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Isolation and structural elucidation of two new compounds Elieaxanthone and Elieaflavonone from *Eliea articulata* Cambess (Clusioid Clade, Family Hypericaceae, and Tribe Cratoxyleae) originated from Madagascar

Fatiany Pierre Ruphin, Robijaona Baholy, Fiatoa Barthelemy, Raharisololalao Amelie, Marie-Therese Martin, Koto-te-Nyiwa Ngbolua

Abstract
From the root bark of *Eliea articulata* Cambess (Hypericaceae), a medicinal plant species endemic to Madagascar, two new compounds typical of Xanthone and flavonone named *Elieaxanthone* and *Elieaflavonone* containing two membered rings in its side chain were isolated by repeated silica gel column chromatography. Their structures were determined by 1D and 2D NMR spectroscopy and spectroscopy High-resolution MS. The results of the present research work is reported for the first time and revealed that a comparative study of the clusioid clade will help to understand the origin and maintenance of diversity among this clade and their implication at Pharmacognosy level (biotaxonomy).

Keywords: Spectroscopic techniques, Phytochemical markers, Biotaxonomy, Clusioid clade, Madagascar

1. Introduction
The island of Madagascar, located in the Indian Ocean, presents all the characters of a small continent. Its flora is of an interest because of his diversity and its very great richness. The current Malagasy flora is marked by the persistence of kinds and very antiquated species belonging to only known families with the state of fossils on the other continents. Madagascar constitutes one of the most important biodiversity hotspots worldwide with more than 90% of its plant species being endemic. This rate of endemism is besides raised on all the taxonomic levels, eight families are regarded as being entirely endemic of the island [1-4].
The plant known under the vernacular name Hela (Malagasy name), and scientifically named *Eliea articulata* Cambess (Hypericaceae) is endemic of the South-eastern part of Madagascar [5-7]. During our ethnobotanical field work, we have been told that the root bark decoction of *Eliea articulata* Cambess is used by the local communities to treat fever and incurable wounds. This plant is well-known and very important recipe in this region because of its therapeutic values in the Malagasy traditional medicine. Extensive phytochemical studies have shown that *Eliea articulata* Cambess is rich in a variety of phenolic compounds especially flavonoids and xanthones [8, 9].
According to available literature, no phytochemical research investigation has been carried out on this plant species. As part of our phytochemical work on Madagascar medicinal plants, we investigated the root bark of this plant. In the present paper, we report for the first time the isolation and structure elucidation of two new compounds from *Eliea articulata* Cambess which have been trivially named Elieaxanthone (fig.1) and Elieaflavonone (fig.3).

2. Materials and Methods
2.1. General
Silica gel 60 and 100, and TLC precoated plates were purchased from Merck, Darmstadt, Germany, Analytical HPLC was performed on a Waters system (Millenium 32 workstation, 2690 separation module, 996 photodiode array detector) equipped with HiChrom Lips 100-5-250D column (4.6 x 250nm: LiChrospher Phase 5 µm, Si 100). A Perkin-Elmer 241 polarimeter was used for measurement of optical rotation. The 1D and 2D vasoconstrictive, hypertensive, and cardiac stimulant action [Beyaoui, *et al*, 2012] [6] and can act as an allergenic substance [Nofal, 2004; Assarehzadegan, 2009] [21, 4].

Salsola species have antioxidant and anti-inflammatory properties [Ahlam & Fatma, 2007] [2]. Alkaloid experiments were performed at 600 MHz and 400MHz for ^{1}H and ^{13}Crespectively, on a Bruker Avance 600MHz instrument equipped with an Ultra Shield Plus magnet and triplet resonance cryoprobe with gradient unit. Sample temperatures were stabilized at 298 K.

The deuteriomethyl^{13}C signal and the residual ^{1}H signal of the solvent (DMSO-D_6) were used as secondary references (δ39.6and δ2.49 from tetramethylsilane, respectively). The exact molecular mass (HRMS) of molecular ion $[M-H]^+$ and fragment F^+ were determined with a Micromass QTof-2 mass spectrometer equipped with an electrospray ion source and a Micromass QTof-2JOEL mass spectrometer equipped with a Direct Analysis in Real Time (DART) atmospheric pressure ion source.

2.2. Plant material

The root bark of *Eliea articulata* Cambess (Hypericaceae) was collected in Lanirano at nearly 12 km from Fort-Dauphin city, Anosy's Region, in the South-eastern part of Madagascar. The plant sample was identified by Benja Rakotonirina, a botanist from the Malagasy Institute of Applied Research, and by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number FDU-034 was deposited at the Herbarium of the Laboratory of Applied Chemistry, Layflaylle Street, University of Toliara, Madagascar.

2.3. Extraction

The plant material (6 kg) of *Eliea articulata* Cambess (Hypericaceae) was kept at room temperature (25 – 30 °C) for air drying (3 weeks). The air dried and powdered root bark of *Eliea articulata* Cambess (1 Kg), were extracted by repeated maceration with ethanol 90° at room temperature (2x 3l, 72 h). The filtered solvent was evaporated under vacuum to afford a crude ethanolic extract (33, 27 g). The residue (30 g) was suspended in water and was partitioned by successive with n-hexane, methylene chloride, ethyl acetate and n-butanol to yield the corresponding soluble extracts.

2.4. Isolation

Ten (10) grams of the methylene chloride extract was first subjected to fractionation using silica gel column chromatography eluted with methylene chloride and a gradient of methanol which resulted into five fractions (F1-F5). The fraction was selected for the following steps. The fraction F2 was checked for its purity by analytical TLC, and the zones were detected both with a UV lamp at 254 nm and 365 nm and by spraying with sulfuric vanillin acid, followed by heating at 120 °C during 1-5 min. The fraction F2 was resubmitted to silica gel column chromatography. The elution was done using cyclohexane and a gradient of ethyl acetate, which resulted into six fractions and 160 mg of F24 was subjected to further purification using a silica gel column chromatography, with hexane and a gradient of ethyl acetate for elution. The latter provided two pure compounds. The purity of Elieaxanthone and Elieaflavonone was then detected by analytical HLPC with the mixture of chloroform and methanol 1:1 (v/v) as mobile phase, and the chromatography was performed with isocratic regime during 25 min. The eluted compound was detected based on its UV absorption in the wavelength range from 190 nm to 315 nm. The purity was respectively 97.98% at λmax=213 nm and [α] = + 22.4° (Eliaxanthone) and 99.92% at λ_{max}=273 nm (1,403), 204 nm (0,73) and [α] = + 50.4 (Eliaflavonone).

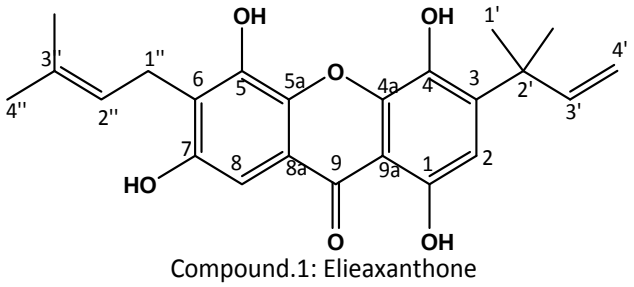
3. Results

The root bark powder of *Eliea articulata* Cambess collected from Lanirano Fort-Dauphin (Madagascar), was extracted with ethanol 90°. The ethanolic crude extract was suspended in water and was partitioned successively with different organic solvents of croissant polarity (Hexane, methylene chloride, ethyl acetate and n-butanol) in order to yield with the corresponding soluble extract.

Repeated silica gel column chromatography of the methylene chloride-soluble extract led to the isolation of Elieaxanthone and Elieaflavonone, in pure forms as proved by HPLC analysis.

The molecular formula of compound 1 was determined to be $C_{23}H_{24}O_6$ by HREISM (m/z = 395.0 $[M-H]^+$,calculated), and 1D,2D-NMR experiments. Its ^{1}H-NMR spectrum exhibited, their four singlets at δ0.99, δ1.25, δ1.65, and δ1.85, characteristic attributed to four methyl groups, and two doublet-doublet between δ3.45 and δ3.65, characteristic attributed to the signals of genime, protons of different chemical environments of the methylene group. Five signals alcens protons between δ5.01(d), δ5.25(m), δ6.01 (d) and two singlets at δ6.45 and δ7.35, attributed to the characteristic of signals alcens proton typical for benzene skeleton, and in the presence of the three hydroxyl protons between δ8.25, δ11.95 and δ12.35 typical for phenolic at the end implying that the compound 1 is di-O-Substituted Xanthone.

Regarding the range of multiplicity, the signals of alcens protons between δ5.01 (d), δ5.25(m), δ6.01 (d) (region of the linear alcens groups) indicate that these alcens protons signals are characteristic The presence of linear chain alcen proton in the compound 1 was indicated by the peaks in this region and their information of range multiplicity.



Compound.1: Elieaxanthone

Fig 1: Structure of compound 1

The 1D ^{13}C broad band-NMR spectrum contained 23 signals of the carbons indicating 13 signals correspond to the carbons of typical Xanthone [10, 11] skeleton including the carbonyl group between δ185.51, is not symmetry in the molecule and 10 signal carbons attributed to the linear chain of alcens groups are present in the compound1.

Examination of 1D ^{13}C and the 2D HSQC spectrum data of the compound 1 revealed that of about 16 alkene carbons (C=C) double bonds indicating two (2) shielded aromatic methine groups at δ95.11 (C-8) and δ129.10 (C-2), ten (10) quaternary carbons of which the characteristic are attributed to the typical carbons of benzene skeleton at δ102.70 (C-5a), δ108.51 (C-4a), δ109.20 (C-6), δ126.45 (C-1), δ139.93 (C-3), δ145.06 (C-4), δ147.51 (C-9a), δ156.40 (C-8a), δ160.01 (C-5) and δ164.02 (C-7), four carbons characteristic attributed to linear chain of

alkene groups between one methylene group atδ115.50(C-4'), two methine groups at δ121.61 (C-2') and δ143.43 (C-3') and quaternary carbon at δ137.13 (C-3'').

In addition to the examination of the 1D ^{13}C and the 2D HSQC spectrum that permitted to reveal the presence of the four methyl groups at δ18.61 (3''-CH_3), δ26.50 (C-4''), δ28.31 (C-1') and δ28.37 (3'-CH3) at the end one carbon quaternary δ40.06 (C-3''). The ^{1}H and ^{13}C chemical shift values of individual spin system were determined by correlation in the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by ^{1}H-^{1}H COSY spectrum and 2D HSQC an HMBC correlation spectra are shown respectively in table1as well the intra-space correlation in the 2 ^{1}H-^{1}H NOESY spectrum.

Intra-space Relevant NOESY correlations for the major rotamer of Elieaxanthone are displayed in figure 2. To the best of our knowledge; this is the first time that a membered ring occurs in the side chain of a typical Xanthone.

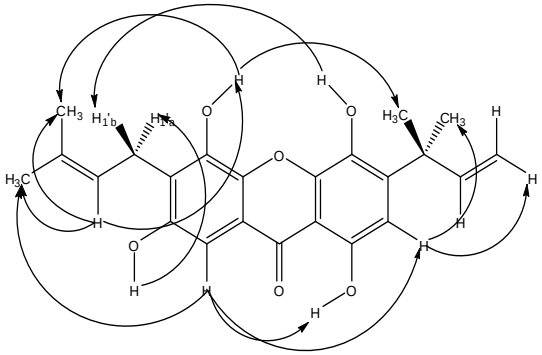


Fig 2: Long-range correlation ^{1}H-^{1}H NOESY

Table1: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H (COSY, NOESY) and important HMBC correlation of Eliaxanthone

Position	1D-NMR experiments		2D-NMR experiments		
			Homonuclear Correlations(^{1}H-^{1}H)		Heteronuclear Correlation
	δ ^{1}H	δ ^{13}C	COSY	NOESY	HMBC
1	11.65 s	126.4			C-2 and C-9a
2	7.35 s	129.1		2'-H, H-8 and H-4'	C-2' ; C-3 ; C-4;C-9a
3		139.1			
4	11.95 (OH) s	145.0		H-1''b	C-3, C-4a
4a		108.5			
5	12.35 (OH) s	160.0		H-3'', 3''-H and H-1'	C-5a , C-6
5a		102.7			
6		109.2			
7	8.25 (OH) s	164.0		H-1''a	C-6
8	6.45 s	95.1		H-4'',H-2	C-5a ; C-6; C-7; C-8a , C-9
8a		156.4			
9		185.5			
9a		147.5			
1'	0.99 s	28.31			C-3; C-2'; C-3', 2'-CH_3
2'		40.6			
3'	6.015 t	147.5	H-4'		C-3; C-2', C-4'
'4	5.015 d	115.5	H-3'		C-2', C-3'
2'-CH3	1.25 s	28.37			C-3; C-1'; C-2',C-3'
1''	3.45 ($H_{1''a}$) dd	21.6	H-2'' ; $H_{1''b}$		C-5; C-6; C-7; C-2'', C-3''
	3.75 ($H_{1''b}$) dd		H-2'' ; $H_{1'a}$		
2''	5.25 m	121.6	$H_{1'a}$; $H_{1''b}$	H-5(OH), 3''-H, H-4''	C-1'', C-3''
3''		137.1		3''-H, H-4'' and H-5	
3''-CH3	1.65 s	18.6			C-2'' , C-3''
4''	1.85 s	26.5		H-2'' and H-8	C-2'' , C-3''

The isolation of compound 2, showed a quasi-molecular ion at m/z= 338.0148 $[M+H]^{+}$, observed in the High-Resolution EIS-MS spectrum which corresponds to the molecular formula $C_{20}H_{18}O_5$.

UV analysis results shown that the absorption band was at λ_{max} 273 nm (1.403) and at 204 nm (0.73), acharacteristic indicating the presence of one dihydroxyfavonone [6]. The information of the basic structure of compound 2 concerning the presence of dihydroxyfavonone was confirmed by IR analysis, because the IR spectra data is between λ = 1619Cm^{-1} and at 3475 Cm^{-1}, respectively indicating the presence of one ketone group and their hydroxyl functions in the compound 2.

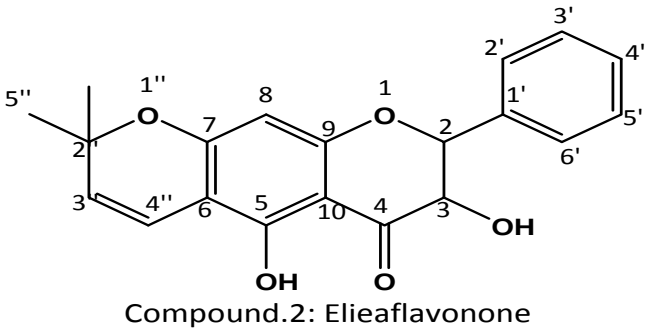


Fig 3: Structure of compound 2

Examination of the 1D ^{1}H-NMR spectra data of the compound 2 revealed of the presence one singlet signal at δ1.43 (H-5''), corresponding to the integration attributed at six protons of the two methyl groups, characteristic indicating acid lonchocarpic, integrated in one 2, 2-dimethyl-cromine cycle [3], two doublet signals respectively at δ4.53 (*J*=12Hz, H-3), and δ5.04 (*J*=12Hz, H-2), attributed to characteristic of the proton dihydroxyfavonone and the presence two alkene proton signals at δ5.53 (J= 7.2 Hz, H-4'') and at δ6.62 (J=7.2Hz, H-3'') attributed to the protons of dimethyl-pyrane.

In addition of the examination of the 1D ^{1}H-NMR, permitted to reveal the presence of four aromatic protons, indicating one of the singlet signal at δ5.96 (H-8), attributed to the aromatic proton in the cycle A, and three alkene protons with one doublet at δ7.53 (J= 7.2 Hz, H-2'), and two pseudo-triplet respectively atδ7.44 (J= 7.2 Hz, H-3') and δ7.35 (J= 7.2 Hz, H-4'), characteristic of the symmetry having the typical protons of the benzene skeleton.

The 1D ^{13}C spectrum contained 20 signal carbon atoms

Examination of the 1D ^{13}C broad band and 2D HSQC-spectra data revealed of about 15 signals corresponding to the carbons of typicalflavonone skeleton, respectively indicating five alkene carbons (C=C), double band signals at δ127.4 x2 (C-2' and C-6'), δ128.3 x2 (C-3' and C-5') and δ128.5 (C-5'), characteristic attributed to the carbons of typical benzene skeleton corresponding to one system AA',BB' formed of ortho coupling, three signals of carbons, a characteristic of methine groups at δ72.36 (C-3), δ83.27 (C-2) and δ96.75(C-8) correspond to the carbons in the cycle a dihydroxyfavonone and at the end seven quaternary carbons at δ100.4 (C-10), δ116.18 (C-6), δ136.1 (C-1'), δ157.7 (C-5), δ162.3 (C-9) and δ162.8(C-7), including the carbonyl group assigned to C-4 resonance at δ195.7.

In addition to the examination of 1D ^{13}C broad band and 2D HSQC-spectrum permitted to reveal the presence of five carbon signals attributed to two methyl at δ28.92 x2 (2'-CH_3 and C-5''), two methine groups at δ114.96 (C-3'') and δ126.62 (C-4'') and at the one quaternary carbon δ78.64 (C-2'') attributed to characteristic of the typical carbons of dimethyl-pyrane substitute pattern of A-ring in the basic structure of flavonone integrated at the C-6 and C-7. The ^{1}H and ^{13}C chemical shift values of individual spin system were determined by correlation in the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by ^{1}H-^{1}H COSY spectrum and 2D HSQC an HMBC correlation spectra are respectively shown in table 2. The compound 2 was identified as 3, 5-dihydroxy-5'', 5''-dimethyl-2-phenyl-2, 3-dihydropyrano [3, 2 *g*]-Chromen-4 [*8H*], it is named Elieaflavonone.

Regarding the mass spectra obtained using to the high resolution ESI-MS analysis permitted to confirm the chemical structure of compound 2. In addition to fragments F^{+} of molecular ion identified by mass spectrum data of about three (3) peaks of molecular ions parents respectively at m/z = 323, indicated to the methyl group parted of the dimethyl-pyrane, the peak at m/z= 203 attributed to 6-formyl-5-hydroxy-2-methyl-*2H*-cChromen-7(6H)one observed at the retro Diels-Alder mechanism of heterocycle in the cycle A has confirmed the presence of dimethyl-pyrane substituted moiety in the cycle A, assigned at C-6 and C-7, and at the end, the peak at m/z = 177 attributed at 3, 5-dihydroxychroman-4one, is obtained for the intra-molecular rupture of the liaisons on α position of the oxygenic function, the ethylenic function and on α position of benzene cycle B. The fragments mechanism molecular was assigned to fig.4.

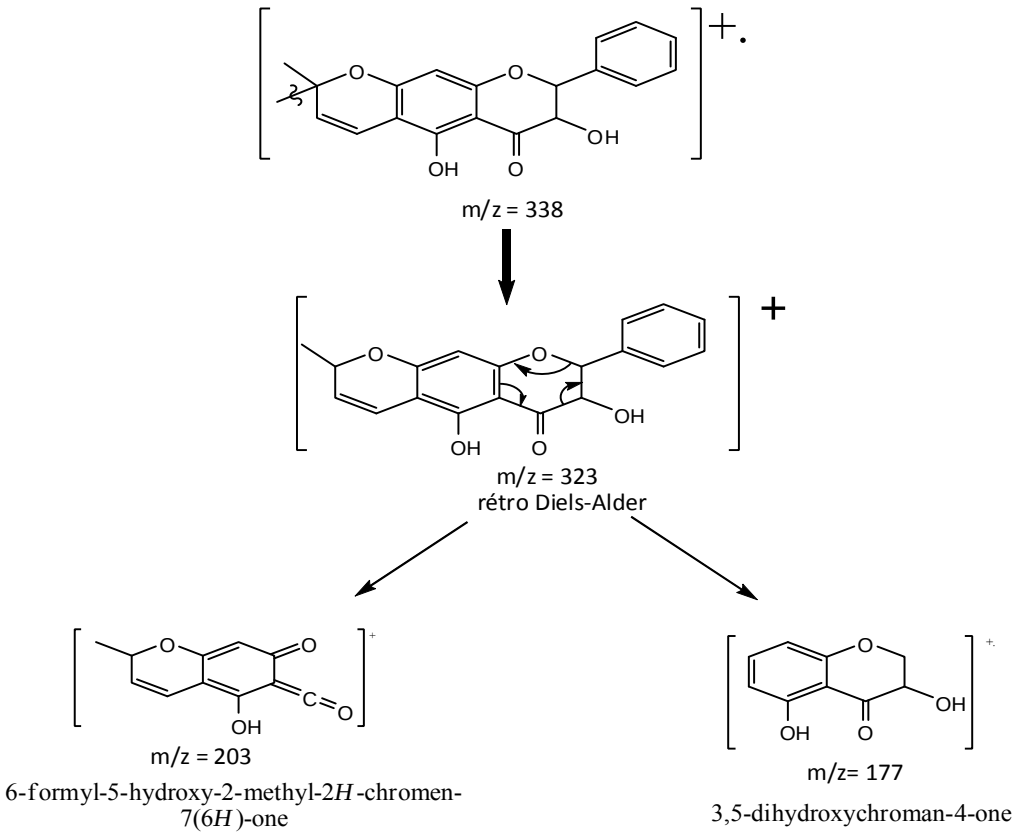


Fig 4: The fragments important for the structure elucidation of Elieaflavonone.

Table 2: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H and important HMBC correlation of Eliaflavonone

Position		1D-NMR experiments		2D-NMR experiments	
				Homonuclear Correlations (^{1}H-^{1}H)	Heteronulcear Correlation
N°	Types	δ ^{1}H	δ ^{13}C	COSY	HMBC
2	CH	5,04 d (*J= 12 Hz*)	83,27	H-3	C-1' ;C-3 and C-4
3	CH	4,53 d (*J= 12 Hz*)	72,36	H-2	C-1' ;C-2 and C-4
		-OH 3,43 s	-	-	C-3 and C-4
4	Cq	-	195,7		
5	C	-11,43 (OH) s	157,7		C-6 and C-10
6	Cq	-	116,18		
7	Cq	-	162,18		
8	CH	5,96 s	96,75		C-7 ;C-6 ;C-9 and C-10
9	Cq	-	162,3		
10	Cq	-	100,4		
1'	Cq	-	136,1		
2'	CH	7,53 d (*J= 7.2 Hz*)	127,4	H-3'	C-2 ; C-4' and C-6'
3'	CH	7,44 Pt (*J= 7.2 Hz*)	129,3	H-2' ; H-4'	C-1' and C-5'
4'	CH	7,35 Pt (*J= 7.2 Hz*)	128,6	H-3' and H-5'	C-2' and C-6'
5'	CH	7,44 Pt (*J= 7.2 Hz*)	129,3	H-4' ; H-6'	C-1' and C-3'
6'	CH	7,53 d (*J= 7.2 Hz*)	127,4	H-5'	C-2 ; C-2' and C-4'
2''	Cq	-	78,64		
3''	CH	6,62 d (*J= 12 Hz*)	114,96	H-4''	C-2'' ;C-4'' and C-6
4''	CH	5,53 d (*J= 12 Hz*)	126,62	H-3''	C-2'' ;C-3'' ;C-5 ; C-6 and C-7
5''	2 xCH_3	1,43 s	28,92		C-2'' and C-3''

4. Discussion

Secondary metabolites of pharmacological relevance can serve as biomarkers and could be used to ascertain plant identity and their presence is of particular important for chemical authentication of plant samples. It was reported that the presence or absence of such compounds is of particular interest in the field of pharmacognosy. Their presence indicates that particular biosynthetic pathways have been conserved within a taxon, or alternatively, have arisen two or more times within a taxon through evolutionary convergence.

The clusioid clade is phytochemically unique within the Malpighiales due to the shared possession of xanthones, compounds related to flavonoids with elements derived from both acetate and shikimate pathways, by its members [12]. Although these phytochemical markers are uncommon in *Eliea articulata* suggesting that the oxygenation and prenylation patterns of xanthones in this plant species could have potential chemotaxonomic value due to their variability.

The clusioids are a clade of flowering plants in the large rosid order Malpighiales [13]. This well-supported clade contains five families [14] representing 94 genera and ~1900: Bonnetiaceae, Calophyllaceae, Clusiaceae, Hypericaceae, and Podostemaceae. Several species have pharmacological activity and are potentially useful for the treatment of tumors, depression and AIDS [15]. The clusioids offer a unique opportunity to study the biogeography of tropical angiosperms with Gondwanan distributions because they are of ancient origin and possess a pantropical distribution. Indeed, various clusioid clades date back to Gondwanan times when Africa and Madagascar were in close proximity [16]. So, the results of the present research work revealed that a comparative study of the clusioid clade will help to understand the origin and maintenance of diversity among this clade and their implication at Pharmacognosy level (biotaxonomy).

5. Conclusion

Two new compounds, typical of Xanthone and flavonone named as Elieaxanthone and Elieaflavonone were isolated from the methylene chloride-soluble extract from the root bark of *Eliea articulata Cambess* (Hypericaceae), collected in Lanirano Fort-Dauphin, in the South-eastern part of Madagascar. The structures of isolated new compounds were identified as Elieaxanthone and Elieaflavonone on the basis of spectroscopic methods (1D, 2D –NMR, HREIS-MS, IR and UV).

6. Acknowledgements

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