and atmospheric pressure of stem bark in ethanol has already been done by Kognou et al to extract the active ingredients of the species *Dichaetanthera africana* (Hooker F.) Jacq. Felix and the scavenging activity of the DPPH radical was estimated at IC_{50} equal to $0.49 \pm 0.02 \mu\text{g/mL}$ [4].

III. METHODOLOGY

Ethnobotanical investigations

Information on the species was collected through ethnobotanical investigations. To allow villagers to freely express their knowledge and opinions on traditional medicine, the surveys took place in the form of free conversation without structure and without rules in order to obtain the necessary information according to the objectives. The pre-established questionnaires were asked to men and women between 18 and 60 years old.

Harvesting and drying

The plant *Dichaetanthera altissima* known under the vernacular name “vatrotrokala” is on a site which is located in the northern part of Madagascar and more particularly in the DIANA Region, district of Ambanja, rural commune Maherivatra, fokontany Maherivaratra. The botanical sample that was the subject of our study was collected in June 2019 at this location with the help of a tradipractitioner called Jôro, as shown on figures 1 and 2.



Figure 1 : *Dichaetanthera altissima* roots cut Figure 2: *Dichaetanthera altissima* leaves and panicle

The roots were cut with a knife and then dried without mold in a well-ventilated place away from sunlight for 1 month. Drying is monitored every 2 days, weighed using a balance, taken from the location temperature using a thermometer during 1 month of drying. Afterwards, these roots are cut into small pieces using a knife, and then, we proceed to grinding using a mortar in the form of a fine powder which was used for the preparation of the extracts. Then for conservation it was put in dry jars previously cleaned and disinfected.

Extractions

This method consists of leaving the root powder in prolonged contact with a solvent to extract the substances until exhausted. It is a maceration which is carried out at room temperature and atmospheric pressure.

The order of polarity of the solvents is chosen according to two parameters including the dipole moment μ and the dielectric constant ϵ in table 1. When there is ambiguity the dielectric constant ϵ wins because ethanol which has μ equal to 1.69 cannot be less polar than ethyl acetate with μ equal to 1.78.

Table 1 : Polarity of solvents

Solvents	Dielectric constant ϵ	dipole moment μ
n-hexane	1.88	1.08
Ethyl acetate	6.02	1.78
dichloromethane	8.93	1.60

Ethanol	25.3	1.69
Water	80.1	1.85

(Source: https://blog_fr.interchim.com/solvant-polaire-vs-apolaire-comprendre-les-differences-et-les-caracteristiques/, 24/07/2024)

60 g of powder were macerated in 330 mL of non-polar solvent such as hexane. After filtration, the dry residues were macerated again in a higher polarity solvent such as ethyl acetate, then in dichloromethane and hydroethanol. After all these series of extractions, different extracts were obtained such as: hexane extract (EHEx), ethyl acetate extract (EAE), dichloromethane extract (EDCM), and the hydroethanolic extract (EE) after drying in an oven. This method allows compounds to be separated according to their polarity. Indeed, the powder is degreased by using hexane; everything that is fat is thus extracted by this solvent. Then, the less polar and medium polar products will be shared between dichloromethane and ethyl acetate. Finally, the most polar compounds are extracted using the binary solvent ethanol/water: 70/30 (V/V). The extraction is schematized in the flowchart in Figure 3 below.

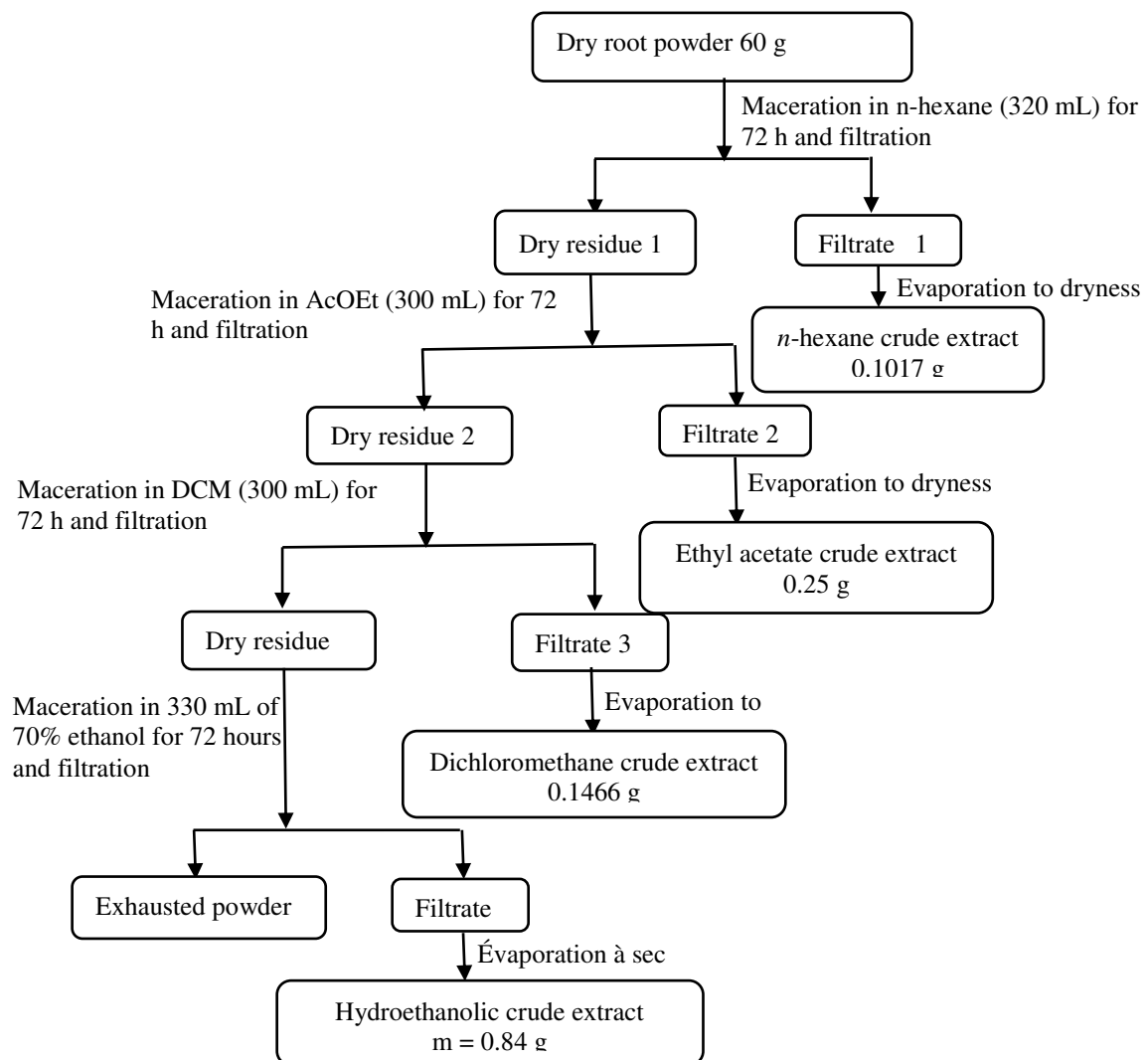
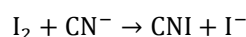


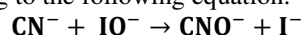
Figure 3: Maceration protocol

Phytochemical screening

The method applied in this part is that which was generally used by Yves-Alain BÉKRO1 et al, Lazureskii G.V.et al, Senov P. L. et al, Melenteva G.A. & Antonova L.A., Tona L. et al, and Longaga A. Otshudi et al, [5-10]. The dosage of cyanogen could have been direct with the hydrogen carbonate. However, the formation of cyanogen iodide which is colorless justifies that the following reaction took place:



The formation of cyanogen oxide is confirmed by the appearance of the yellow-brown coloring as indicated by Jean-lui Burgot and other authors in [11-15] according to the following equation:



To properly manage the quantity of extracts obtained, it was imperative to calculate the mass of the equivalent test portion before carrying out the different tests using the following formula.

$$\text{Mass of test portion} = \frac{\text{Powder mass required}}{\text{Total mass of root powder}} \times \text{Total extract mass}$$

Alkaloids: The equivalent of 5 g of plant powder extract was dissolved in 6 mL of 2 N hydrochloric acid, then the solution was heated in a water bath for 15 min then filtered. The filtrate is placed in 4 test tubes:

- The first tube served as the witness;
- 5 drops of Mayer's reagent were poured into the second tube;
- In the third tube, 5 drops of Wagner reagent were added; - In the fourth tube, 5 drops of Dragendorff reagent were added. The appearance of a precipitate or flocculation with a change in color characteristic of each of these reagents would indicate the presence of alkaloids: cream color for Meyer, reddish brown for Wagner.

Saponins: The equivalent of 1 g of plant powder extract was dissolved in 100 mL of distilled water, then the solution was heated in a water bath at 100 °C for 30 min. The solution is filtered after cooling. After filtration, the solution was then introduced successively into 10 series of test tubes of 1.3 cm diameter and the volume in each tube was shaken lengthwise or vertically for 15 s at a rate of 2 shakes per second. . After this, the tubes were left to rest for 15 min. At the end of this period, the foam height was measured and the foam index I was calculated by the following formula:

$$I = \frac{10}{0,0X} h$$

With h: foam height in cm; X: volume of the stock solution

Unsaturated sterols and triterpenes: There are two methods for screening unsaturated sterols and triterpenes: Liebermann-Burchard test and Salkowski test. Initially, the equivalent of 2 g of plant extract powder is dissolved in 10 mL of maceration solvent. After stirring, the mixture was filtered then distributed into 3 test tubes, the first of which served as the control. Liebermann-Burchard test: 2 drops of acetic anhydride were added to the second tube. The mixture was stirred slightly, then a few drops of concentrated H₂SO₄ were added to it. Blue-green coloring denotes the presence of steroids, red-purple indicates the presence of triterpenes. Salkowski test: 2 mL of concentrated H₂SO₄ is poured onto the internal wall of the third tube by tilting it 45°. The appearance of a red color indicates the presence of unsaturated sterols.

Flavonoids and leucoanthocyanins: The equivalent of 5 g of vegetable powder of the extract is taken up in 20 mL of maceration solvent. The solution thus obtained is distributed into 3 test tubes. The first tube served as the control and the other 2, each containing 3 mL of solution, were used for the aforementioned tests. Wilstater test: 0.5 mL of HCl and 0.5 g of Zinc were added to the solution in the second tube. After 10 min, the change to red indicates the presence of flavones, while a change in color to red-purple denotes the presence of flavones and red-purplish shows the presence of flavanones and flavanols. Bâte-Smith test: 0.5 mL of concentrated HCl was poured into the third tube, then the solution obtained was heated in a water bath for 30 min. The appearance of a purplish red color after cooling indicates the presence of leucoanthocyanins and purple red denotes the presence of flavonoids.

Tannins and polyphenols: 0.1 g extract to be tested was dissolved in 3 mL of hot distilled water at 96°C, then distributed into 4 test tubes. The first tube was used as a control, while the other 3 were intended for the following 3 tests: Gelatin test, in the second tube 4 drops of 1% gelatin were added. The appearance of a cloudiness with white precipitate indicates the presence of water-soluble catechic tannins. Salted gelatin test, four drops of salt gelatin (10 g NaCl in 100 mL 1% aqueous gelatin) were added to the third tube. The presence of condensed pyrogalllic tannins results in the formation of a precipitate. Ferric chloride test, four drops of 10% ferric chloride (FeCl_3) in methanolic solution are added to the fourth tube. The appearance of a greenish-colored precipitate indicates the presence of catechic tannins, while a blackish-blue color denotes the presence of gallic tannins.

Digital glycoside: The equivalent of 2g of plant powder extract was dissolved in 5 mL of distilled water, then 2 mL of glacial acetic acid, 3 drops of 10% FeCl_3 , 1 mL of concentrated sulfuric acid were added. The formation of a ring on the interface of brown or purple or green color indicates the presence of digital glycosides.

Heterosides: The equivalent of 2 g of plant powder extract was dissolved in 2 mL of acetic anhydride and stirred for 2 minutes, then filtered. Then 0.5 mL of concentrated H_2SO_4 was poured into the filtrate. The presence of a red-brown colored ring at the interface denotes the presence of glycoside.

Polysaccharides: Lugol's reagent is a solution containing iodine and potassium iodide. 20 g of KI and 10 g of iodine were poured into 1 L of distilled water, mix the solution and dilute it 10 times (at the time of use) and store it in a brown bottle. Polysaccharides adsorb iodine differently depending on their size and give rise to colored complexes. The appearance of a blue-violet color going to black in the presence of starch, brown characterized by dextrans and mahogany brown for glycogen

Reducing sugar: The application of the Ekhert reaction to a liquid was the subject of this part. It consists of pouring 5 drops of NaOH (2 N) into 5 mL of the extract and adding 2 palettes of NaOH. Expected results: - Dark red coloring with levulose - Yellow coloring with arabinose - Light yellow coloring with glucose, maltose, xylose - Lemon yellow coloring with lactose. The tube on the left serves as the indicator.

Quinones: 0.5 mL of 10% NaOH was added to 2 mL of the hydroalcoholic solution. Expected result: a color that turns yellow, red or purple in the aqueous phase.

Coumarins: 0.5 mL of 10% NaOH was poured into 2 mL of the hydroethanolic extract, then the solution was heated in a water bath at 60 °C for 30 min and 4 mL of distilled water was added to the mixture after cooling. . The solution became transparent by adding it to the control, then 3 to 5 drops of concentrated HCl caused it to lose its yellow color to make it cloudy or form a precipitate.

Cyanide dosage: 5 mg of dry hydroalcoholic extract were dissolved in 10 mL of distilled water and divided into two tubes of equal volume. Tube No. 1 served as the control and the contents of tube No. 2 were titrated with the 3% (m/V) diode I_2 solution until the color changed. The cyanogen iodide being colorless and was in turn titrated with the 0.1% (m/V) NaHCO_3 solution until the color changed to yellow-brown.

Antimicrobial activity tests: Preliminary antimicrobial test: The extracts to be tested were prepared at 10 mg / L per 100 mL of inoculated milk previously boiled and cooled. Inoculation lasts for 30 min using a strain sensitive to antibiotics (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) at the rate of 1 g in 250 mL of milk. The control test contains an antibiotic and the blank test does not. The inoculated sample was shaken well on a magnetic stirrer to solubilize these extracts. After 2 hours of incubation at 40°C, the extract is active if the milk does not coagulate [16].

Antibiogram test: The strains used come from the strain collection of the Environmental Microbiology Laboratory (LME)/CNRE. These germs include 3 Gram negative bacteria, and 2 Gram positive bacteria and a yeast as indicated in Table 2.

Table 2 : Characteristics of the germs to study

	Strain	Gram stain
BACTERIA	Enterobacter cloacae	-
	Staphylococcus aureus ATCC 11632	+
	Streptococcus pneumoniae ATCC 6301	+
	Escherichia coli ATCC 25922	-
	Salmonella enteridis ATCC 13076	-
YEAST	Candida albicans	

The method includes three steps such as preparation of the inoculum, seeding and deposition of the discs as mentioned by the following authors: Yala JF, et al, Mostafa AA, et al, Gbogbo KA, et al, Javid T, et al, in [17-22]. Each extract was distributed on an antibiogram blotting disk with a standard diameter of 6 mm then placed on the medium in a radial manner. The concentration decreases with distance from the source.

Preliminary antioxidant test: A separation on a conventional chromatographic column by gravity of 1 g of the ethyl acetate extract was carried out before the antioxidant test with a glass column of height 20 cm and diameter 1 cm, filled with 8 g of gel silica. The extract was deposited by liquid route then eluted with the binary solvent system n-hexane/ethyl acetate: 50/50 (V/V) in isocratic mode and then with another eluent system DCM-ethanol in gradient mode. The separation on a silica column of the ethyl acetate extract made it possible to obtain seven fractions 1, 2, 3, 4, 5, 6, 7 after grouping the different fractions according to their profile on TLC. These fractions, the dichloromethane extract and the hydroethanolic extract were subjected to the antioxidant test by revelation of the DPPH radical in methanolic solution.

Antioxidant test by dose effect on UV-Vis spectrophotometry: This method consists of measuring the absorbance under UV-Visible by Genova Bio Jenway Spectrophotometer in the presence of a DPPH solution at 6.10⁻⁵ mol/L, an application of that described by Berset C. et al, Brand-Williams W et al, Choe E and Min D.C, Cuvelier ME and Maillard MN, Dangles O., Mc Clements D and Decker E, in [23-29]. If the extracts to be analyzed do not absorb at 515 nm the recording of the kinetic curve at different concentrations 3; 2; 1.5; 1 and 0.5 mg.L⁻¹ can be taken place. 3 mL of the DPPH solution at 6.10⁻⁵ mol/L were mixed with 77 µL of ethanol or AcOEt to serve in the control tank and 77 µL of each of them. After stirring by inversion, recording the spectrum at 515 nm from the kinetic control for 18,000 seconds with a measurement interval of 60 seconds makes it possible to output the kinetic curve of each of the solutions.

IV. EXPERIMENTAL RESULTS

TABLEAU 3 : SUIVI DE SECHAGE DE LA RACINE

Table 3 : Root Drying Monitoring

Jours	Masses(g)	Températures (°C)
1	233	28,5
2	192	32,5
4	152	29,5
6	126	34
10	104	33,5
12	90	28
14	81	32
16	75	31
18	74	29
20	73,5	30
22	73	33
24	72,5	29
26	72	32,5
26	72	32

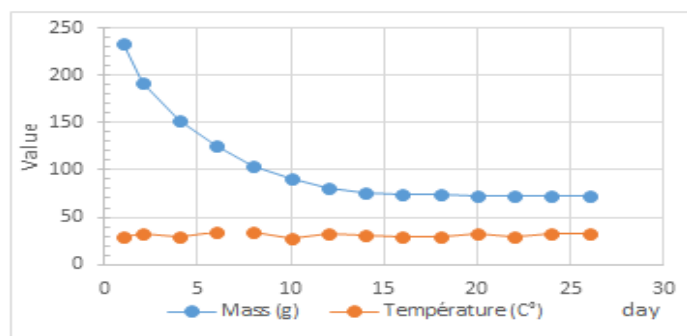


Figure 2 : Daily variation of mass depending on temperature

The drying of the root, previously cleaned under running water, lasted exactly 24 days before the mass stagnated. Daily mass monitoring is presented in Table 3.

The root powder has lost 69% of humidity at the end of drying, only 41% of the dried material remains which, in fact, allows its conservation. Macerations by increasing polarity 60 g of the plant material were macerated by increasing polarity with four solvents, namely n-hexane, AcOEt, DCM, hydroalcoholic. After filtration and evaporation, the yields of each extract obtained are grouped in Table 4.

Table 4 : Maceration assessment by increasing polarity

Extract	Masse (mg)	Yield (%)
EHEX	101.7 ± 0.1	0.1695 ± 0.0002
EAE	250.0 ± 0.1	0.4167 ± 0.0002
EDCM	146.6 ± 0.1	0.2443 ± 0.0002
EE	840.0 ± 0.1	1.4 ± 0.0002

In this table 4, polar compounds are the majority in the root of this plant.

Phytochemical screening

The results of the phytochemical screening carried out on the roots of *Dichaetanthera altissima* are grouped in tables 5 and 6.

Alkaloids detection of both extracts with hexane and ethyl acetate reacted cloudily and changed with the Mayer and Wagner reagent. According to Table 5, the root alkaloids are not polar because they are found in nonpolar solvents such as n-hexane and ethyl acetate.

Coumarins test was carried out on the hydroalcoholic extract with which the addition of a few drops of concentrated HCl had no effect on its coloring. However, a very fine precipitate is formed. This test is, in fact, positive.

Cyanide dosage was conducted on the hydroalcoholic extract because cyanide is a polar compound. During the dosage two balances were brought into play, notably between I₂ / CNI and CN⁻ / CNO⁻. The first is reached when the solution turns very pale yellow, the second when the coloring changes to yellow-brown.

Flavonoids and leucoanthocyanins tests gave a bright red color with precipitate on the EE extract and less pronounced on the EAE extract which denotes a moderate presence of leucoanthocyanin. The same is true for flavonoids.

The green rings which characterize Glycoside digital are present on the interfaces in the tubes containing the EHex, EAcoEt and EDCM extracts. They are non-polar because they are carried by non-polar solvents such as n-hexane, ethyl acetate and dichloromethane.

The red-brown ring characteristic of glycosides exists with a varied thickness depending on the tubes, the widest which suggests a higher concentration in the hydroethanolic extract, on the other hand it is absent in the hexane extract. An average thickness can mean the presence of glycosides in average concentration in the Dichloromethane extract, a thinner ring a priori translates to a low concentration in the ethyl acetate extract.

The presence of quinones in the hydroethanolic extract is confirmed by the turning yellow of the solution with a fairly dark color which was generated by an average concentration. After shaking different tubes containing the daughter solutions of each extract, the foams are almost non-existent or dissolve instantly. Foam indices do not reach 100%. Indeed, the root of this plant does not contain saponin. Table 6 shows that the root of this plant contains triterpenes and steroids. They are mainly concentrated in EDCM and EE. As the Ekhert reaction turns dark red, the hydroethanolic extract contains, in fact, levulose in considerable concentration. Tannins are slightly found in DCM and EE.

Table 5 : Results of phytochemical screening on the four crude extracts

Chemical family	Tests	Reagents	Expected results	EHEX	EAE	EDCM	EE
Alcaloids	Mayer	HgCl ₂ /IK	Creamy, white precipitate	+	+	-	-
	Wagner	I ₂ /IK	Orange-red precipitate	+	-	-	-
Coumarins	Observation under UV	0.5 mL of 10% NaOH	Intense fluorescence				++
Cyanide		3 % (m / V) I ₂ + 0,1 % (m / V) NaHCO ₃	Light yellow turn + brown yellow turn				+
Flavonoids and leucoanthocyanins	Wilstater	1 g of Zn + 0,5 mL of concentrated HCl	Turning red: flavones Turning purple: flavonones Change to purplish red: flavanones and flavanols	-	-	+	+++
	Bâte-Smith	0,5 mL of concentrated HCl	Red or reddish coloring	-	-	+	+++
Digital glycoside		2 mL of glacial acetic acid, + 3 drops of FeCl ₃ 10% + 1 mL of concentrated sulfuric acid	Brown or purple or green ring on the interface	++	++	+	-
Heterosides		2 mL of acetic anhydride + 0.5 mL of concentrated H ₂ SO ₄	Red-brown colored ring at the interface	-	+	++	+++
Polysaccharides	Lugol	20 g of KI in 1 L of water + 10 g of iodine	Blue violet to black: starch Brown: dextrin Mahogany brown: glycogen	-	-	-	+++

(-): Absence; (+): troubled turn; (++) : presence of precipitate; (+++): presence of precipitate with deposit;

Table 6 : Suite of phytochemical screening results on the four crude extracts

Chemical family	Tests	Reagents	Expected results	EHEX	EAE	EDCM	EE
Quinones		0.5 mL of 10% NaOH	Color that turns yellow, red or purple in the aqueous phase.	-	-	-	++
Saponin	Foam	Foam height	Foam index > 100	-	-	-	-
Sterols-Triterpenes	Liebermann Burschard	1 mL of acetic anhydride + 1 mL of concentrated H ₂ SO ₄	Red violet to pink: triterpenes Blue green: steroids Red: unsaturated sterols	-	-	++	++
	Salkowski	2 mL of H ₂ SO ₄	Red or reddish color	-	-	+++	+++
Reducing sugar	Ekhert	NaOH (2 N) + 2 palettes of NaOH	Dark red coloring with levulose Yellow coloring with arabinose Light yellow coloring with glucose, maltose, xylose – Lemon yellow coloring with lactose				+++
Taninns and Polyphenols		1 mL of 1% gelatin	White precipitate	-	-	+	+
		1 mL of salted gelatin	White precipitate	-	-	+	+
		1 mL of 10% FeCl ₃	Greenish or blackish-blue ² coloring	-	-	+	+

(-): Absence; (+): troubled turn; (++) : presence of precipitate; (+++) : presence of precipitate with deposit;

According to this result, the various tests on the chemical families of *Dichaetanthera ariculata* roots are exposed to the existence of flavonoids and leuconthocyanins, glycosides, dextrin type polysaccharides and unsaturated sterols, levulose type reducing sugar, at high concentration, at the low concentration of alkaloids, coumarins, saponosides, Tannins and Polyphenols. Steroids, triterpenes and digitalis glycosides, quinone are present in medium concentration. However, alkaloids, polyphenols and tannins (gallic, catechic) are present in low concentrations.

Results of biological activities

Preliminary antimicrobial activity test According to the antimicrobial test carried out on the different extracts at 10 ppm, we found that only the extracts with ethyl acetate and dichloromethane inhibit *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in milk, in fact, they have antimicrobial activity.

Antibiogram

The sensitivity halo diameters are reported in Table 8 below.

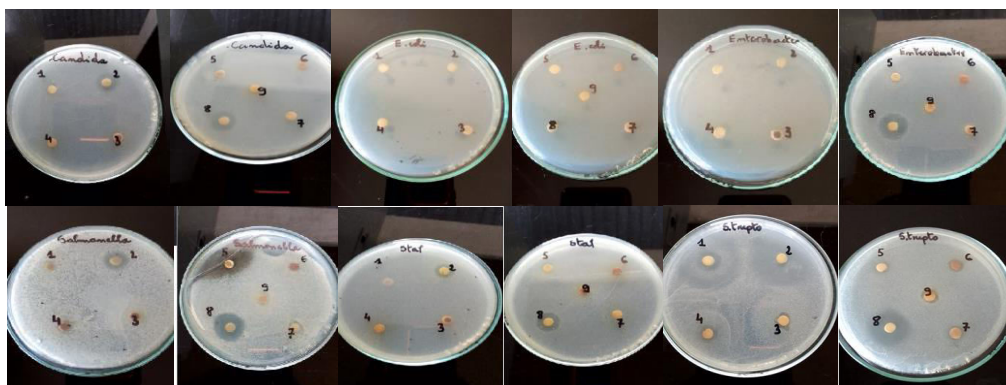


Figure 4: Antibiogram of 9 extracts tested on the 6 strains

In Table 7 and Figure 3, as mentioned 5/6 strains tested are sensitive to dichloromethane extract namely Enterobacter cloacae, Candida albicans, Salmonella enteridis, Staphylococcus aureus, Streptococcus pneumoniae. While only the strain Streptococcus pneumoniae ATCC 6301 is sensitive to ethyl acetate extract.

Table 7 : Halo diameter in cm after antimicrobial testing of crude extracts

Crudes extracts and reference antibiotics	E. cloacae ATCC 700323	E. coli	S. enteridis	S. aureus	S. pneumoniae ATCC 6301	C. albicans
1	6	6	6	6	12	7.5
2	6	6	11	11	33	13
3	6	6	6	6	9	6
4	6	6	6	6	8	6
5	7	6	9	6	8	7.5
6	6	6	6	6	8	6
7	6	6	6	6	7.5	6
8	16	6	17	12.5	21	13
9	6	6	6	6	6	6
NA30	15	16	26	-	-	-
SXT25	-	-	-	23	-	-
FA10	-	-	-	-	28	-
Butanolic extract of streptomyces sp.	-	-	-	-	-	31

AEA: 1, 4 and 7; EDCM : 2, 5 and 8 ; EE : 3, 6 and 9

Samples 1, 2 and 3 were collected on March 23, 2023 at the same time as 7, 8 and 9 but from two different trees. The other samples 4.5 and 5 were collected on September 15, 2023.

Preliminary antioxidant test results

The DPPH radical revelation results of the 3 extracts were shown in Figures 4, 5 and 6.

Eluent : Hex/AcOEt : 5/5 (V/V)
Revealer : DPPH radical



Figure 5 : TLC of the antioxidant test of crude extract fractions with ethyl acetate

Eluent : AcOEt/ EtOH
90° : 9/1 (V/V)
Revealer : DPPH radical

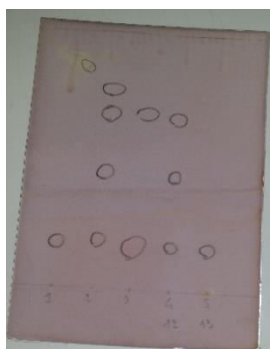


Figure 6: TLC of the antioxidant test of EDCM fractions

Eluent: EtOH 90° /H₂O /
acetic acid: 5/4/1 (V/V/V)
Revealer : DPPH radical

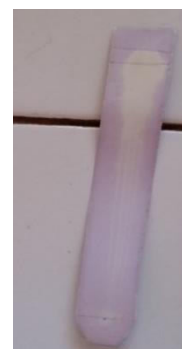


Figure 7 : TLC of the antioxidant test on the EE

Likewise, 0.35 g of the dichloromethane extract was subjected to chromatography on a column 22 cm long and 2.5 mm in diameter, filled with 3 g of silica gel. The extract was deposited dry and eluted isocratically with the solvent mixture AcOEt / EtOH 90°: 90 / 10 (V / V) then in graduated mode up to 90% ethanol. This separation made it possible to obtain five fractions 1, 2, 3, 4, 5 after grouping the different fractions according to their TLC profile. From Figures 5 to 7, the ethyl acetate extract and the hydroethanolic extract show yellow spots on a purple background after the revelation with radical DPPH, on the other hand on the dichloromethane extract no yellow spot was visible. Therefore, we found that the hydroethanolic extract and the ethyl acetate extract have an anti-radical activity, unlike the dichloromethane extract.

Antioxidant power: The kinetic study of the measured solutions is crucial for any effectiveness due to the slow evolution of the DPPH^o radical reduction reaction. The two extracts EAcOEt and EDCM were the subject of this study following the positive preliminary test.

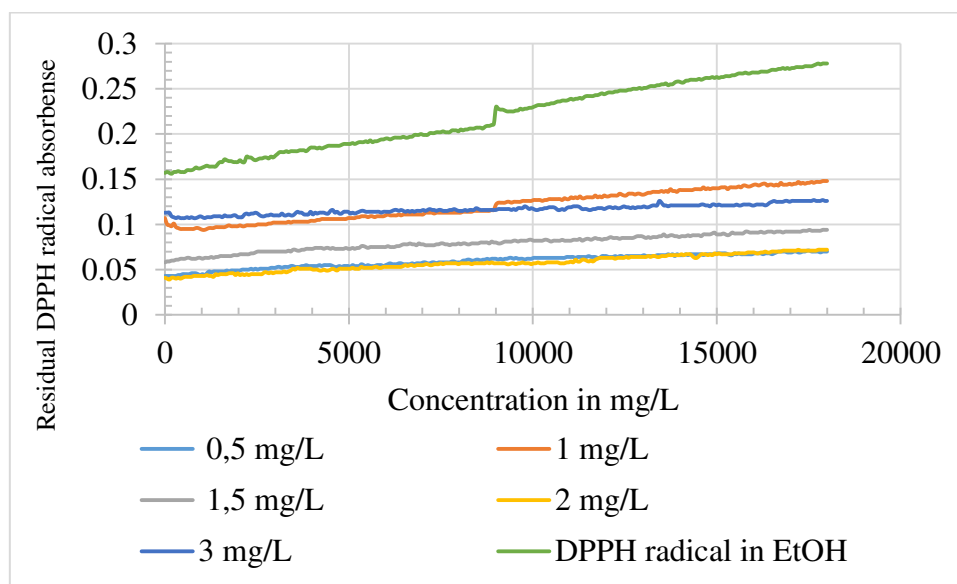


Figure 8 : Kinetics of DPPH radical reduction as a function of EE concentration

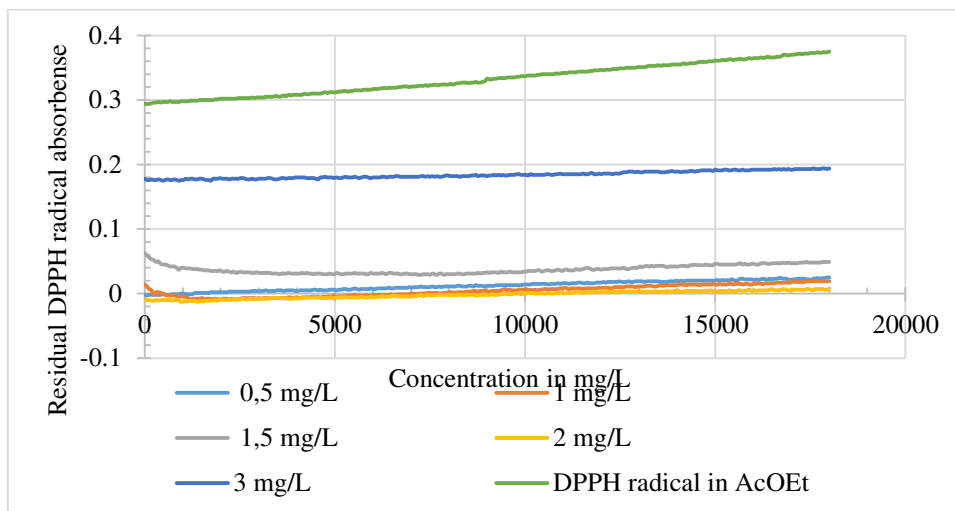


Figure 9 : Kinetics of DPPH radical reduction as a function of EAE concentration

Table 8 Percentage of residual DPPH radical

Concentration in mg/L	EAE	EE
3.0 ± 0.1	51,73 ± 0.02	45,32 ± 0.01
2.0 ± 0.1	2,13 ± 0.01	25,90 ± 0.01
1,5 ± 0.1	13,07 ± 0.01	33,81 ± 0.01
1.0 ± 0.1	5,07 ± 0.01	53,27 ± 0.02
0,5 ± 0.1	24,51 ± 0.01	25,18 ± 0.01
0	100.00 ± 0.02	100.00 ± 0.02

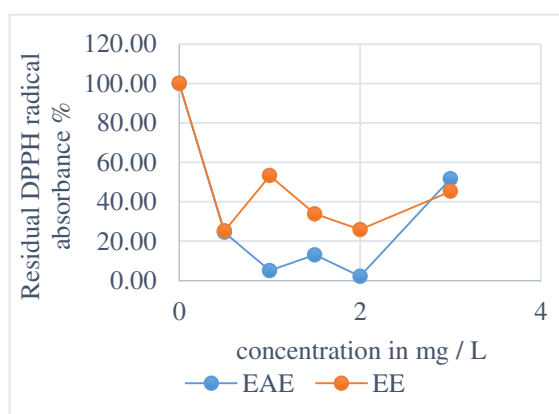


Figure10 : Dose-effect curve

The absorbance value stabilizes after 18000 seconds and will be applied to the calculation of the percentage of the DPPH° radical. This value is presented in Figures 7 and 8 for EAcOEt and EDCM respectively.

Calculation of IC50 of the residual DPPH radical using the kalythos command of the R software gave (0.363 ± 0.009) mg/L for the ethyl acetate extract, and (1.113 ± 0.008) mg/L for the hydroethanolic extract.

V. CONCLUSION

The species is characterized by a pyramidal panicle, usually sparsely flowered, bearing calyxes 5 mm high, with an obtusely 4-sinuate limb. It is clear that polar compounds are predominant in the root because they are soluble in water and transported with nutrients from the soil. According to the study done on this plant, they have a DPPH radical scavenging activity. Some apolar compounds that have accumulated or produced by metabolism at the root level are divided into two, those that are soluble in ethyl acetate (EAE) with scavenging activity and others that are soluble in dichloromethane (EDCM) do not have despite their antimicrobial activity against *Enterobacter cloacae*, *Candida albicans*, *Salmonella enteridis*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*.

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