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Mineral and phenolic compositions, antioxidant activity and GC-MS analysis of the leaves of *Anchomanes difformis (Blume)* Engl. from Côte d'Ivoire

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Abstract

The main objective of this study is to contribute to the valuation of *Anchomanes difformis*, a plant whose leaves are used for its oxytocic effect at the end of pregnancy and its use in the treatment of rheumatism in Côte d'Ivoire. The X-ray fluorescence spectrometer assay showed that the leaves of *Anchomanes difformis* contain (mg / 100g): K / 2061; P / 315.2; Ca / 1268; Na / 206.9; Mg / 653.8; Fe / 116.4; Cu / 0.79; Zn / 10.03 and Mn / 68.59. The quantitative spectrophotometric analysis of the phenolic compounds of the hydromethanolic crude extract of the leaves made it possible to determine the contents of total polyphenols (6.12 mg EAG / g), total flavonoids (mg EC / g), condensed tannins (0.637%) and hydrolyzable (0.312%). The evaluation of the antioxidant activity, measured by spectrophotometry against the stable free radical DPPH, revealed that the study extract (0.598 mg / ml) is less effective than vitamin C (0.032 mg / ml) taken as a reference. GC-MS analysis of the hydromethanolic extract revealed the existence of several phenolic compounds, the presence of which would be responsible for the therapeutic virtues of the leaves of the leaves of *Anchomanes difformis*.

Keywords: Anchomanes difformis; Mineral and phenolic compositions; Antioxidant activity; GC-MS

1. Introduction

Medicinal plants take an important place in the therapeutic arsenal of humanity. According to the World Health Organization (WHO), approximately 80% of the world's population uses medicinal plants for their primary health care needs [1]. Indeed, most plant species have therapeutic virtues, because they contain active ingredients, which act directly on the body [2]. Among these, mineral elements and phenolic compounds, whose biological role is currently not in doubt. Minerals are involved in many biochemical processes, they stimulate and normalize metabolism, and act on enzymes and the genetic apparatus of cells. Many macro- and trace elements perform strictly defined functions, being a kind of catalyst for various reactions in humans and animals, their deficiency is often the cause of pathological processes and diseases [3, 4]. As for phenolic compounds, they are the most important constituents of plants. The synthesis of polyphenols in human cells is not possible, so they enter the body mainly with plant foods, while having a generally beneficial effect on it. They are above all renowned for their antioxidant properties, by fighting against the formation of free radicals, which in excess in the body can promote cellular aging and the early onset of certain diseases [5, 6]. In this regard, the search for new plant sources rich in bioactive compounds such as polyphenols and macro- and trace elements is topical to obtain new highly active drugs, having antioxidant, anti-inflammatory, anticancer, antiviral, antiparasitic and antibacterial.

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The leaves of *Anchomanes difformis* (Araceae) were selected for this study. *A. difformis* is a large herbaceous plant about 2 m tall, erect on a huge horizontal tuber, often reaching 50-80 cm long and 10-20 cm in diameter [7]. Its leaves are broad umbrella-shaped (hence its name monkey umbrella) with multi-parted blades, connected to the fleshy stem by thorny petioles [8, 9]. *A. difformis* grows in the tropical forests of West Africa (Nigeria, Ghana, Cote d'Ivoire, Sierra Leone, Senegal and Togo) [10]. It is known by various names in vernacular languages. In Côte d'Ivoire, it is called topi in Agni, bédro-bédro in Beté, kohodié in Abouré, dé in Kulango, niamé kwanba in Baoulé, don in Guéré and diri dobli dobli in Gouro [11]. In Nigeria, *A. difformis* is known as ogirisako in the Igo language, olumahi by the Igbos, ebaenan by the Efik, chakara by the Hausa [12]. In addition, it is called nau in the Tem language in Togo and eken in Diola in Senegal [13]. *A. difformis* is a versatile plant, the different parts of which are widely used in the traditional treatment of a variety of ailments in many countries of West and East Africa [8-21]. The use of its leaves in traditional medicine is also diverse. In Côte d'Ivoire, the leaves are used at the end of pregnancy for its mild oxytocic effects and to facilitate childbirth [11, 14], and also for the treatment of rheumatism [15]. In Ghana, the leaves and bark of the stems are used to treat snakebites and burns [18].

Despite the fact that *A. difformis* is a plant used in the Ivorian pharmacopoeia, the Ivorian species has not been sufficiently studied and there are no chemical studies. This is why we undertook this study on the mineral and phenolic composition, the antioxidant activity and the GC-MS analysis of the hydromethanolic extract of the leaves of *A. difformis* from Côte d'Ivoire.

2. Material and methods

The plant material consists of the leaves of *Anchomanes difformis*, which after identification by Dr Malan Djah François, systematic botanist of the NANGUI ABROGOUA University (Abidjan, Côte d'Ivoire) were collected on June 16, 2018 in the forests of Kokumbo an locality in the center of Côte d'Ivoire (Toumodi department in the lakes region (6 ° 33'N, 5 ° 15'W). The leaves were cleaned and then dried in an air-conditioned room for 7 days. Were pulverized with an electric mill (RETSCH, type SM 100) to obtain a powder which was used for the preparation of the study extracts.

2.1. Mineral composition

Elemental chemical analysis was performed using an AMETEK spectro Xepos ED2000 type X-ray fluorescence spectrometer coupled to a computer. Four grams of powder, obtained from the leaves by grinding using a RETSCHMM400 vibro-mill, are taken to make pellets using a hydraulic press (pressure 10 tons). The pellets introduced into the spectrometer are bombarded by photons emitted from an X-ray tube. The primary radiation is absorbed, the atoms of the ionized sample are filled by internal electrons emitted (secondary X-ray beam). This electronic relaxation releases energy (X-ray fluorescence) in the form of the characteristic photons for each atom. These photons are detected by a counter, which identifies the atom based on its energy. From the flow of photons received, the analyzer deduced the corresponding mass concentration.

2.2. Preparation of extracts

10 g of the crushed leaves are macerated for 24 h in 100 ml of 80% methanol with permanent stirring. This operation is repeated twice with the same marc. After filtration on Büchner, the macerates obtained are combined and then concentrated at 40°C on a rotary evaporator (BÜCHI Waterbath B-480). The aqueous extract is stored in the refrigerator for 48 hours to precipitate lipophilic compounds. After decanting, the hydromethanolic extracts were obtained which were used for the quantification of polyphenols, flavonoids, flavonic aglycones, anthocyanins and tannins and to evaluate their antioxidant activity.

2.3. Quantitative analysis

2.3.1. Determination of polyphenols

The total polyphenol content of the leaves of *A. difformis* was determined according to the colorimetric method of Folin-Ciocalteu [22]. To 1 ml of the extract diluted to 1 / 10th with distilled water, are added 1.5 ml of Na₂CO₃ (17%, m / v) and 0.5 ml of Folin-Ciocalteu reagent (0.5N). The whole is incubated for 30 min at 37°C and then the absorbance read at 720 nm against a blank. The quantification of the total polyphenols was made according to the linear calibration line (y = ax + b) carried out with gallic acid at different concentrations (0 to 1000 µg/ml) under the same operating conditions. The content of total polyphenols (TP) is calculated according to the following formula:

$$TP = (V \times C \times d) / m$$

With V: final volume of the extract (ml); C: concentration of the extract (μ g / ml); d: dilution; m: mass of dry plant matter (g).

The results are expressed in micrograms equivalent of gallic acid per gram of dry plant matter (μ g EAG / g DM).

2.3.2. Determination of total flavonoids

The content of total flavonoids (TF) is determined with aluminum trichloride (AlCl₃) [23]. To 500 μ l of each extract are added 1500 μ l of distilled water and 150 μ l of 5% sodium nitrate. The mixture is left to stand for 5 min in the dark, then 150 μ l of 10% AlCl₃ are added to it. After 11 min of incubation in the dark, 500 μ l of NaOH (1 M) is added to the mixture. The whole is vortexed and the absorbance of the solution read at 510 nm (WPA S800 spectrophotometer) against a blank. A calibration curve is produced in parallel, using catechin as positive control, under the same operating conditions and at different concentrations (2.5; 5; 10; 20; 40; 80 mg/l). The total flavonoid content of the plant extracts studied is expressed in milligram (mg) equivalent of catechin per gram of dry plant material (mg EC/g DM).

2.3.3. Determination of hydrolyzable tannins

The content of hydrolyzable tannins (TH) is determined by the colorimetric method of with iron trichloride (FeCl₃) [23]. 0.2 g of the crushed leaves is macerated in 10 ml of 80% methanol for 18 h. The mixture is filtered and 1 ml of the filtrate is added to 3.5 ml of a solution of FeCl₃ (0.01 M) in hydrochloric acid (HCl) at 0.001 M. After 15 seconds, the absorbance of the mixture is read at 660 nm. The hydrolyzable tannins are expressed according to the formula:

TH (%) = (Abs \times M \times V) / E mole \times P

With Abs: absorbance, E mole: 2169 of gallic acid (constant expressed in mole), M: mass = 300, V: volume of the extract used, P: mass of the sample

2.3.4. Dosage of condensed tannins

The quantification of condensed tannins (TC) is carried out according to the method based on the condensation of phenolic compounds with vanillin in an acidic medium [23]. 0.2 g of the crushed leaves are macerated for 18 h in 10 ml of 80% methanol. After filtration, 1 ml of the filtrate is added to 2 ml of a vanillin solution prepared at a base of 1% in 70% sulfuric acid H_2SO_4 . The mixture is placed in a water bath for 15 min at 20°C, protected from light, and the absorbance (A) is read at 500 nm with a spectrophotometer. The contents of condensed tannins (TC) are expressed by the formula:

TC (%) =
$$(5.2 \times 10^{-2} \times A \times V) / P$$

With 5.2 x 10⁻²: constant expressed in cyanidine equivalent; A: absorbance; V: volume of the extract used; P: sample weight.

2.4. Evaluation of antioxidant activity (DPPH test)

The antioxidant activity of the crude extract of the leaves of *A. difformis* is evaluated by the spectrophotometric method using 2,2'-diphenyl-1-picrylhydrazyl (DPPH). The DPPH is dissolved in methanol, to obtain a solution of concentration 0.3 mg / ml. Different concentration ranges (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg / ml) of the extract are prepared in methanol. 2.5 ml of extract and 1 ml of methanolic solution of DPPH are introduced into dry tubes. After shaking, the tubes are placed in the dark for 30 min. The absorbance of the mixture is read at 517 nm against a blank composed of 2.5 ml of the methanol and 1 ml of the DPPH solution. Vitamin C is used as an antioxidant control. The percentage reduction (PR) of DPPH by the crude extract is calculated according to the following formula [24]:

With Ab: absorbance of the blank and Ae: absorbance of the sample

2.5. GC-MS analysis

To 10 g of the ground leaves of *A. difformis* previously treated with petroleum ether, 50 ml of 2N HCl are added. The whole is heated at reflux for 2 hours. After cooling, the hydrolyzate is treated with 3 × 50 ml of ethyl acetate. The ethyl acetate fractions are combined, dried with anhydrous MgSO₄, then concentrated to dryness on a rotary evaporator. 1 mg of the dry extract is taken up in 2 ml of ethyl acetate (AcOEt), filtered using a disposable filter with a porosity of 0.2

 μ m. The GC-MS analysis was carried out on a GC-MS chromatograph (SHIMADZU, model QP2010SE) equipped with a Zbron ZB-5ms column (7HG-G010-11) of 20 m × 0.18 mm internal diameter, and 0.18 μ m film thickness in stationary phase. The carrier gas is helium with an overhead pressure of 108.3 Kpa, a flow rate of 0.6 ml/min and a linear velocity of 38.2 cm/s. The injector and detector temperatures are 280 and 290°C, respectively. The temperature program is 70°C for 4 min then increased to 270°C at the rate of 4°C / min and maintained for 20 min. The mass spectra are recorded by a quadrupole type detector and the ionization is carried out by electronic impact under a potential of 70 eV at 230°C, 50 scans / s for the scanning speed and 10.000 amu / s for the speed acquisition. The volatile compounds are identified after comparison, on the one hand, of their mass spectrum with those of the database (NIST 98. LIB and Wiley 275), and on the other hand, by referring to data from the literature [25].

3. Results and discussion

3.1. Mineral element content

The contents obtained in mineral elements in the leaves of *A. difformis* are presented in Table 1. 28 elements are found in this part of the plant, among which 7 macroelements, 6 trace elements and 15 other elements. As shown in Table 1, the sum of all mineral elements represents about 5.53% of the leaf mass of *A. difformis*, with the higher contents of macroelements such as K, Ca, Mg, S, P, N / A. They are also found to be rich in trace elements, in particular Si, Fe, Mn and Zn.

Macroelements		Tra	ce elements	Other elements			
К	2061 ± 3	Si	234.9± 0.6	Al	84.25± 0.67	Ag	0.26± 0.07
Са	1268 ± 4	Fe	116.4± 0.3	Ti	10.074± 0.54	Zr	0.17±0.03
Mg	653.8 ± 2.7	Mn	68.59± 0.31	La	3.47± 0.62	Cd	0.12±0.05
S	463.8 ± 0.4	Zn	10.03±0.05	Cs	2.98± 0.58	Th	0,12±0,01
Р	315.2 ± 0.4	Se	0.19±0.01	Rb	2.04± 0.01	Ga	0.05±0.02
Na	206.9 ± 5.5	Cu	0.79± 0.000	Sr	2.47± 0.01	Ni	0.55± 0.03
Cl	25.19 ± 0.05			Та	0.42± 0.01	V	0.98± 0.98
			Br	0.38± 0.01			

Table 1 Mineral composition of the leaves of Anchomanes difformis (mg / 100g)

The contents of K, Ca, Na, P, S, Fe, Mn and Zn are higher than those of tea leaves and nettle [26-28], which are widely used as a source of trace elements. The importance of these elements in supporting and maintaining human health has been recognized [29]. Indeed, Ca, Mg and P represent the mineral mass of the bone skeleton. In addition, Ca and Mg participate in muscle and cardiac contraction, blood coagulation, cellular exchanges, membrane permeability, hormone release and the transmission of nerve impulses [30, 31]. P contributes to the maintenance of the acid-base balance (pH) and participates in most of the biochemical reactions of the organism. Na plays a major role in the regulation of osmotic pressure, water-electrolyte balance and body water mass. In the body, Fe plays a major role in the production and functioning of hemoglobin and myoglobin. Zn is of importance in cell renewal, wound healing and immunity [32]. All this indicates the prospects for further research on the leaves of *A. difformis* in order to consider the possibility of creating new therapeutic preparations.

3.2. Quantitative analysis

3.2.1. Phenols, total flavonoids and tannins content

The content of phenolic compounds in a plant is a very important factor. Numerous studies have shown that the content of phenolic compounds determines the antioxidant activity of a plant material [5, 6]. The total phenol content of the leaves of *A. difformis*, obtained from the following linear regression equation: $y = 0.0013x-0.0081^{\circ}$; $R^2 = 0.9887$ (for gallic acid), is 6.12 ± 0.26 mg EAG / g DM (Table 2). This content is found to be significantly higher than that obtained (2.19 mg EAG / g DM) in the leaves of the species from Benin [9] and lower than that found in the leaves of *A. difformis* collected in Cameroon (10.45 ± 0.07 mg EAC / g) [33].

Table 2 Total contents of phenols, flavonoids, hydrolyzable and condensed tannins in the hydromethanolic extract of the leaves of *Anchomanes difformis*

TP (mg EAG/g MS)	TF (mg EC/g MS)	TH (%)	TC (%)	
6.120 ± 0,260	2.557 ± 0.452	0.637 ± 0.029	0.312 ± 0.007	

* TP: total phenol content; TF: flavonoid content; TH: content of hydrolyzable tannins;

The results obtained with the leaves of *A. difformis* are comparable with those of N'Guessan et al. which showed the high levels of phenolic compounds for the 10 medicinal plants of Côte d'Ivoire (3.493 - 7.818 mg EAG/g DM) [22]. Flavonoids are an important class among phenolic compounds, as it is this class of substances that exhibit various types of biological activity [34, 35]. AlCl₃ is a standard reagent widely used to assess total flavonoid content. Its content obtained using the linear regression equation: y = 0.0013x-0.0081; $R^2 = 0.9887$ (for gallic acid) presents the value equal to 2.557 ± 0.452 mg EC / g DM (Table 2), which is lower than that found (4.594 mg EC / g DM) in the leaves collected in Benin [9].

In plants, hydrolyzable and condensed tannins occur simultaneously, with the predominance of one class. In this study, the content of condensed tannins (0.637%) in the leaves of *A. difformis* is greater than that of hydrolyzable tannins (0.312%) (Table 2). The content of condensed tannins obtained is similar to that determined in the leaves of *A. difformis* from Nigeria (0.66%) [36]. However, this content is lower than that of the leaves of *A. difformis* from Benin (0.974%) [9]. Moreover, the tannin contents found are comparable with those noted on the leaves of *Marrubium vulgare* used in the manufacture of cough remedies, they contain 0.65% of condensed tannins and 2.8%) of hydrolyzable tannins [37].

3.3. Antioxidant activity

Free radicals are super active substances which have a maximum destructive effect on the human cell. Their trapping is extremely important. It is the level of Free radicals trapping that characterizes the capacity of an antioxidant. The antioxidant activity of the hydromethanolic crude extract of the leaves of *A. difformis* was evaluated by the method of studying the anti-free radical activity of 2,2'-diphenyl-1-picrylhydrazyl (DPPH•). We note that the percentage reduction of DPPH• by the extract is only greater than 50% at the concentration equal to 1 mg / ml, the CR_{50} of which is estimated at 0.598 mg / ml, which is much greater than that of vitamin C ($CR_{50} = 0.032$ mg / ml), taken as a reference antioxidant (Table 3). However, the anti-free radical activity determined in this work is similar to that of extracts from the leaves of *A. difformis* published by other researchers [9, 33, 36, 38].

Concontration (mg/ml)	PR (%)	CR50 (mg	g/ml)
Concentration (mg/ml)	FK (%)	Extrait	Vit C
1	64.576		
0.5	42.804	0.598	0.022
0.25	29,890		
0.125	9.594	0.598	0.032
0.0625	3.690		
0.03125	1.476		

Table 3 Antioxidant activity of the raw extract of the leaves of *A. difformis*

PR: percentage reduction; CR50: reducing concentration 50%

3.4. GC-MS analysis

For the first time, the raw extract of the leaves of *A. difformis* is analyzed by GC-MS. The chromatogram shows 49 peaks (Figure 1) which represents 98.42% of the overall composition. Twenty-seven (27) compounds have been identified, including phenolic compounds (16.63%), carboxylic acids and their derivatives (39.53%) and substances of furan nature (2.38%), including structures are confirmed by fragment ions recorded on the MS spectra (**Table 4**).

Most of the molecules identified in the extract of the leaves of *A. difformis* are antioxidant phenolic substances. These are hydrocinnamic (sinapic, ferulic and p-coumaric) and syringic acids which have, in addition, anticancer, anti-inflammatory, antifungal and antidiabetic properties [39-42]. Also, vanillin and its derivative (apocynin), having aphrodisiac, anti-arthritic, antibacterial, anti-asthma [43, 44] activities have been identified. In addition, the presence of an allylphenol (eugenol) with antiseptic, analgesic, anti-inflammatory and antimicrobial properties [45] and another flavoring phenol (p-vinylguaiacol) has been demonstrated [46].

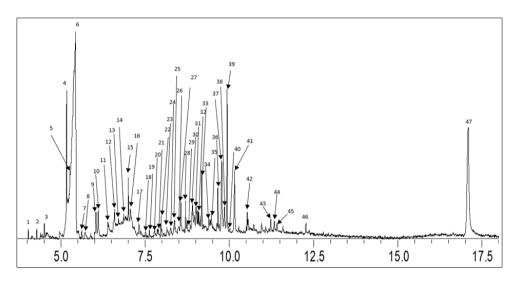


Figure 1 GC chromatogram of the raw extract of the leaves of Anchomanes difformis

Table 4 Compounds identified by GC-MS	in the leaves of Anchomanes difformis
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Peak	T _R (min)	Percenta ge (%)	Molecular formula	Molar mass (g/mol)	Fragmentation, m/z (%)	Identified compound
1	4.027	0.30	$C_5H_6O_2$	98	98[M] ⁺ ; 83[M-CH ₃] ⁺ ; 69[M-CO ₂] ⁺ 56(100); 35	5-méthylfuran- 2(5 <i>H</i>)-one
2	4.280	0.25	$C_6H_6O_2$	110	110(100)[M] ⁺ ; 81[M-CHO] ⁺ ; 67[M - CHO-CH ₃] ⁺ ; 53; 39	5-méthylfuran-2- carbaldéhyde
3	4.508	0.31	C6H10O3	130	130[M]+; 115[M-CH ₃]+ 99[M-OCH ₃]+ 88 71[M-C ₂ H ₃ O ₂]+;59[MC ₄ H ₃ O ₇]+; 48(100)	4-oxopentanoate de méthyle
4	5.166	4.27	C7H12O3	144	144[M]+; 129[M-CH ₃]+; 99[M-C ₂ H ₅ O]+; 74[M-C ₄ H ₇ O+H]+; 56[M-C ₃ H ₅ O-H]+; 48(100);	4-oxopentanoate d'éthyle
5	5.267	5.38			99(100); 71; 59; 45	Non identifié
6	5.437	30.59	$C_5H_8O_3$	116	116[M] ⁺ ; 101[M-CH ₃] ⁺ ; 73[M-C ₂ H ₃ O] ⁺ ; 56[M-C ₂ H ₃ O-OH] ⁺ ; 48(100)	Acide 4- oxopentanoique
7	5.520	0.45	C5H8O4	132	132[M] ^{+;} 114[M-H ₂ O] ⁺ ; 105[M-C ₂ H ₅ - 2H] ⁺ ; 97(100); 60[M-C ₃ H ₅ O ₂ +H] ⁺ ; 43	Acide 3-éthoxy-3- oxopropanoique
8	5.620	0.28	C5H6O4	130	99(100)[M-CH ₃ O] ⁺ , 85[M-CHO ₂] ⁺ 81 59[M-C ₃ H ₃ O ₂] ⁺ , 53, 45[M-C ₄ H ₅ O ₂] ⁺	Acide (2E)-4- Methoxy-4-oxo-2- buténoïque
9	6.036	1.49	C7H6O2	122	122(90) [M] ⁺ ; 105(100) [M-OH] ⁺ ; 77(70); 51(30) [C ₆ H ₅] ⁺	Acide benzoïque
10	6.107	1.06	$C_6H_8O_4$	144	$\begin{array}{l} 144[M]^{+;} & 127(12)[M-OH]^{+;} & 117(10); \\ 99(100)[M-C_2H_5O]^{+;} & 82(20); & 60(8); \\ 56(10); 45(18) [CHO_2]^{+;} \end{array}$	Ester monoéthylique d'acide fumarique
11	6,393	1.08	C ₈ H ₈ O	120	120(100)[M] ⁺ ; 103(2) [M-OH] ⁺ ; 90(20) [M-CH ₂ O] ⁺ ; 77 [C ₆ H ₅] ⁺ ; 65(20) [C ₆ H ₅] ⁺ ; 51(5)	2,3- Dihydrobenzofurane (Coumarane),
12	6,595	2.12	C8H8O2	136	136(35) [M ⁺]; 91(100) [M-CHO ₂] ⁺ ; 77(2) $[C_6H_5]^{+}$; 65(15) $[C_6H_5]^{+}$; 39(10)	Acide phénylacétique

13	6.707	1.69				Non identifié
14	6.860	4.88			429; 341; 98; 79; 77; 67; 56; 41	Non identifié
15	6.999	1.82	$C_8H_8O_4$	168	$ \begin{array}{c} 168[M]^{+}; 125(100)[M-C_{2}H_{3}O]^{+}, 109(20) \\ [M-C_{2}H_{3}O_{2}]^{+}; & 97(10)[M-C_{2}H_{2}O-CHO]^{+}; \\ 79(30); 43(40) \ [C_{2}H_{3}O]^{+} \end{array} $	5-Acetoxymethyl-2- furaldehyde
16	7.053	2.71	$C_9H_{10}O_2$	150	$\begin{array}{cccc} 150(98)[M]^+; & 135(100) & [M-CH_3]^+;107(60) & [M-CH_3O-CH_2]^+; \\ 77(56)[C_6H_5]^+ & \end{array}$	2-Methoxy-4- vinylphenol (p-vinyl guaiacol)
17	7.173	0.60			140 139 101 88 77 56 43 39	Non identifié
18	7.320	0.48	C10H12O2	164	$\begin{array}{c} 164(100)[M]^{+}; & 149(30)[M-CH_{3}]^{+}; \\ 103(25) & [M-CH_{3}O-OH-CH_{2}]^{+} & 91(17)[M-CH_{3}O-C_{3}H_{5}-H]^{+}; \\ 77(23)[C_{6}H_{5}]^{+} \end{array}$	4-Allyl-2- méthoxyphénol (eugénol)
19	7.627	0.28	$C_8H_8O_3$	152	152(100) [M] *; 137(5) [M-CH ₃]*; 121(20) [M-CH ₃ O]*; 109(15)[M-CHO- CH ₂]*; 81(20)	4-Hydroxy-3- méthoxybenzaldéhyd e (vanilline)
20	7.781	0.47			429; 367; 281; 249; 193; 147; 119; 98; 85; 73; 69; 55; 41	Non identifié
21	7.860	0.53	C ₈ H ₈ O ₂	136	136 [M] +; 121 (100); 93; 65; 39	m- Hydroxyacetophenon e
22	7.973	0.55			266; 252; 251; 133; 125	Non identifié
23	8.140	0.44	C9H10O3	166	166(40)[M]*; 151(100)[M- CH ₃]*;136(10); 123(28)[M-C ₂ H ₃ O]*; 108(10) [M-C ₂ H ₃ O-CH ₃]*	1-(4-hydroxy-3- methoxyphenyl)etha n-1-one (apocynine)
24	8.255	0.41	C7H6O3	138	138(70) [M] ⁺ ; 121(100) [M-OH] ⁺ ; 93(21) [M-CHO ₂] ⁺ ; 65(25)[C ₅ H ₅] ⁺ ; 39(18)	Acide p- hydroxybenzoique
25	8.369	0.88			180; 137; 122; 97; 73; 69; 55; 41	Non identifié
26	8.473	0.46			180; 137; 107; 87; 77; 67; 39;	Non identifié
27	8.555	1.33	C ₈ H ₈ O ₄	168	168(100) [M] ⁺ ; 153(70) [M-CH ₃] ⁺ ; 137[M-CH ₃ 0] ⁺ ; 108[M-CH ₃ 0-CH ₃] ⁺ ; 125(25); 97(30); 91[M-CH ₃ 0-CH ₃ -OH] ⁺	Acide 3-hydroxy-4- méthoxybenzoique (Isovanillique)
28	8.699	1.26			151(100); 136; 121; 108; 95; 77; 65; 43	Non identifié
29	8.834	0.25			188; 173(100); 144; 129; 115; 99; 79; 59; 43	Non identifié
30	8.904	1.49			152; 136; 124; 111; 83; 60; 55(100); 41	Non identifié
31	9.009	1.79			267; 210; 192; 171; 166; 149; 135; 109 ; 97; 83; 69; 48 (100)	Non identifié
32	9.060	0.98	C ₁₃ H ₁₈ O ₂	206	150[M-C ₃ H ₄ O] ⁺ ; 108(100)[M-C ₄ H ₅ O- 2CH ₃ +H] ⁺ ; 77[C ₆ H ₅] ^{+;} 43	(E)-5,5-trimethyl-4- [3-oxobut-1-en-1- yl]cyclohex-2-en-1- one
33	9.181	1.54			222; 152; 137; 109; 95; 82; 69(100); 55 41	Non identifié
34	9.387	0.41		210	210(87)[M] ⁺ ; 195(25)[M-CH ₃] ⁺ ; 167(10); 151(100)[M-C ₂ H ₃ O ₂] ⁺ ; 119(35)[M-C ₂ H ₃ O ₂ -CH ₃ -OH] ⁺	Méthyl 2-(3,5- dimethoxyphenyl)ac étate

35	9.465	1.04	C ₁₀ H ₁₂ O ₄	196	196(35) [M] ⁺ ; 181(20) [M-CH ₃] ⁺ ; 167(1); 150(2) [M-CHO ₂ -2H] ⁺ ; 137(100)[M-CHO ₂ -CH ₃];122(18); 77 [C ₆ H ₅] ⁺	Acide (4-hydroxy-3- methoxyphenyl) propionique
36	9.660	1.50			126(100); 103(40); 97(10); 79(30)	Non identifié
37	9.772	3.20	C9H8O3	164	164(100) [M] ⁺ ; 147(48)[M-OH] ⁺ ; 136(6); 119(30)[M-CHO ₂] ⁺ ; 107(15); 91(30)[M-CHO ₂ -OH-2H] ⁺	Acide 3-(4- hydroxyphényl)- prop-2-ènoïque (acide trans-p- coumarique)
38	9.854	1.06	C9H10O5	198	198(100)[M] ⁺ ; 183(31)[M-CH ₃] ⁺ ; 153(5) [M-CHO ₂] ⁺ ; 138[M-CHO ₂ -CH ₃] ⁺ ; 127(20); 109(20); 93(10); 77 [C ₆ H ₅] ⁺	Acide 4-hydroxy-3,5- diméthoxy benzoïque (acide syringique),
39	9.932	1.06			126; 99(100); 71; 41	Non identifié
40	10.013	1.06			152; 137(100); 121; 98; 81; 69; 43	Non identifié
41	10.153	2.36	C ₁₀ H ₁₀ O ₄	194	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Acide 3-(4-hydroxy- 3-méthoxyphényl) prop-2-ènoïque (acide férulique)
42	10.530	0.80			256(45); 227(5); 213(29); 199(10); 129(49); 115(20); 73(100)	Non identifié
43	11.227	0.45	C ₁₁ H ₁₂ O ₅	224	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Acide 3-(4-hydroxy- 3,5-diméthoxy- phenyl) prop-2- ènoïque (acide sinapinique)
4444	11.334	0.45			216; 173; 145; 120; 105; 97(100;) 77	Non identifié
4545	11.413	0.45	C ₁₈ H ₃₆ O ₂		$\begin{array}{c} 284[M]^{+}; 241[M-C_{3}H_{7}]^{+}; 185[M-C_{3}H_{7}-\\ C_{4}H_{8}]^{+}; 143[M-C_{10}H_{21}]^{+}; 129[M-\\ C_{11}H_{23}]^{+}; 73[M-C_{15}H_{31}]^{+}; 60; 43(100) \end{array}$	Acide octadecanoïque
46	12.269	0.45			290; 195; 150(100); 135; 95; 77; 65	Non identifié
47	17.087	9.67			386(100); 309(25); 282(8); 279(5); 193(15); 153(5); 78(15)	Non identifié
48	18.270	1.86			386(100); 309(25); 282(8); 279(5); 193(15); 153(5); 78(15)	Non identifié
49	18.417	0.63			375(100); 319; 167	Non identifié
		98,42				

4. Conclusion

Elemental chemical analysis by X-ray fluorescence spectrometry has shown that the leaves of *Anchomanes difformis* contain most of the mineral elements necessary for human health. The spectrophotometric determination of phytophenols in the raw leaf extract revealed the significant presence of polyphenols, flavonoids, hydrolyzable and condensed tannins. Evaluation of the antioxidant potential of said extract compared to DPPH showed that it exhibits antioxidant activity, which is lower than that of vitamin C. GC-MS analysis made it possible to identify 27 phytoconstituents in the crude extract leaves, the majority of which are phenolic compounds with therapeutic effect. The present study is, therefore, a rational explanation of the use of *Anchomanes difformis* leaves in traditional medicine in Côte d'Ivoire.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

References

- [1] World Health Organization (WHO). Traditional medicine (report by the secretariat). Fifty-sixth World Health Assembly. 2003; 18: 5.
- [2] Isérin P. Encyclopedia of Medicinal Plants: Identification, Preparation, Care. Edition Larousse. Paris. 2001; 335.
- [3] Martin A, Azais-Braesco V, Bresson JL, Couet C. Recommended dietary allowances for the French population. (CNERNA-CNRS et AFSSA). Third Edition, Tec and Doc. 2001; 605.
- [4] Chappuis P, Favier A. Trace elements in nutrition and therapy. Edition Lavoisier. 1995; 1: 474.
- [5] Kabran GRM, Ambeu NC, Mamyrbekova-Bekro JA, Bekro YA. Total phenols and flavonoids in organic extracts of ten plants used in the traditional therapy of breast cancer in Côte d'Ivoire. European Journal of Scientific Research. 2012; 68: 182-190.
- [6] Makarova NV. Antioxidant properties of fruits: influencing factors, application, products. Samara (Russie). 2015: 471.
- [7] Ahmed HA. *Anchomanes difformis*: A Multipurpose Phytomedicine. IOSR Journal of Pharmacy and Biological Sciences. 2018; 13(2): 62-65.
- [8] Burkill HM. The Useful Plants of West Africa. Family *Anchomanes difformis*. Second ed. Ibadan: Royal Botanic Gardens. 1985; 648.
- [9] Akibou OML, Agbangnan DCP, Bossou A, Yedomonhan HP, Avlessi F, Sohounhloue KCD. Antiradical activities and study of volatile compounds of three plants of traditional medicine in Benin: *Anchomanes difformis, Parkia biglobosa* and *Polyalthia longifolia*. International Journal of Innovation and Applied Studies. 2014; 9(4):1609-1619.
- [10] Ataman JE, Idu M. Renal Effects of *Anchomanes Difformis* Crude Extract in Wistar Rats. Avicenna Journal of Phytomedicine. 2015; 5(1): 17-25.
- [11] Malan D, Neuba DFR. Traditional Practices and Medicinal Plants Use during Pregnancy by Anyi-Ndenye Women (Eastern Côte d'Ivoire). African Journal of Reproductive Health. 2011; 15(1): 85-93.
- [12] Eneojo AS, Egwari LO, Mosaku TO. *In vitro* Antimicrobial Screening on *Anchomanes difformis* (Blume) Engl. Leaves and Rhizomes Against Selected Pathogens of Public Health Importance. Advances in Biological Research. 2011; 5 (4): 221-225.
- [13] Bello OM, Jagaba SM, Bello OE. A wild edible vegetable *Anchomanes difformis* (Blume) Engl: its ethnomedicinal, phytochemistry, nutritional importance and other uses. Eurasia Journal of Biosciences. 2019; 13: 1137-1147.
- [14] Bouquet A, Debray M. Medicinal plants of Côte d'Ivoire. Works and Documents O.R.S.T.O.M., Paris. 1974; 32: 232.
- [15] Vroh Bi TA, Ouattara D, Kpangui KB. Availability of spontaneous plant species for traditional use in the locality of Agbaou, Center-West of Côte d'Ivoire. Journal of Applied Biosciences. 2014; 76: 6386-6396.
- [16] N'guessan K, Konan EK, Kouassi KK. Ethnobotanical Study of Plants Used to Treat Diabetes, in Traditional Medicine, by Abbey and Krobou People of Agboville (Côte d'Ivoire). American Journal of Scientific Research. 2009; (4): 45-58
- [17] Kerharo J, Adam JG. The traditional Senegalese pharmacopoeia. Medicinal and poisonous plants. Vigot brothers, Paris. 1974; 512.

- [18] Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensl A. An Ethnopharmacological survey and in vitro confirmation of ethno-pharmacological use of medicinal plants used for wound healing in Gosomtwi-Atwima-Kwanwoma area, Ghana. Journal of Ethnopharmacology. 2009; 125: 393- 403.
- [19] Dibong SD, Mvogo Ottou PB, Vandi D, Ndjib RC, Tchamaha FM, Mpondo ME. Ethnobotany of anti-haemorrhoidal medicinal plants from markets and villages in the Center and Littoral region of Cameroon. Journal of Applied Biosciences. 2015; 96: 9072-9093.
- [20] Adomou AC, Yedomonhan H, Djossa B, Legba SI, Oumorou M, Akoegninou A. Ethnobotanical study of medicinal plants sold in the Abomey-Calavi market in Benin. International Journal of Biology and Chemical Science. 2012; 6(2): 745-772.
- [21] Fah L, Klotoé JR, Dougnon V, Koudokpon H, Fanou VBA, Dandjesso C, Loko F. Ethnobotanical study of plants used in the treatment of diabetes in pregnant women in Cotonou and Abomey-Calavi (Benin). Journal of Animal & Plant Sciences. 2013; 18(1): 2647-2658.
- [22] N'Guessan AHO, Dago DCE, Mamyrbékova-Békro JA, Békro YA. Contents of phenolic compounds of 10 medicinal plants used in the traditional therapy of arterial hypertension, an emerging pathology in Côte d'Ivoire. Industrial engineering journal. 2011; 6: 55-61.
- [23] Dif MM, Benchiha H, Mehdadi Z, Benali-Toumi F, Benyahia M, Bouterfas K. Quantitative study of polyphenols in the different organs of *Papaver rhoeas* L. Lavoisier. Phytothérapie. 2015; 13(5): 324-319.
- [24] Kadja AB, Mamyrbékova-Békro JA, Benie A, Boua BB, N'Gaman KC, Bekro YA. *Erythrophleum africanum* Afzel. (Caesalpiniaceae), an African toothpick: Phytochemical screening, total flavonoid content and antioxidant activity. Journal of Medicinal Plants Research. 2011; 5(27): 6273-6277.
- [25] N'guessan PA, Kadja AB, Ouattara LH, Kouadio ATG, Mamyrbékova-Békro JA, Békro YA. Phytochemical Identification by TLC and GC-MS of Crude Hydroethanolic Extracts of Four Medicinal Plants from Côte d'Ivoire. International Journal of Current Research and Academic Review. 2020; 8(1): 11-21.
- [26] Amal AHS, El Otmani IS, Sanae D, Benmoussa A. Highlighting the nutritional and therapeutic potential of stinging nettle (*Urtica dioïca* L.). Hegel. 2016°; 6(3): 280-292.
- [27] Xie M, Von Bohlen A, Klockenkaemper R, Jian X, Guenther K. Multielement analysis of Chinese tea (*Camellia sinensis*) by total-reflection X-ray fluorescence. Food Research and Technology. 1998°; 207(1): 31-38.
- [28] Matsuura H, Hokura A, Katsuki F, Itoh A, Haraguchi H. Multielement determination and speciation of major-totrace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. Analysis Sciences. 2001; 17 (3): 391-398.
- [29] Pasteur JL. All the vitamins to live without drugs. Edition Bien Être Paris. 1996; 270.
- [30] Jeroen HF. de Baaij JGJH. Magnesium in Man: Implications for Health and Disease. Physiological Reviews. 2015; 95(1): 1-46.
- [31] Vasson M. Food supplements: the keys to advising them at the pharmacy. Paris Defense: the Editions « Moniteur des pharmacies ». (Pro-officina). 2015.
- [32] Eureka Santé by VIDAL [Internet]. Eureka Santé.
- [33] Guetchueng ST, Gbaweng JY, Tchinda AT. Phenolic content and DPPH scavenging activity of *Carpolobia lutea* and *Anchomanes difformis*. International Journal of Pharma Sciences and Research. 2015°; 6(5): 909-912.
- [34] Del Rio E, Costa LG, Lean ME, Crozier A. Polyphenols and health: What compounds are involved? Journal of Nutrition, Metabolism & Cardiovascular Diseases. 2010; 20: 1-6.
- [35] Cardona F, Andrés-Lacueva C, Tulipani S, Tinahones FJ, Queipo-Ortuño MI. Benefits of polyphenols on gut microbiota and implications in human health. Journal of Nutrition and Biochemistry. 2013°; 24(8): 1415-1422.
- [36] Oghale OU, Idu M. Phytochemistry, anti-asthmatic and antioxidant activities of *Anchomanes difformis* (Blume) Engl. leaf extract. Asian Pacific Journal of Tropical Biomedicine. 2016.
- [37] Bouterfas K, Mehdadi Z, Latreche A, Hazem Z, Bouredja N. Quantification of some polyphenols of *Marrubium vulgare* L. from Mount Tessala (Western Algeria) during the two periods of vegetation and flowering. Les technologies de laboratoire. 2013; 8(31): 34-41.

- [38] Idowu D, Adebiyi A, Olajide O, Afolayan M, Orishadipe A, Omojola M. Phytochemical, antioxidant and cytotoxicity properties of *Anchomanes difformis* (Bl.) Engl. tuber extract. International Journal of Applied Chemistry. 2012°; 8(3): 173-181.
- [39] Mori H, Kawabata K, Yoshimi N, Tanaka T, Murakami T, Okada T, Murai H. Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. Anticancer Research. 1999°; 19(5A): 3775-3778.
- [40] Yong SL. Role of NADPH oxidase-mediated generation of reactive oxygen species in the mechanism of apoptosis induced by phenolic acids in HepG2 human hepatoma cells. Archives Pharmacal Research. 2005°; 28(10): 1183-1189.
- [41] Ferguson LR, Shuo-tun Z, Harris PJ. Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 », Molecular Nutrition & Food Research. 2005°; 49(6): 585-693.
- [42] Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Kumar CS. Syringic acid (SA). A Review of Its Occurrence, Biosynthesis, Pharmacological and Industrial Importance. Biomedicine & Pharmacotherapy. 2018°; 108: 547-557.
- [43] Palmen MJHJ, Beukelman CJ, Mooij RGM, Pena AS, Van Rees E. Anti-inflammatory effect of apocynin, a plantderived NADPH oxidase antagonist, in acute experimental colitis. The Netherlands Journal of Medicine. 1995°; 47(2): 41-47.
- [44] Stefanska J, Sarniak A, Wlodarczyk A, Sokolowska M, Pniewska E, Doniec Z, Nowak D, Pawliczak R. Apocynin reduces reactive oxygen species concentrations in exhaled breath condensate in asthmatics. Experimental Lung Research. 2012°; 38(2): 90-99.
- [45] Ulanowska M, Olas B. Biological Properties and Prospects for the Application of Eugenol, A Review. International Journal of Molecular Science. 2021°; 22(3671): 1-13.
- [46] Morata A, González C, Suárez-Lepe JA. Formation of vinylphenolic pyranoanthocyanins by selected yeasts fermenting red grape musts supplemented with hydroxycinnamic acids. International Journal of Food Microbiology. 2007°; 116: 144-152.