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Research Paper

Antioxydant activity, isolation and structural elucidation of two new bioactive compounds typical of isobenzofuro-[2,3*c*]-Xanthone named Chiovisobenzofuroxanthone-A and Chiovisobenzofuroxanthone-B from aerial parts of *Cymbopogon giganteus Chiov var. madagascariensis* (POACEAE)

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Abstract
The Cymbopogon giganteus (Hochst.) Chiov. var. madagascariensis (Poaceae), known by the vernacular name "Verompoly," is a plant with therapeutic properties that is widely recognized in western and southwestern Madagascar. According to ethnobotanical studies conducted in western Madagascar, the aerial parts of this plant are used by the local population to treat several diseases, including high blood pressure, general fatigue, infectious diseases, and fever.
The objectives of this study were threefold: to verify the ethnomedical data on this plant using scientific methods, to isolate the bioactive principles, and to elucidate their chemical structures.
The application of the cold maceration extraction technique, followed by partition extraction, enabled the identification of the active extract. The antioxidant activity of various extracts (n-hexane, chloroform, ethyl acetate, and n-butanol) from the most frequently cited plant, C. giganteus (Hochst.) Chiov. var. madagascariensis, was tested on 2,2-diphenyl-1 picrylhydrazyl(DPPH) assay method using UV spectrometry, showing that the ethyl acetate extract has very interesting antioxidant activity with IC50 = 09.21±0.04 µg/ml (n=6). Bioguided fractionation of the ethyl acetate extract led to the isolation of two pure antioxidant products, designated PG-01 and PG-02, with respective IC50 values of 07.11±0.04 µg/ml and 04.31±0.04 µg/ml. The chemical structures of these two compounds were elucidated using spectral analysis methods such as NMR (1D and 2D), HREIS-MS, IR, and UV. These compounds were identified as isobenzofuro-[2,3c]-xanthone derivatives and were designated as Chiovisobenzofuroxanthone-A (PG-01) and Chiovisobenzofuroxanthone-B (PG-02), respectively. These novel molecules have been isolated for the first time in the Cymbopogon genus.
The findings of this study are being presented for the first time, thereby confirming the traditional uses of the plant in question and demonstrating that Malagache medicinal plants have particular potential, particularly with regard to bioactive molecules. These findings offer hope for the discovery of a new precursor for drug development.

Keywords: *Ethnomedicine, C. giganteus (Hochst.) Chiov., bioactives compounds, antioxidant activity, Chiovisobenzofuroxanthone-A and Chiovisobenzofuroxanthone-B.*

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I. Introduction

Madagascar is the fourth largest island in the world, boasting an exceptional ecosystem and an extremely diverse flora, with an endemism rate of around 85% of listed plants found nowhere else. This

significant plant endemism is a valuable resource for research into new bioactive molecules derived from medicinal plants. It also contributes to the preservation of this flora, which is a significant heritage asset [1-10].

The empirical use of these medicinal plants has already been studied [11-15], but given the rate of endemism, there is still a great deal of work to be done. Studies on plant-based natural substances have revealed a significant number with strong biological activity [16-17]. These substances can serve as precursors or models for developing drug candidates. Furthermore, plants play critical roles in human society, particularly within traditional medicine systems. According to the World Health Organization, two-thirds of African and Asian women use plants to treat their families, and human dependence on plants continues to grow [19-24].

Ethnobotanical surveys conducted in the Menabe region of western Madagascar have revealed that the aerial parts of the plant known locally as "Verompoly" in Malagasy and scientifically named *Cymbopogon giganteus (Hochst.) Chiov. var. madagascariensis* has several therapeutic properties. Local populations use this plant to treat a number of illnesses, particularly high blood pressure, general fatigue, infectious diseases, and fever. The Cymbopogon genus is well-known on the African continent for its therapeutic properties and scent. It is used in traditional African medicine to treat various illnesses, most often on its own and sometimes in combination with other plants [25-36].

This prompts the question of how to leverage the untapped potential of traditional medicinal plants to address current public health challenges. We chose to take an in-depth scientific approach, combining Phytochemistry and biology, to scientifically justify and validate the traditional uses of *Cymbopogon giganteus Chiov var. madagascariensis* (Poaceae).

The objective of this project is to isolate the antioxidant principle(s) from the plant's aerial parts using bio-guided fractionation methods and combined chromatography analysis techniques. The goal is to elucidate the chemical structures of these isolated active principles using spectroscopic methods and to scientifically demonstrate the traditional uses of this plant.

II. Materials and methods

2.1. Selection and collection of the plant

The ethnobotanical data on the plant known by the vernacular name Verompoly, which was collected from traditional healers, traditional practitioners, and traditional therapists during field surveys conducted in the southwestern and western regions of Madagascar, prompted me to select and scientifically study this plant.

The aerial part of this plant was collected in the village of Ankidona, Anontsibe-Centre commune at nearly 18 km from Manja district, Menabe's region in the Western part of Madagascar, on July 20, 2022. The plant sample was identified by MBOLA Versène Balzaque, a botanist-teacher-researcher at the University of Toliara and by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo, 101Antananarivo- Madagascar. The reference specimens with assigned sample number CG-01, was deposited at the herbarium of the Laboratory of Applied Chemistry at the Faculty of Science and Technology, Layflaylle Street, University of Toliara, Madagascar.

2.2. Extraction and preliminary separation

Three kilograms (3kg) of the aerial parts of *C. giganteus madagascariensis* were collected, dried, and then ground into a powder using a grinder. 2.5 kg of this powder was extracted by cold maceration using a water-ethanolic mixture (20/80) three times (3x5 hours). Following filtration, the solutions obtained were evaporated to dryness under reduced pressure using a rotary evaporator. The extracts were then dried with Na_2CO_3, yielding crude extract of 44.12 g.

40 g of the crude extract was recovered using demineralized water, with the addition of 5 microliters of methanol. The resulting solution was then separated using immiscible organic solvents of increasing polarity, starting with n-hexane (3 x 200 milliliters). After decanting, the aqueous solution obtained was recovered with chloroform (3 x 200 ml), followed by ethyl acetate (3 x 200 ml) and n-butanol (3 x 200 ml). All corresponding solutions obtained were evaporated to dryness and dried with Na_2CO_3. Four distinct extracts were obtained according to their polarities. All extracts were stored at a temperature of 4°C, ensuring optimal freshness and quality.

2.3. Phytochemical screening[37-38]

The first step in the phytochemical study of a plant is to determine the main classes of secondary metabolites that it has developed during its evolution. This information is crucial for chemical analysis because it allows the extraction method to be selected in order to avoid molecular degradation and artifact formation, and to focus on the differences in chromatographic analyses used during the isolation of active ingredients. The subsequent step involves comparing the results of phytochemical screening with ethnobotanical and chemotaxonomic data. This allows us to hypothesize about the basic structure of the active ingredient.

Phytochemical screening is a qualitative chemical analysis that utilizes color reactions or precipitation patterns. These reactions are more or less specific to the chemical classes present in the plant matrix.

a. Screening for Alkaloids

10g of plant powder were macerated in water that had been acidified with 5% HCl for a period of five minutes. The mixture was then filtered. The resulting solution was divided into equal volumes in four test tubes:
The first tube served as a control, the second tube had four drops of Mayer's reagent added, the third tube had four drops of Wagner's reagent added, and the fourth tube had four drops of Dragendorff's reagent added. The appearance of precipitation or flocculation with the three reagents would indicate the presence of alkaloids.

Table 1: Reagents used to detect alkaloids in the sample

Reagents	Chemical formula
WAGNER	I_2/KI
VALSER-MAYER	$HgCl_2$
DRAGENDORFF	$(Bi(NO_3))_3$/KI

b. Saponins screening

This is a foam test. Approximately two grams of plant powder is macerated in 10 ml of distilled water. The mixture is then shaken vigorously for one minute. The tube is then placed vertically to measure the variation in foam height in the interval between five and thirty minutes after shaking. If the foam height exceeds three centimeters after thirty minutes of rest, this indicates the presence of saponins.

c. Screening for Anthraquinones

The presence of anthraquinone chemical families was identified using the BORNTRAGER test. To do so, a volume equivalent to 1 g of the hydro-alcoholic extract was evaporated, followed by the addition of 30 mL of distilled water. The solution obtained was then subjected to liquid-liquid extraction using 10 milliliters of benzene. After decanting, the benzene phase obtained was transferred to a test tube. Then, 5 milliliters of 25% NH4OH was added, and the mixture was stirred for one minute using a vortex mixer. The presence of a red color in the alkaline phase (lower phase) indicates the presence of anthraquinones.

d. Screening for Unsaturated Sterols and Triterpenes

Two methods were used to detect the presence of unsaturated sterols and triterpenes in the plant matrix: the LIBERMAN-BRUCHARD test and the SALKOWSKI test.
First, the dry residue was dissolved in 3 milliliters of chloroform. Following the stirring process, the solution was allocated into three test tubes: the first served as a control, the second was utilized for the LIBERMAN-BRUCHARD test, and the last was designated for the SALKOWSKI test.

❖ **Liberman-Bruchard Test**

Two drops of acetic anhydride are added to the second tube and gently shaken. Then, a few drops of 36N sulfuric acid are added. Observe the color change for one hour. The presence of a greenish hue signifies the existence of steroids, while the appearance of a red or purple ring indicates the presence of triterpenes. Both results can be observed simultaneously.

❖ **Salkowski test**

Tilt the third tube, then add one milliliter of 36N sulfuric acid. The appearance of a red ring at the interface is an indication of the presence of unsaturated sterols.

e. Screening for Flavonoids and Leucoanthocyanins

The WILSTATER test is used to perform phytochemical screening for flavonoids, while the BATTE-SMITH test is used to screen for leucoanthocyanins. The dry residue was dissolved in ten milliliters of 80% ethanol. The solution obtained is then divided into four test tubes. The first tube serves as a control, and the three tubes containing 3 ml of solution each will be used for the aforementioned tests.

❖ **Wilstater test**

The second tube was added with 0.5 ml of 12N HCl and magnesium turn, and the previous protocol was repeated for the third tube, but with the addition of one milliliter (1 ml) of distilled water and 1 ml of isoamyl alcohol. After 10 minutes, the appearance of a red color change in the second tube indicates the presence of flavones, while the change to a red-purple or purplish-red color in the third tube indicates the presence of flavonols or flavonones.

❖ **Batte-smith test**

In the fourth tube, add 0.5 milliliters of concentrated HCl (12N). Then, heat the resulting solution in a water bath at 100°C for thirty minutes. The presence of leucoanthocyanins is indicated by the appearance of a red color after cooling the solution.

f. Screening for Tannins and Polyphenols

The dry residue from evaporating the extract of the plant to be tested is dissolved in 3 milliliters of distilled water heated to 40°C. The mixture obtained is then divided into test tubes. The first of these is used as a control, while the other three are used for the following three tests.

❖ **Gelatin Test**

Four drops of 1% gelatin by mass per volume are added to tube number 2. The appearance of white flocculation indicates the presence of water-soluble catechin-type tannins.

❖ **The salted gelatin test**

Four drops of salted gelatin (10 g of NaCl in 100 ml of 1% aqueous gelatin) are added to the third tube. The formation of a precipitate indicates the presence of condensed pyrogallic tannins.

❖ **Ferric chloride test**

Four drops of ferric chloride (FeCl3) at 10% in methanol solution are placed in tube N° 4.

The presence of a greenish-black precipitate indicates the presence of catechol-type tannins, while a bluish color indicates the presence of pyrogallol-type tannins.

If the green color appears in the ferric chloride test, it indicates the presence of another phenolic compound in the extract, even if both tests yield negative results.

g. Screening for unsaturated lactones.

The dry residue is dissolved in 1 ml of methanol and mixed with 0.5 ml of 1N alcoholic potassium hydroxide (KOH).

Two drops of 3,5-dinitrobenzoic acid (KEDDE reagent) are then added to the solution obtained. The solution is then placed in a boiling water bath for several minutes. The presence of a purple coloration following cooling of this solution is an indication of its unsaturated lactone content.

2.4. Isolation of antioxidant principles

The results of the biological test carried out on the four extracts obtained from the partition extraction of the crude water-ethanolic extract of the aerial part of C. giganteus madagascariensis show that the ethyl acetate extract exhibits very interesting antioxidant activity. Ten grams (10g) of ethyl acetate extract were separated using chromatography on a Sephadex LH-20 gel column eluted with a chloroform/methanol mixture (v/v) which resulted into five fractions (F1-F5). The results of the tests carried out on these five fractions indicate that fraction F3 has the strongest antioxidant activity. TLC analysis of the active fraction (F3) indicates that it was not pure, as the fraction contains multiple spots (fig.1).

Figure.1: Result of the TLC analysis F3

2.5 g of the fraction F3 as resubmitted to silica gel column chromatography. The elution was done using cyclohexane and a gradient of ethyl acetate, which resulted into six fractions. Antioxidant testing on these fractions demonstrated that fractions F34 and F35 exhibited very strong antioxidant activity. The purity of these fractions was verified 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 TLC analysis results indicate that these two fractions, F34 and F35, are not pure and, furthermore, both have the same

chromatographic profiles. By combining these fractions (labeled F35) and 300mg of this fraction was subjected to further separation by Sephadex LH-20 gel column chromatography eluted with a mixture of methylene chloride/methanol (V/V) and gave us five fractions. Fraction F352 showed the strongest antioxidant activity and was purified.

45 mg of fraction F352 were purified using preparative chromatography with a mixture of n-hexane/dichloromethane/methanol (2/7.75/0.25) as the solvent. This process resulted in the isolation of two pure products, which are designated as follows: PG-01 (6.478 mg) and PG-2 (7.362 mg). The purity of these two compounds was detected by analytical HPLC using a mixture of chloroform and methanol (1:1 by volume) as the mobile phase. Chromatography was performed in isocratic mode for 30 minutes.

2.5. Evaluation of the antioxidant activity of different extracts from the aerial part of *Cymbopogon giganteus madagascariensis*

The literature describes two methods for evaluating the antioxidant activity of a compound: bioautography [39-40] and DPPH assay[41-43]. The DPPH (2,2-diphenyl-1 picrylhydrazyl) assay method using UV spectrometry was used to evaluate the antioxidant activity of different extracts from samples (aerial part) of *Cymbopogon giganteus madagascariensis*.

a. The method is straightforward: use a spectrophotometer to measure DPPH[44-48]

We quantified the antioxidant power of different extracts from samples (aerial part) of C. giganteus madagascariensis using the method described by Brand and Sanchez Moreno, with some modifications. DPPH (25 mg) dissolves perfectly in 100 ml of methanol. This preparation is guaranteed to stay fresh when stored away from light. Add ten milliliters of this solution to 45 milliliters of methanol. We have prepared concentrations ranging from 2 mg/mL to 0.125 mg/mL of various extracts of C. giganteus madagascariensis with the utmost rigor. In dry tubes, 200 µL of each concentration was mixed with 3800 µL of the 4.5% DPPH solution. I prepared blanks consisting of 3800 µL of the 4.5% DPPH solution and 200 µL of methanol. The test was meticulously executed six times, ensuring consistency and accuracy. Then, it was incubated in the dark for one hour. The same procedure was applied to the vitamin E (α-tocopherol) control, and their absorbances were measured using a spectrophotometer at a wavelength of 517 nm.

The antioxidant activity, defined as the capacity to capture free radicals, is quantitated by the extent of discoloration of DPPH in a methanol solution. The expression (1) is the means by which this is determined:

Percentage of inhibition:

$\% = [(Ac-As)/ (As)] \times 100$

Ac: The absorption of the DPPH at a wavelength of 517nm was measured

As: absorbance of the test extract

III. Results and discussion

3.1. Extraction and rough separation

The aerial part powder of C. giganteus madagascariensis collected from Ankidona Village Manja Western part of Madagascar was extracted with the mixture water/ethanol (20/80). The ethanolic crude extract was suspended in water and was partitioned successively with different organic solvents of croissant polarity (Hexane, chloroform, ethyl acetate and n-butanol) in order to yield with the corresponding soluble extract (Table.2).

Table.2: Extraction results

Part examined	Solvents	Extract yield (g)	Yields (%)
Powder of Areal part (2kg)	Mixture (water/ethanol)	44.12	2.206
Crude extract (40g)	Hexane	6.27	15.675
	Chloroform	11.35	28.375
	Ethyl acetate	14.34	35.85
	Butanol	4.65	11.625
	Water	3.18	7.95

3.2. Phytochemical Screening

The results of the phytochemical screening (Table 3) conducted on the extract of the aerial parts of C. giganteus Chiov var. madagascariensis indicate that this plant is abundant in complex phenolic compounds and their derivatives, including flavonoids, coumarins, quinone compounds and their derivatives, and xanthones. The presence of terpenic compounds was also revealed, but in small proportions, and this plant does not contain chemical compounds such as saccharides and their polymers, alkaloids, or tannins.

Table 3: Results of phytochemical screening

Chemical families	Characterization reagent	Results
Alkaloids	Mayer, Wagner et Dragendorff	-
Flavonoids	MeOH, Mg, HCl concenter	++
Coumarins	NaOH, UV-lamp at λ 254nm and 365nm	+++
Anthraquinones et Xanthone	$CHCl_3$ et NH_4OH	+++
Leucoanthocyanes	Hot HCl	+
Tannins	H_2O, NaCl, gelatin, $FeCl_3$	-
Saponins	H_2O	-
Polyphenols	Gelatin	+++
Triterpènes	H_2SO_4, CH_2Cl_2, $C_4H_6O_3$, HCl concenter	++
Steroids	H_2SO_4, CH_2Cl_2, $C_4H_6O_3$, HCl concenter	+
Polysaccharides	Ethanol	-
Iridoïdes		+

3.3. Isolation of bioactive compound

The antioxidant activity was only found in the ethyl acetate extract. Chromatographic bio-guided fractionation of the ethyl acetate extract, using repeated silica gel column chromatography, LH-20 sephadex gel column, and preparative TLC, resulted in the isolation of two new bioactive compounds named PG-1 and PG-2 (table.2), as evidenced by analytical TLC and at the end, in pure forms as proved by HPLC analysis. The purity was respectively 99.18% at λ_{max} =214nm and [α] =23.2°C (compound 1) and 99.78% at λ_{max} =217nm and [α]=22.6°C (compound 2)

Table.4: Isolated pure products characteristics

Pure products	Weight (mg)	Yields (%)	Color	Solubility
PG-1	6.478	0.06478	Crystallisez	DMSO
PG-2	7.362	0.07362	Colorless	Methanol

3.4. Antioxidant test result

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable radical that exhibits maximum absorption at 517 nm. It has been utilized to assess the capacity of diverse extracts from plant matrix samples to function as free radical scavengers or hydrogen donors. Additionally, it has been employed to evaluate the antioxidant activity of various extracts of Canarium madagascariensis, employing α tocopherol (vitamin E) as a reference product. The results of the DPPH radical scavenging activity test on the different extracts of the samples of Cymbopogon giganteus madagascariensis are presented in Tables 5 and 6.

Table.5 clearly shows that all different extract of Cymbopogon giganteus madagascariensis exhibit anti-radical activity. However, at a concentration of 06.25 µg/ml, the chloroform extract and n-hexane extract are inactive. Both extracts are still active. The acetate ethyl extract's anti-radical effect is particularly noteworthy (see Table 6)

Table.5: percentage inhibition of different extracts from the areal part of the Cymbopogon giganteus Madagascariensis

Concentration (µg/ml)	Percentage inhibition of the different extract (%) at n=6				
	Crude extract	Ethyl acetate extract	Chloroform extract	n-hexane extract	Witness
150	86.13		80.56	84.93	
100	75.45		69.37	73.49	
75	63.05		48.79	52.14	
50	59.91		29.47	38.54	
25	43.75	96.42	18.72	21.91	97.78
12.5	39.25	88.72	05.91	03.62	92.47
6.25	30.39	81.15			85.26
3.12	21.78	63.10			75.01
1.5	19.56	43.75			60.38
1	11.23	31.02			47.61
0.5	04.61	18.45			26.58

Table.6: The IC_{50} value of the different extracts from the areal part of the Cymbopogon giganteus Madagascariensis

Extracts	IC_{50} (µg/ml) at n=6
Water-ethanol (20/80)	13.38±0.03
Ethyl acetate	09.21±0.04
Chloroform	41.53±0.06
n-Hexane	37.68±0.05

Compound-1	07.11±0.04
Compound-2	04.31±0.04
α-tocopherol	06.57±0.08

3.4. Structural elucidation

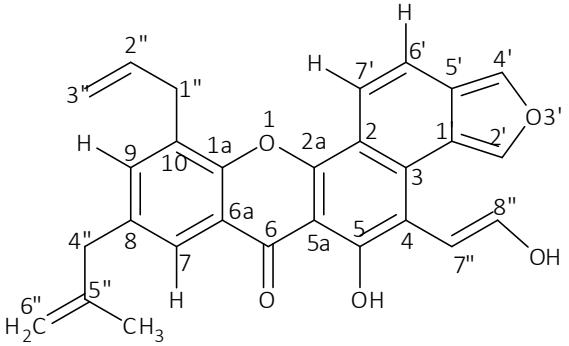
The positive-ion high resolution electrospray ionization (HRESI) mass spectrum of compound-1 (PG-1) displayed a protonated molecular ion peak at m/z = 439.03762 corresponding to the molecular formula $C_{28}H_{22}O_5$ required for $[M+H]^+$, with eighteen (18) of insaturation degree.

In addition to fragments F^+ of molecular ion identified bay mass spectrum data of about two peaks of molecular ions parent at m/z = 398, 355 and m/z = 300, indicated to the parted respectively at m =41 , 43 and m = 55, corresponding to the allyl group, hydroxyl-vinyl and methyl-allyl group. These fragments that confirm to the presence of the allyl group, hydroxyl-vinyl and methyl-allyl group in the compound-1 (PG-1).

Les résultats des analyses de la RMN-1D du proton de composé PG-1 a permis d'identifier que leur bande spectrale sorte entre 1 à 13ppm. L'allure générale de cette bande spectrale a permis de relever les caractéristiques, les attributions et les propriétés spécifiques de chaque proton présent dans la molécule du composé PG-1.

Les signaux des protons sortant respectivement à δH 3.21 (d, 2H), 3.24 (s, 2H), quatre signaux de protons géminés des environnements chimiques différentes à δH 4.88 (dd, 1H), 4.92 (d, 1H), 5.02 (dd, 1H), 5.04 (d, 1H) et δH 5.92 (d, 1H) sont tous attribués aux caractères des protons de groupes allyl. Le signal sort à 1.32ppm (s, 3H) est attribué au proton du groupe méthyl, de plus, on a identifié deux pics de protons sortent à 5.64ppm (d, 1H) et 6.68 (d, 1H) correspondent aux caractères de protons du groupe vinyl. Ensuite, les trois signaux de protons alcèniques très particuliers sortant à δH 7.38 (s, 2H asymétriques), 7.68 (d, 1H) et 8.45 (d, 1H) correspondant aux caractéristiques de protons du noyau isobenzofurone et de plus deux pics de protons sortent à 7.25ppm (s, 1H) et 7.26 ppm (s, H) sont attribués aux protons alcéniques typiquement du squelette du noyau benzénique et afin les deux signaux sortant à δH 11.84 et 12.34 possèdent des caractères acides sont attribués aux protons labiles du phénols.

The 1D ^{13}C broad band-NMR spectrum contained 27 signals of the carbons indicating 13 signals correspond to the carbons of typical Xanthone [49-52] skeleton including the carbonyl group between δ179.9, is not symmetry in the molecule, six signals carbons attributed to the typical for isobenzofuro skeleton, seven signals carbons attributed to the allyl groups, and at the end in the presence of the two carbons characteristic to the signals of the vinyl group. In addition, the DEPT spectrum (Distorsionless Enhancement by Polarization Transfer) revealed the presence of one methyl carbon, four methylene carbons and eight methine carbons. However, the 1D ^{13}C-NMR spectrum recorded in DEPT mode does not allow the identification of quaternary carbons. Based on the interpretation of the 1D ^{13}C-NMR spectra in both BB (broadband decoupling) and DEPT modes, compound-1 (PG-01) contains twelve (12) quaternary carbon atoms.



(*E*)-10-allyl-5-hydroxy-4-(2-hydroxyvinyl)-8-(2-methylallyl) -6*H*-isobenzofuro[2,3*c*]Xanthen-6-one

Figure.2: Chiovisobenzofuroxanthone-A (PG-01)

Examination of 1D ^{13}C and the 2D HSQC spectrum data of the compound 1 revealed that of about twenty-three (23) alkene carbons (C=C) double bonds indicating two (2) shielded aromatic methine group at δC 127.2 (C-7) and 129.7 (C-9), ten (10) quaternary carbons of which the characteristic are attributed to the typical carbons of Xanthone skeleton at δC 108.2 (C-5a), 110.6 (C-4), 120.7 (C-6a), 123.1 (C-2), 130.1 (C-8), 130.6 (C-10), 134.4 (C-3), 148.4 (C-5), 152.2 (C-1a) and δC 155.2 (C-2a), six carbons characteristic attributed to the typical carbons for the isobenzofuro skeleton between four methine groups at δC 124.5 (C-6'), 126.2 (C-7') and δC 134.2 x2 (C-2' and C-4'), two quaternary carbons at δ129.2 (C-5') and δ121.2 (C-1'), four signals of carbons characteristic attributed to the two allyl groups between one methine group at δC 136.5 (C-2"), one quaternary carbon at δC 145.8 (C-5") and two methylene groups at δC 112.1 (C-6"), and δC 115.9 (C-4"). In

addition to the examination of the 1D ^{13}C and the 2D HSQC spectrum that permitted to reveal the presence of the one methyl group at δC 23.2 (C-5”a) at the end two methylene groups at δC 34.2 (C-1”) and 43.9 (C-4”). 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 table1. To the best of our knowledge; this is the first time that a membered ring occurs in the side chain of a typical isobenzofuro [2, 3-c]-Xanthone.

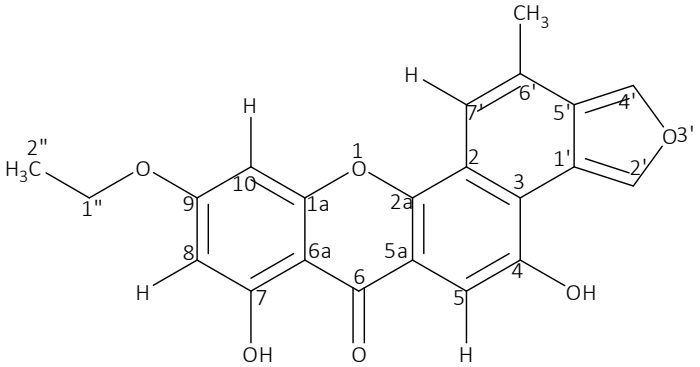
Table 7: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H (COSY) and important HMBC correlation of compound-1 (PG-1).

Position		Expériences RMN 1D		Expériences RMN 2D	
N°	Types	δH et multiplicité	δC	COSY	HMBC
1	-O-	-	-	-	-
1a	Cq	-	152.2	-	-
2	Cq	-	123.1	-	-
2a	Cq	-	155.2	-	-
3	Cq	-	134.4	-	-
4	Cq	-	110.6	-	-
5	=COH-	12.43 mal résolu	148.4	-	C-4 et C-5a
5a	Cq	-	108.2	-	-
6	C=O	-	179.9	-	-
6a	Cq	-	120.7	-	-
7	=CH-	7.26 (s)	127.2	-	C-1a, C-6, C-4’’ et C-9
8	Cq	-	130.1	-	-
9	=CH-	7.25 (s)	129.7	-	C-1a, C-7, C-1’’ et C-4’’
10	Cq	-	130.6	-	-
1’	Cq	-	121.2	-	-
2’	=CH-O-	7.38 (s)	134.2	-	C-1’, C-3, et C-5’
3’	-O-	-	-	-	-
4’	=CH-O-	7.38 (s)	134.2	-	C-1’, C-5’, et C-6’
5’	Cq	-	129.2	-	-
6’	=CH-	7.68 (d)	124.5	$H_{7'}$	C-2, C-2a, C-3, et C-5’
7’	-CH=	8.45 (d)	126.2	$H_{6'}$	C-2, C-1’, et C-5’
1’’	$-CH_2-$	3.21 (d)	34.2	$H_{2''}$	C-2’’, C-3’’, C-8, et C-10
2’’	=CH-	5.92 (m)	136.5	$H_{1'}$, $H_{3''a}$ et $H_{3''b}$	C-1’’, C-3’’ et C-10
3’’	$=CH_2$	4.97 (dd)	115.9	$H_{2'}$ et $H_{3''b}$	C-1’’, C-2’’
		5.02 (dd)		$H_{2'}$ et $H_{3''a}$	C-1’’, C-2’’
4’’	$-CH_2-$	3.22 (s)	43.9	-	C-9, C-8, C-7, C-5’’, C-5’’a et C-6’’
5’’	Cq	-	145.8	-	-
5’’a	$-CH_3$	1.32 (s)	23.2	-	C-5’’
6’’	$=CH_2$	4.88 (d)	112.1	$H_{6''b}$	C-4’’, C-5’’ et C-5’’a
		5.04 (d)		$H_{6''a}$	C-4’’, C-5’’ et C-5’’a
7’’	=CH-	6.83 (d)	104.4	$H_{8''a}$	C-3, C-4, C-5 et C-8’’
8’’	-HCOH-	5.64 (d)	165.0	$H_{7''}$	C-4 et C-7’’
		11.84		-	C-7’’

The molecular formula of compound-2 (PG-2) was determined to be $C_{22}H_{16}O_6$ by HREISM (m/z = 377.9307 $[M-H]^+$,calculated), with fifteen (15) of insaturation degree and 1D,2D-NMR experiments. Its ^{1}H-NMR spectrum exhibited, the one singlet at δH 2.70 characteristic attributed to the one methyl group, three signals alcens protons between δH 6.36 (s), 6.39(s) and δH 6.46 (s) attributed to the characteristic of signals alcens proton typical for benzene skeleton, and in the presence of the two hydroxyl protons between δH 9.61 and δH 11.85 typical for phenolic at the end implying that the compound-2 is di-O-Substituted Xanthone.

Regarding the range of multiplicity, the two signals of linear protons between δH 1.32 (t) and δH 4.09 (q) characteristic attributed to the protons signals of the ethoxy group.

The presence of linear chain alkyl proton in the compound-2 was indicated by the peaks in this region and their information of range multiplicity. In addition of the examination of the 1D ^{1}H-NMR, permitted to reveal the presence of the two aromatic signals alcens protons, between δH 7.38 (s, 2H resonance) and δH 8.41 (s) indicate that these alcens protons signals are characteristic the presence typical for isobenzofuro skeleton in the compound-2 (PG-02).



9-ethoxy-4,7-dihydroxy-6'-metyl-6*H*-isobenzofuro[2,3-*c*] Xanthen-6-one

Figure.3: Chiovisobenzofuroxanthone-B (PG-02)

The 1D ^{13}C broad band-NMR spectrum contained twenty one (22) signals of the carbons indicating 13 signals correspond to the carbons of typical Xanthone [49-52] skeleton including the carbonyl group between δC 179.9, is not symmetry in the molecule, six signal carbons attributed to the carbons typical for isobenzofuro skeleton and two (2) carbons picks attributed to the linear chain of ethoxy group are present in the compound-2.

Examination of 1D ^{13}C and the 2D HSQC spectrum data of the compound-2 (PG-2) revealed that of about 18 alkene carbons (C=C) double bonds indicating three (3) shielded aromatic methine groups at δC 106.2 (C-10), 107.6 (C-5) and δC 114.9 (C-8), nine (9) quaternary carbons of which the characteristic are attributed to the typical carbons of benzene skeleton at δC 162.5 (C-7), δC 162.2 (C 9), δC 157.6 (C-1a), δC 150.2 (C-4), 145.5 (C-2a), δC 128.8 (C-2), δC 128.4 (C-3), δC 116.7 (C-5a) and δC 101.6 (C-6a), six (6) carbons characteristic attributed to the typical carbons of isobenzofuro skeleton between three carbons of methine group at δC 122.0 (C-7') and δC 134.2 x 2 (C-2' and C-4'), and three (3) quaternary carbon at δC 119.8 (C-1'), δC 131.0 (C-5') and δC 137.6 (C-6').

In addition to the examination of the 1D ^{13}C and the 2D HSQC spectrum that permitted to reveal the presence of the two carbons characteristic attributed to linear chain of ethoxy group at δC 14.8 (C-2") and δC 64.6 (C-1") at the end one carbon of methyl group at δ19.8 (6'-CH_3). The 1H 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 table 8.

Table 8: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H (COSY) and important HMBC correlation of compound-2 (PG-2).

Position		Expériences RMN 1D		Expériences RMN 2D	
N°	Types	δH et multiplicité	δC	COSY	HMBC
1	-O-	-	-	-	-
1a	Cq	-	157.6	-	-
2	Cq	-	128.9	-	-
2a	Cq	-	145.5	-	-
3	Cq	-	128.4	-	-
4	-COH	9.61 (mal résolu)	150.2	-	C-3 and C-5
5	CH	6.48 (s)	107.6	-	C-2a, C-3, C-5a and C-6
5a	Cq	-	116.7	-	-
6	C=O	-	179.9	-	-
6a	Cq	-	101.5	-	-
7	COH	11.85	162.5	-	C-6a and C-8
8	CH	6.36 (s)	114.9	-	C-6a, C-9 and C-10
9	Cq	-	162.2	-	-
10	CH	6.39 (s)	106.2	-	C-6a, C-8, C-9 and C-1a
1'	Cq	-	-	-	-
2'	=CH-O-	7.38 (s)	134.2	-	C-1', C-3, et C-5'
3'	-O-	-	-	-	-
4'	=CH-O-	7.38 (s)	134.2	-	C-1', C-5', et C-6'
5'	Cq	-	131.0	-	-
6'	Cq	-	137.6	-	-
6'-CH_3		2.70 (s)	19.8		C-5', C-6' and C-7'
7'	-CH=	8.14 (s)	122.0	-	C-2, C-2a, and C-5'
1''	-CH_2-	4.09 (q)	64.8	$H_{2''}$	C-2'', C-3'', C-8, et C-10
2''	CH_3-	1.32 (t)	14.8	$H_{1''}$	C-1'', C-3'' et C-10

IV. Discussion

Plants have developed a variety of molecules through different metabolic pathways [53], with different physicochemical characteristics and various biological properties. These molecules have been used by plants in different ways, particularly as a defense mechanism against predators. These secondary metabolites, produced by plants, have a natural effect on predators and can serve as precursors for the discovery of new drugs to combat the threat of (multi)drug resistance in diseases or pandemics. Consequently, researchers are now exploring the potential of plants to identify their secrets and healing properties, with the aim of determining the specific molecule(s) responsible for their biological activity [54-60].

A survey of the ethnomedicine data of the populations of western Madagascar revealed that the plant known by the vernacular name Verompoly is utilized by the local population for the treatment of infections, fevers and blood pressure problems. A review of the existing literature on this plant reveals that it exerts vasorelaxant effects, with its pharmacological mechanisms of action being NO-dependent and involving the β2-adrenergic receptor [61]. Furthermore, the essential oils derived from this plant have been shown to possess antibacterial properties [62].

The application of the cold maceration extraction technique using hydro-ethanol on the powder of the aerial part of the plant collected in July 2022 in Ankidona-Manja yielded a crude hydro-alcoholic extract designated EB. A series of biological screening tests were conducted on the extract, yielding several notable findings. Firstly, the extract demonstrated vasorelaxant activity, which refers to its ability to relax blood vessels. Secondly, it exhibited noteworthy antibacterial properties, suggesting its potential to combat bacterial infections. Finally, the extract exhibited antioxidant activity, a property that plays a crucial role in protecting cells from oxidative stress. These results lend further support to the ethnomedical data and bibliography on this plant.

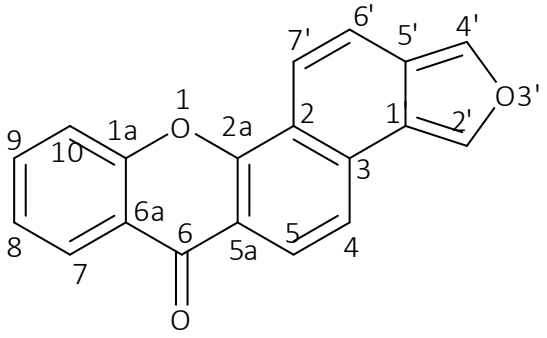
Phytochemical screening of extracts from the aerial parts of C. giganteus Chiov var. madagascariensis shows that it is rich in phenolic compounds and their derivatives, namely flavonoids, coumarins, and quinone compounds and their derivatives. The presence of terpenic compounds has also been revealed, but in small proportions, and it does not contain chemical compounds such as saccharides and their polymers, alkaloids, or tannins. These preliminary study results have provided valuable insights into the biological activities of this plant and their ethnobotanical data concerning the empirical uses of C. giganteus Chiov var. madagascariensis.

The extraction process, involving liquid-liquid partitioning between two immiscible solvents (water and an organic solvent of increasing polarity), was carried out on the crude extract of the plant in question. This process yielded five distinct extracts. The results of the biological tests on these different extracts demonstrate that the chloroform extract exhibits antibacterial properties, while the ethyl acetate extract demonstrates antioxidant activities. However, applying this method to the crude extract of the raw material sample allows the extracts with the highest biological activity to be identified. These activities vary depending on the polarity of the molecule and their solubility in the extraction solvents.

The aim of this study is to isolate the principle(s) responsible for the biological activities of this plant. C. giganteus Chiov var. madagascariensis has two distinct biological activities. Additionally, previous scientific studies on this plant, as referenced in the literature, demonstrate a paucity of research on the identification of antioxidant principles in the extract of the aerial parts of C. giganteus Chiov var. madagascariensis. In view of the results obtained, it was decided to refocus the research on the isolation of antioxidant compounds from the ethyl acetate extract.

The application of bioguided fractionation techniques to the ethyl acetate extract of this plant, using various series of chromatography analyses combined with antioxidant testing, enabled the isolation of two pure active products. The chemical structures of these isolated compounds were elucidated using NMR spectroscopy (1D and 2D) and mass spectrometry (Figs. 2 and 3). These compounds were identified as derivatives of isobenzofuro-[2,3c]-xanthone, specifically named Chiovisobenzofuroxanthone-A (Fig. 2) and Chiovisobenzofuroxanthone-B (Fig.3).

A comprehensive review of the extant literature, including both bibliographic research and comparative studies, has revealed a paucity of information regarding the chemical structures and biological activities of these two molecules. Additionally, it has been determined that the two molecules in question share a common basic structure, attributed to isobenzofuro[2,3c]-xanthone derivatives, as illustrated in Figure 4.



Isobenzofuro[2,3*c*]Xanthen-6-one

Figure.4: The basic structure of these two molecules

Research conducted on the structure-activity relationships of these two molecules reveals the presence of antioxidant activities in both compounds. However, these compounds exhibit different levels of inhibition, with compound 1 demonstrating higher activity compared to compound 2. Therefore, it is reasonable to hypothesize that the activity of the compounds is attributable to the substituent groups rather than the basic structure.

V. Conclusion

The plant known under the vernacular name of Verompoly (Malagasy name) and scientifically named as Cymbopogon giganteus Chiov var. madagascariensis (Poaceae) is well known in Western part of Madagascar for its healing properties. It has been used by the population of this region to treat infections, fevers, and blood pressure problems. Biological screening carried out on the water-alcoholic extract of the aerial parts of the plant confirms the traditional uses of this plant.

Two new bioactive compounds typical of the derived isobenzofuro-[2,3*c*]-Xanthone named Chiovisobenzofuroxanthone-A and Chiovisobenzofuroxanthone-B were isolated from the ethyl acetate-soluble extract from aerial parts of *Cymbopogon giganteus Chiov var. madagascariensis* (POACEAE), collected in the village of Ankidona, Anontsibe-Centre commune, Manja district, Menabe's region in the Western part of Madagascar. The structures of isolated new compounds were identified as named Chiovisobenzofuroxanthone-A and Chiovisobenzofuroxanthone-B on the basis of spectroscopic methods (1D, 2D –NMR, HREIS-MS, IR and UV).

All of our results demonstrate the potential of medicinal plants, particularly their bioactive molecules, in the discovery of drug candidates.

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