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Phytochemical investigation of *Cissus aralioides* stems from Côte d'Ivoire

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ABSTRACT

As part of research on plants of interest for the floristic biodiversity of Côte d'Ivoire, a phytochemical investigation of the stems of *Cissus aralioides*, a species with medicinal properties of the botanical family Vitaceae, was produced. The mineral and organic compositions of said extract were determined, respectively by X-ray fluorescence spectrometry and by GC-MS. Elemental chemical analysis revealed considerable levels of trace elements (Fe, Mn, Zn), macro elements (Ca, Mg, P, K), and the presence of other elements (Ba, Ti, La, Cs, Rb, Ta, Cd). Analysis by GC-MS shows the existence of several active ingredients such as phenol acids, flavonoids, stilbenes and phytosterols. A major phytosterol (E-resveratrol) has been isolated from C. aralioides growing in Ivory Coast.

Keywords: *Cissus aralioides*, extract, stem, phytochemical investigation.

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INTRODUCTION

Plants are an excellent reservoir of molecules of interest, which find use in various fields (phytomedicine, plant protection, nutrition, etc.). The use of plants in traditional medicinal practices is known. *Cissus aralioides* (Baker) Planch. is a plant belonging to the botanical family Vitaceae. It is a woody climbing plant with large succulent stems, in the shape of cones tightened at the nodes. The leaves are sometimes sub-succulent. The greenish flowers are sometimes whitish, relatively large, and the reddish fruits turning blue-purple[1]. This plant is known by various local names: Ewo Toma (in Agni - Ivory Coast)[2] ; Kindamina (common name - Cameroon) and Eriri agwo (in Igbo - Nigeria)[3]. It is widespread in the forests of tropical Africa[4]. In African traditional medicine, the preparations made from its leaves and roots are used as febrifuge, analgesic, antimicrobial, anti-inflammatory, anti arthritic, anti rheumatic, antimalarial. On the other hand, organs are used against dropsy, gout, gonorrhea, edema, pulmonary disorders. The sap treats eye disorders and venereal diseases[5-7]**.** In Congo-Brazzaville, stems are used as analgesic, antiseptic, cough suppressant; and to relieve body pain[8]. In Côte d'Ivoire, *C. aralioides* stems are used in prenatal care to facilitate delivery[2]. Phytochemical screening of *C. aralioides* leaves showed the presence of alkaloids, flavonoids, proteins, saponins, terpenoids, coumarins, reducing sugars and cardiac glycosides[4, 8-9]. Scientific studies have shown that the leaves exhibit analgesic, antibacterial, anti-inflammatory and antioxidant properties; which validates their use in traditional medicine in the treatment of many diseases[6, 10-12]**.** Although *C. aralioides* is widely used in the African pharmacopoeia, few studies have been carried out on the chemical composition of the stems. The objective of the present study is to determine the elemental and organic chemical compositions of *C. aralioides* stems growing in Côte d'Ivoire.

MATERIALS AND METHOD

After authentication of the plant by Dr. Malan Djah François, systematic botanist from Nangui Abrogoua University (Abidjan, Ivory Coast), a sample was deposited in our laboratory. *C. aralioides* stems were collected on June 16, 2018 in the Kokumbo forests, a central locality of Côte d'Ivoire (Toumodi department in the lakes region (6° 33′N, 5° 15′W). The stems were cleaned and dried under air conditioning during 30 days. Stems were pulverized by using an electric mixer (RETSCH, type SM 100). The powders obtained were used for the preparation of the organic extracts.

Determination of elemental chemical composition

X-ray fluorescence spectrometry on a spectrometer (AMETEK spectro Xepos ED 2000) coupled to a computer was performed from elemental chemical analysis. 4g of vegetable powder obtained

by grinding the dried stems using a vibro-mill (RETSCHMM 400) was used for making pellets using a hydraulic press (pressure of 10 tonnes). The pellets were subjected to X-rays. The latter emit energy in the form of X-rays, the spectrum of which has made it possible to determine the elemental chemical composition[13].

Preparation of organic extracts

100 g of powder were macerated in 1000 ml of methanol (MeOH) (80%) with permanent stirring during 24 h. After filtration and concentration on a rotary evaporator (BÜCHI RII), the concentrate is successively exhausted with 3 × 100 ml of hexane, chloroform, ethyl acetate and n-butanol. The organic fractions were concentrated with a rotary evaporator, then kept in an oven (40°C) for 24 h to successively provide the dry extracts. For the rest, only the dry extract with ethyl acetate was retained.

GC-MS analysis

To 3 mg of dry extract with ethyl acetate, 0.5 ml of distilled dichloromethane (CH_2Cl_2) and 0.2 ml of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) are added. After 12 h of incubation with stirring in the dark at room temperature, the mixture was concentrated on a rotary evaporator to dryness. The dry extract was taken up in 2 ml of ethyl acetate (AcOEt), filtered through a disposable filter (0.2 µm porosity) and an aliquot injected into 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 µm d 'film thickness in stationary phase. The carrier gas is helium with a pressure of 108.3 Kpa, a flow rate of 0.6 ml / min and a linear speed 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 during 20 min. A quadrupole type detector recorded the mass spectra and the ionization was 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 acquisition speed. The volatile compounds were identified after comparison, on the one hand, of their spectral configurations obtained with those of the databases (NIST 98. LIB and Wiley 275), and on the other hand, by referring to data drawn from the literature[14].

Phytoconstituent from ethyl acetate dry extract: Isolation and characterization

The dry extract (817 mg) was fractionated in column containing silica gel (50 g). The elution was carried out with a ternary mixture of dichloromethane (DCM) / AcOEt / MeOH by varying the proportions (Table 1). Four fractions (I-IV) were collected according to the similarity of the chromatographic profiles. The first fraction (22.4 mg) was subjected to preparative plate chromatography. After development by means of a ternary mixture, DCM / AcOEt / MeOH

(76/19/5), drying and collection of the band of interest, a phytocompound (7.11 mg) was isolated by scraping. Its molecular structure was determined by GC-MS and 1H NMR (Bruker 400 MHz acetone-d6 spectrometer).

Table 1: Solvent gradients

DCM (ml)	AcOEt (ml)	MeOH (ml)
76.8	19.2	4
76.0	19.0	5
75.2	18.8	6
74.4	18.6	7
73.6	18.4	8
72.8	18.2	9
72	18	10

RESULTS AND DISCUSSION

Stem bark mineral profile

Minerals differ in weight and functional diversity and their content in plants is variable. The results of *C. aralioides* stems mineral chemical analysis revealed the presence of macro elements (Ca, K, Mg, Cl, S, Na, P) and microelements (Si, Fe, Mn, Zn, Cu, Ni,) (Table 2). The presence of macroelements and microelements, among which the nutrients for the constitution and maintenance of tissues (Ca, P) and the regulation of metabolism (Na, K, P, Mg, Fe, Zn, Cu, Mn), and other mineral elements (Sr, Ti, La, Cs, Rb, Ta, Cd) have been identified[15] (Table 2). The coexistence of these minerals proves that *C. aralioides* stems have therapeutic properties[16-18].

Table 2: Elemental chemical composition of *Cissus aralioides* stems (mg/100g)

Macroéléments		Microéléments		Autres éléments	
Ca	3082 ± 3	Si	50,84 ± 0,4	Sr	8,90 ± 0.02
K	1135 ± 2	Fe	8.92 ± 0.09	Ti	5,46 ± 0,51
Mg	761,6 ± 3	Mn	8.58 ± 0.15	La	4,44 ± 0.07
Cl	354,2 ± 0.3	Zn	7,87 ± 0.04	Cs	3,71 ± 0.59
S	300,0 ± 0.3	Cu	0,70 ± 0.03	Rb	1,31 ± 0.01
Na	236 ± 6.7	Ni	0,49 ± 0.03	Ta	0,39 ± 0.01
P	176,3 ± 0.3			Cd	0,26 ± 0.06

Ethyl acetate extract GC-MS profile

According to Figure 1 and Table 3, GC-MS after derivatization of *C. aralioides* stems AcOEt dry extract showed at least 28 phytoconstituents, which 22 were identified. These are phenolic compounds (25.09 %; peaks 2, 3, 4, 7, 10, 15, 26, 27), stilbenoids (29.29 %; peaks 5, 16, 18, 21, 25), phytosterols (26.13 %; peaks 19, 20, 23, 24) a triterpene (1.80%°; peak 11) and various other compounds (7.27 %; peaks 1, 9, 13, 14).

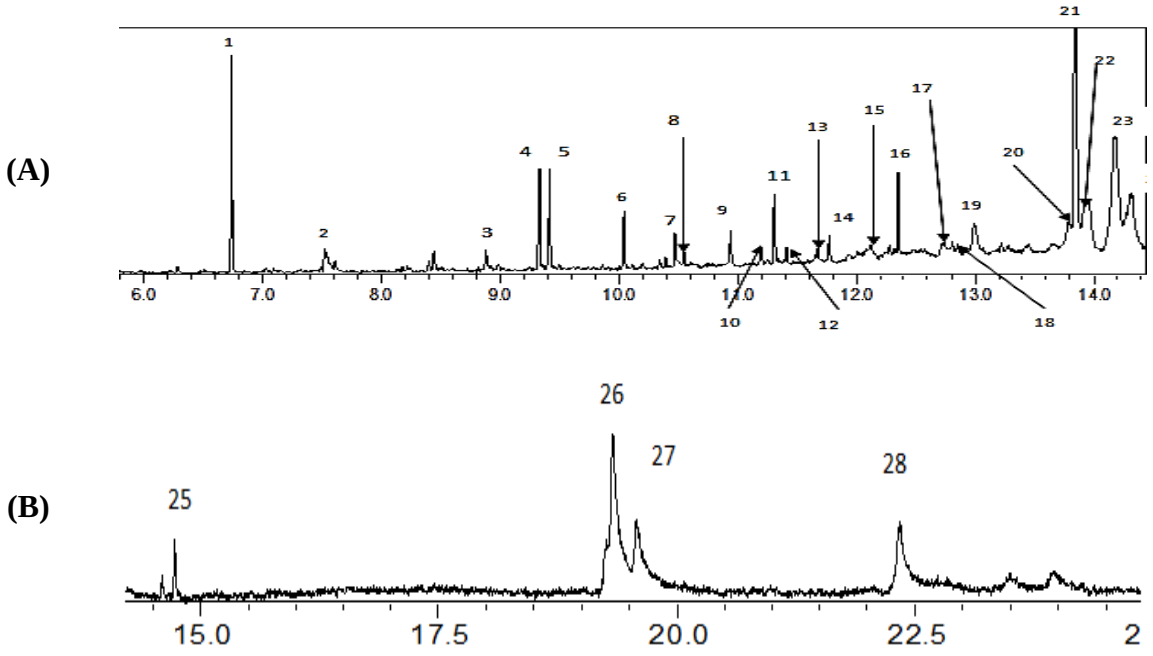

Figure 1: GC-MS chromatogram of the dry extract with ethyl acetate of the stems of *Cissus aralioides* (A) (from 0 to 14.5 min), (B) (from 14.5 to 30 min)

Fragmentation analysis (Table 3) revealed the existence of a peak at m/z 73 in almost all mass spectra, representing the trimethylsilyl (TMS) moiety. The $[M-89]^+$ and $[M-15]^+$ fragments corresponding respectively to the loss of a trimethylsiloxy [-O-Si(CH_3)] and of a methyl (CH_3) were recorded. These fragmentations are characteristic of oximes-TMS derivatives[19]. On the other hand, the presence of the fragment at m/z 147 [(CH_3) 2Si = O-Si (CH_3)$_3$]$^+$ also indicates that two or more TMS groups are present in a molecule[20]. The main ion of the compound (peak 10) at m/z 193 $[M-177]^+$ is generated according to the main fragmentation pathway of phenolic acids[21]. The compound (peak 15) at m/z 650 [M^+] whose fragment at m/z 368 resulting from the Diels-Alder retro cleavage of the B characteristic cycle of flavonoids[22], corresponds to catechin. Thus, GC-MS analysis of the ethyl acetate dry extract of *C. aralioides* stems (Table 3) showed the presence of secondary metabolites with numerous activities beneficial to human health. Indeed, polyphenols are involved in the prevention and treatment of cancer, cataracts, atherosclerosis, diabetes, high blood pressure, neurodegenerative diseases and arthritis[30-32]. Stilbenes have a wide range of therapeutic properties (anti carcinogenic, cardioprotective, neuroprotective, antioxidant, anti-inflammatory, antidiabetic)[33-36]. Sterols play an important role in lowering LDL cholesterol[37]. In addition, they show anticancer and antioxidant activities[38, 39]. The presence of these phytocompounds in the stems of *C. aralioides* could justify their various uses in traditional medicine

Table 3: Compounds identified by GC-MS in ethyl acetate dry extract

Peak	T_R (min)	Percentage (%)	Molecular formula	Molar mass (g/mol)	Fragmentation, m/z (%)	Identified compound	Reference
1	6.74	4.49	$C_{12}H_{16}O_2Si$	22	220$[M]^+$ (21), 205 $[M-CH_3]^+$(52), 147$[M-TMS]^+$ (78), 131 (8), 117(30), 103$[M-COOTMS]^+$ (30), 89(5), 73(100)	E-cinnamic acid	[19]
2	7.524	1.55	$C_{10}H_{14}O_2Si$	194	194(60), 179(100), 151(60), 135(10), 105(5), 89(22), 73(38)	4-Hydroxybenzaldehyde	NIST08.LIB
3	8.880	1.14	$C_{13}H_{22}O_3Si_2$	282	282(30), 267(100), 223(80) 193(45), 147(5), 103(2), 73(65),	p-hydroxybenzoic acid	NIST08.LIB
4	9.331	2.79	$C_{20}H_{32}O_4Si_2$	392	392(70), 377(10), 288(2), 267(90), 217(18), 179(8), 179(5), 129(2), 73(100)	3-methyl-2-butenyl caffeate	[23]
5	9.416	2.83	$C_{20}H_{28}O_2Si_2$	356	356(100),341(49),267(52),251(21),193(10), 178(5), 147(30), 103(15),73(90)	Pinosylvine (3,5-dihydroxystilbene)	[24]
6	10.044	1.67		481	481(48), 466(3), 355(80), 339(3), 281(10), 267(15), 134(5) ; 73(100)	Not identified	
7	10.470	1.70	$C_{16}H_{28}O_3Si_2$	324	324(5),309(1),288(5),235(3),217(50),205 (40);146(10),147(20),117(40),73(100)	E-Coniferol (4-hydroxy-3-methoxycinnamyl alcohol)	[25]
8	10.550	0.55		299	299(100), 284(0.5), 261(0.5), 204(1), 147(1), 75(40)	Not identified	
9	10.942	1.05	$C_{19}H_{40}O_2Si$	328	328(15),313(80),285(5),145(30)132(59), 117(100), 73(81)	Palmitic acid	NIST08.LIB
10	11.199	0.75	$C_{16}H_{30}O_4Si_3$	370	370(60), 355(30), 311(20), 281(10), 267(5), 193(100), 147(4), 73(75)	Protocatechic acid (3,4-dihydroxybenzoic)	NIST08.LIB
11	11.309	1.80	$C_{22}H_{32}OSi$	340	340 (10), 325 (15), 297(8), 265 (3), 250 (10), 221(1), 73 (100),	12-Hydroxysimonellite	[26]
12	11.410	0.63		357	357(10), 327(100), 267(12), 241(1), 177(5), 159(15), 75(42)	Non identified	
13	11.667	0.89	$C_{21}H_{42}O_2Si$	354	354(10),339(60),265(20),222(10),199(20),166(10),129(40), 117(80), 73(100)	(9E)-octadecénoic acid	NIST08.LIB
14	11.773	0.84	$C_{21}H_{44}O_2Si$	356	356(15), 341(85),313(5),293(5),201(15), 145(32), 132(52), 117(100),73(100)	Stearic acid	NIST08.LIB
15	12.120	1.18	$C_{30}H_{54}O_6Si_5$	650	650(15),383(10),368(100),355(40),294(	Catechin (2-(3,4-	[19]

					1), 267(10),249(2),179(5),147(1),73(60)	dihydroxyphenyl) chromane -3,5,7-triol)	
16	12.353	2.23	$C_{23}H_{36}O_3Si_3$	444	444(100), 429(5), 340(1), 251(1), 236(1), 207(2) 165(2), 147(2), 73(62)	Z-Resveratrol	[27]
17	12, 733	1.45		708	708(50),693(12),618(15),587(30),515(20),498(20),354(50),239(15), 209(15),73(100)	Not identified	
18	12.857	0.78	$C_{26}H_{44}O_4Si_4$	532	532(100), 517(10), 443(1), 429(1), 355(1), 192(1), 147(1), 73(65)	Z-Piceatannol (4-[(*Z*)-2-(3,5-dihydroxyphenyl)ethenyl]benzene-1,2-diol)	[28]
19	12.993	3.04	$C_{32}H_{56}OSi$	484	484 (45), 469 (10), 394(50), 379 (18), 355(12), 351 (10), 297 (30), 255 (32), 129 (55), 83(100).	Stigmasterol	NIST08.LIB [29]
20	13.784	2.87	$C_{32}H_{56}N_2O_4Si_2$	576	576(22), 561(8), 547(10), 531(2), 487(50), 486(100), 487(50), 398(35), 383(10), 368(12), 323(8), 264(5), 207(15), 73(65)	Corticosterone diéthoxime,	[26]
21	13.844	20.09	$C_{23}H_{36}O_3Si_3$	444	444(100), 429(8), 371(1), 341(1), 355(2), 341(2), 267(1), 251(0.5), 207(5), 191(0.5), 179(0.5), 147(4), 73(59)	E-resveratrol (E-3,5,4'-trihydroxystilbene)	[27]
22	13.923	7.42		461	461(7),394(1),365(1)337(1),309(1),282(0.5),267(1),239(1),211(6),169(6),113(20),57(100)	Not identified	
23	14.177	14.88	$C_{32}H_{36}OSi$	484	484(55),469(25),394 (5), 379 (15), 355 (10),343(100), 239(10), 255(75),129 (35),73(60).	23-Déhydrositosterol	[29]
24	14.313	8.21	$C_{29}H_{46}O$	410	410(15), 367(12), 312(3), 297(10), 282(2), 269(100), 245(15), 229(10), 147(15), 109(11), 81(30), 69(20)	α-Spinasterone	NIST08.LIB
25	14.730	3.36	$C_{26}H_{44}O_4Si_4$	532	532(100), 517(10), 443(1), 429(1), 355(1), 192(1), 147(1), 73(65)	E-Piceatannol (4-[(*E*)-2-(3,5-dihydroxyphenyl)ethenyl]benzenè-1,2-diol)	[28]

26	19.330	15.98	$C_{13}H_{22}O_3Si_2$	282	282(2), 267(100), 209(1), 193(1), 179(1), 163(1), 117(1), 73(20)	2,4- Dihydrobenzaldehyde	NIST08.LIB
27	19.580	4.68	$C_{13}H_{22}O_3Si_2$	282	282(1), 267(100), 251(1), 223(1), 193(1), 179(2), 165(1), 136(1), 73(20)	2,5-Dihydrobenzaldehyde	NIST08.LIB
28	22,343	7.88		798	798; 721; 707; 647; 619; 559; 455; 436 ; 431 ; 360 ; 344 ; 267 ; 179, 147 ; 73	Not identified	

(E)-resveratrol structure

(E) -resveratrol, isolated from AcOEt dry extract, is a light yellow powder. Its GC-MS profile (Figure 3A) showed a purity of 96.6 %, and a retention time of 13.797 min. Its mass spectrum (Figure 3B) showed a molecular peak at m/z 228.24 $[M+H]^+$, corresponding to the crude formula $C_{14}H_{12}O_3$.

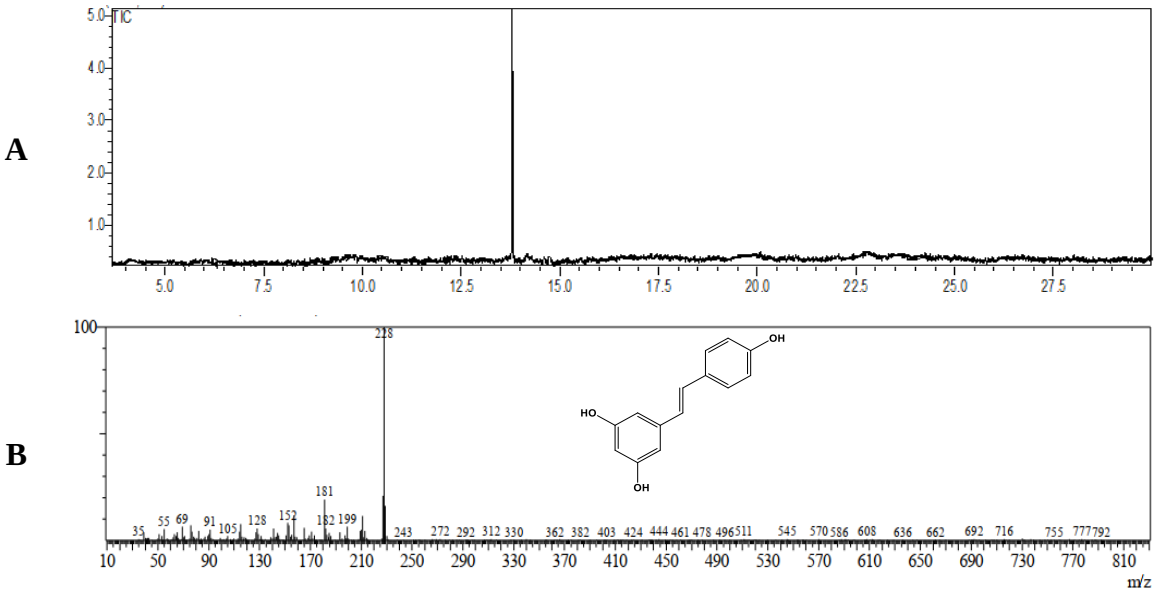

Figure 3: GC-MS chromatogram (A) and (E) -resveratrol mass spectrum (B)

The ^1H NMR spectrum revealed 2 ethylenic protons [δ_H7.04 (1H, d, J = 16.3 Hz, H_C), δ_H 6.90 (1H, d, J = 16.3 Hz, H_D), whose constant coupling $^3J_{HH}$ indicates that the double bond is of configuration E (Figure 4)**.**

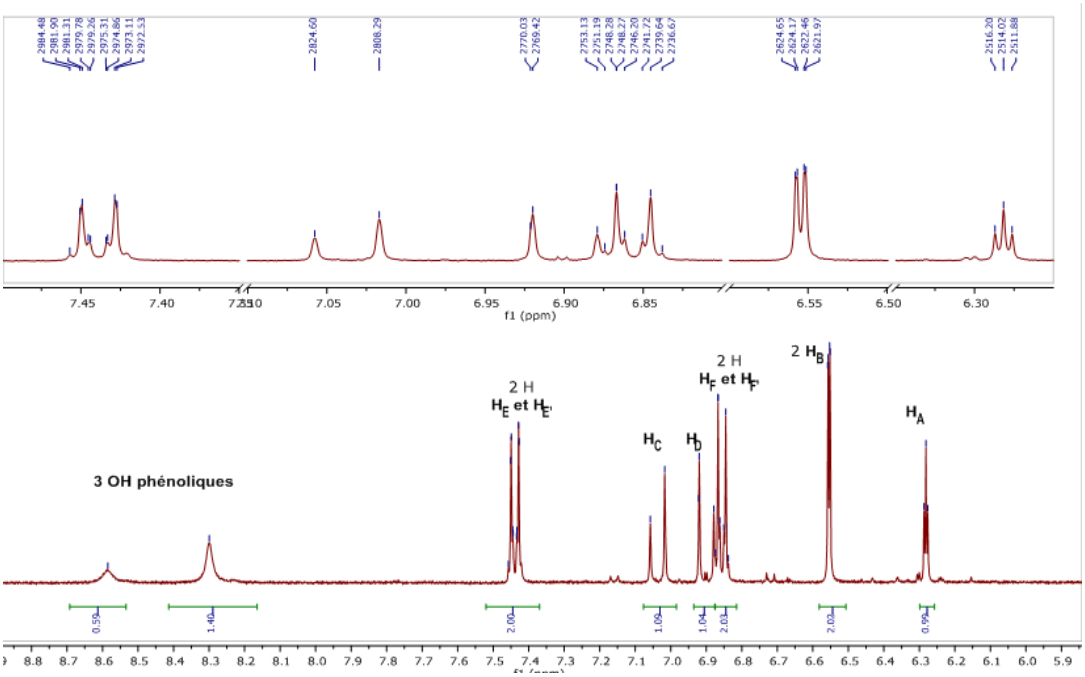

Figure 4: ^1H NMR spectrum of (E)-resveratrol

The signals of the seven aromatic protons constitute two independent systems. The first consists of the two groups of complex signals [δ_H7, 46-7, 42 (2H, m system AA'XX ', H_E) and δ_H 6, 88-6.82 (2H, m system AA'XX', H_F). Depending on the nature of the signal and the large difference in chemical shift, the signals are assigned to the protons of the para-disubstituted cycle. The second aromatic system also consists of two groups of protons [δ_H 6.55 (2H, dd, J = 2.2 and 0.5 Hz, H_B), δ_H 6.28 (1H, t, J = 2.2 Hz, H_A). This motif is compatible with a trisubstituted aromatic system, symmetrical and particularly rich in electrons. It can be noted that there is a weak coupling constant between the HB signals corresponding to a distant zigzag coupling, indicating the presence of a plane conjugate system. Finally, 2 broad singlets [δ_H 8.58 (s, 1H, OH), δ_H 8.30 (s, 2H, OH) represent the 3 protons of the phenolic OH of resveratrol (Figure 5).

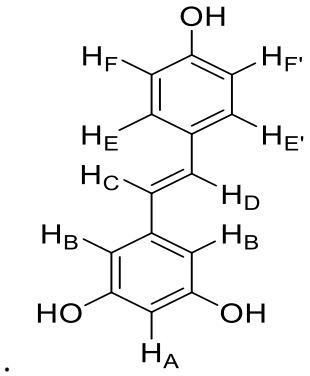

Figure 5: (*E*)-resveratrol molecular structure

Resveratrol is the best known stilbene. It is a phytoalexin from the vine[34]. It manifests anti-inflammatory and anticancer properties. Resveratrol is considered as a powerful antioxidant whose potential has been linked to beneficial effects in cardiovascular disease. Finally, it would have anti-aging properties, and could prevent certain neurodegenerative diseases[34, 36, 40].

CONCLUSION

Medicinal plants virtues depend on its mineral and organic composition. The mineral and GC-MS profile of *C. aralioides* was established and (E) - resveratrol was isolated at the first time in this study. The presence of secondary bioactive principles and minerals seems to explain its medicinal properties. The results obtained support the use of the plant in traditional medicinal practices to treat several diseases. *C. aralioides* can serve as an isolation matrix for phytoconstituents of interest.

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