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ANTIMICROBIAL ACTIVITY, ISOLATION AND ELUCIDATION OF THE THREE NEW BIOACTIVE MOLECULES WERE DERIVED OF ISOBENZOFURO[4,5C]XANTHONE, FROM THE AERIAL PARTS OF *CYMBOPOGON GIGANTEUS CHIOV VAR. MADAGASCARIENSIS* (POACEAE)

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ABSTRACT

In Madagascar, traditional medicine holds an important place in Malagasy society, thanks to its customs and rich biodiversity, particularly the endemicity of plants and their therapeutic virtues. Based on ethnobotanical data, a plant known by the vernacular name Verompoly (Sakalava name) and scientifically called Cymbopogon giganteus Chiov var. madagascariensis (Poaceae) is used by the local population to treat fever, infections and blood pressure problems. The results of biological screening tests show that the dichloromethane extract has very interesting antimicrobial activity. The aim of this study is to isolate the principles responsible for this plant's antibacterial activities. The application of bioguided fractionation methods, using the chromatography technique on dichloromethane extract followed by antimicrobial tests to locate products, enabled us to isolate three pure active ingredients noted (CG-01, CG-02 and CG-03). The chemical structures of these three products have been elucidated using mass spectrometry and nuclear magnetic resonance spectroscopy (1D and 2D NMR). They are all attributed to Isobenzofuro-[4,5c]-Xanthone derivatives. The overall results of studies on this plant show that the empirical uses of Verompoly have been scientifically proven, and their active principles are attributed to new molecules included in the family of complexes phenolic compounds derived from Isobenzofuro-[4,5c]-Xanthone.

KEYWORD: traditional medicine, *C. giganteus Chiov*, antibacterial, Isobenzofuro-[4,5c]-Xanthone.

INTRODUCTION

Madagascar is classified as a country of biodiversity hot spot.[1,2] Its flora consists of approximately 12.000 plant species, of 80 % inventory species are endemic.[3-6] The Malagasy forests host a large number of medicinal plants used by local people to à variety of illnesses.[7- 9] This biological richness may constitute a reservoir of natural products of great significance as drugs and leads

structures.[10-11] Several plants in Malagasy were reported to have pharmacological relevance for the local communities.[12] It is also known that in Africa, the first line of treatment for poor people is the use of herbal medicine at home.[13-16]

During an ethno-botanical survey conducted in the South-western regions of Madagascar, it was reported that the aerial part decoction of Cymbopogon giganteus (Hochst) Chiov var. madagascariensis (A. Camus), including in genus Cymbopogon (Poaceae) known under the vernacular name of "Ahibero" [Malagasy name] is traditionally used by the local communities to treat fatigue, fever, hypertension and bacterial infections.[17]

The genus Cymbopogon (Poaceae) consists of herbaceous trees, and comprises about 56 species distributed in the Madagascar, in Africa, in Asia, in Australia and in the tropical and subtropical regions[18,19], among which four species Cymbopogon citratus, Cymbopogon flexuosus, Cymbopogon martini and Cymbopogon giganteus grow wild in Madagascar. Cymbopogon giganteus (Hochst) Chiov var. madagascariensis (A. Camus) is an herb up to 0.5-2m tall which in encountered in arid South-western regions of Madagascar. The variety differs from the African Cymbopogon giganteus by its lower height (0.5 – 1.5m instead of 2 – 3m). Leaves are glaucous green, linear and flexible, with little rounded or no rounded at the base. Conversely, in African species, leaves are flat and their edges are rough to the touch. Finally, the inflorescences are smaller in diameter (10 – 30 cm instead of 30 – 40 cm).[20-22] This plant is well-known and very important recipe in the Africa mainland, above all in the tropical region because of its therapeutic values in the traditional medicine. This plant could be promising source of bioactive molecule. The present work aims at isolating phytochemicals from the aerial part of Cymbopogon giganteus madagascariensis and evaluating the antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Selection and collection of plant materials

Ethno-botanical information about the plant species selected for this study was obtained by interviewing traditional healers during field work which was conducted in the South-west and Western regions of Madagascar. The aerial part of Cymbopogon giganteus madagascariensis was collected in Ankidona village, Commune Anontsibe-center, district of Manja Western part of Madagascar on Jolay 2022. The plant sample was identified by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number CG-01, was deposited at the Herbarium of the Laboratory of Applied Chemistry, Layflaylle Street, University of Toliara.

2.2. Extraction

The aerial part of C. giganteus madagascariensis (2kg) was powdered and extracted by repeated maceration with a mixture of water-ethanol (20/80) (3x5H) at room temperature. After filtering the mixture, aqueous ethanol filtrates were pooled dried over Na_2CO_3 and evaporated to dryness under reduced pressure using a rotary evaporator to yield crud extract (35.28g). A portion (25g) of the crude extract were suspended in 95% aqueous methanol (200mL) and extracted with n-hexane (3x200mL). The aqueous MeOH layer was then diluted to 75% aqueous MeOH by addition of water before sequentially partitioning with chloroform, ethyl acetate, n-butanol to yield the corresponding extract fractions. The different extracts were evaporated to dryness on an evaporator apparatus to yield the different crud extract. All extracts were stored at 4°C.

2.3. Isolation

Bioassay-guided extraction revealed interesting activity only with chloroform fraction. This fraction displayed a good antimicrobial activity.

Fifteen (15g) of the chloroform-soluble fraction was first chromatographed over silica gel column with different solvents of increasing polarity (n-hexane, n-hexane/dichloromethane (1:1); dichloromethane; dichloromethane/methanol (v/v) and methanol) to yield seven (7) fractions (F1-F7). These two fractions F1 and F5 showed strong antimicrobial activities.

Then 850mg of the fraction F1 was further fractionated over silica gel column eluted with cyclohexane and gradient of ethyl acetate resulting into six (6) fractions. Two fractions F13 and F14 showed strong antimicrobial activities. These fractions were checked for purity by analytical TLC, and the zone was detected with a UV lamp 254 and 365 nm and spaying with sulfuric vanillin acid, followed by heating at 120°C for 1-5 min. These F13 and F14 were combined on the basis of TLC profile similarity and subjected to further separation by LH-20 sephadex gel column chromatography eluting with mixture of chloroform/methanol (V/V) and the column in isocratic regime and at end, it resulted into four fractions. The fraction F142 showed the highest antimicrobial activity which was further investigated. Then 35 mg of the fraction was subjected to further purification by preparative TLC using n-hexane/dichloromethane/methanol (2/7.75/0.25) as the solvent affording compounds CG-1 (7.5mg), CG-2 (4.22mg) and CG-3 (5.18 mg).

Purity of compounds was checked on normal phase silica gel TLC or RP-18 silica gel TLC. TLC plates were developed with suitable eluents. Spots were first visualized under UV light at 254nm and 365nm and then by using the vanillin sulphuric spray reagent.

2.4. Biological screening

2.4.1. Microbial strains

The activity of the chloroform fraction and purse compounds were tested toward 9 different microorganisms: Gram positive bacteria represented by B. subtilis ATCC 6633, S. aureus ATCC 25923, B. cereus ATCC 10876, and Gram negative bacteria: E. coli ATCC 25922, S. typhii ATCC 13311, P. aeruginosa ATCC 27853, and E. cloacae ATCC 13047. The tested strains were obtained from the American Type Culture Collection (ATCC, Rockville MD, USA).

2.4.2. Antimicrobial activity

2.4.2.1. Disc diffusion

The agar disc diffusion method was used to determine the antibacterial activity of the chloroform fraction and pures compounds are following: A 1 mL of suspension of any tested bacteria containing about 10^6 CFU/mL were spreaded on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 20 µL of products and placed on the inoculated plates and allowed to dry for 15 min, then incubated at 37 °C for 24 h. The diameters of the inhibition zones were measured in millimeters. Chloraphenicol or cycloheximid (10 µg/mL) was included as control.

2.4.2.2. Minimum inhibitory and minimum bactericidal concentration

An aliquot (10 µL) of a 10^6 CFU/mL overnight culture was added to wells of a sterile 96-well micro-titre plate. Each product was diluted in Mueller Hinton broth (MHB) containing 0.1% (v/v) Tween 80 and added to wells to give final concentrations ranging from 0.03 to 10 µL/ mL. The positive control wells contained MHB and bacteria suspension without purse compounds while negative control wells contained MHB only. Optical density (OD) was measured at 630 nm using a microplate reader (Titertek Twin-reader, Finland) and again after incubation for 24 h at 37°C. The minimum inhibitory concentration (MIC) was determined as the lowest essential oil concentration at which the optical density after 24 h of incubation of the inoculum remained the same or reduced compared with the initial reading. MTT (30 µL) in aqueous solution (0.01%) was used to evaluate the microorganism viability. For minimum bactericidal (MBC) determination, 10 µL was taken from each well after incubation and spot inoculated on to MHB and incubated for 24 h at 37 °C. The concentration at which no growth observed on subculture was determined as the MBC.[24-29] The mean MBC/MIC ratio was evaluated for each sample.

2.5. Structural elucidation

The chemical background of the genu and the Rf value, fluorescence and color on TLC of the studied compound grave a first idea of the chemical class to which it belongs. Structures were elucidated by means of spectroscopies (NMR (1D and 2D) and HRMS). Spectra were carefully interpreted and data obtained were compared with those published in the literature.

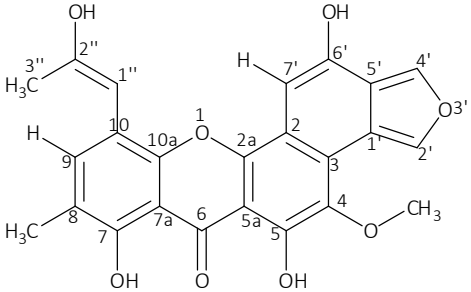
3. RESULTS

3.1. Isolation and structural elucidation

The chloroform fraction of the ethanolic crude extract from the aerial part powder of C. giganteus madagascariensis collected from Ankidona Village Manja Western part of Madagascar were subjected to repeated column chromatography over silica gel, LH-20 sephadex gel, RP-18 gel, analytical TLC and preparative TLC. The fractionation procedure led to the isolation of the three (3) new bioactive compounds named CG-1, CG-2 and CG-3. Three compounds (CG-1, CG-2 and CG-3) own of antimicrobials activities.

The positive-ion high resolution electrospray ionization (HRESI) mass spectrum of compound-1 (CG-1) displayed a protonated molecular ion peak at m/z = 435.18564 corresponding to the molecular formula $C_{24}H_{18}O_8$ required for $[M+H]^+$, with sixteen (16) 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 = 404 and m/z = 351, indicated to the parted respectively at m =31 and m = 57, corresponding to the méthoxy group and 2-hydroxyprop-1-enyl. These fragments that confirm to the presence of the methoxy group and 2-hydroxyprop-1-enyl in the compound-1 (CG-1). The IR spectrum showed a typical absorption band at 3452 Cm^{-1} and 1703Cm^{-1} which were suggestive of one ketone group and their hydroxyl functions in the compound-1 (CG-1).

The ^{1}H-NMR spectrum exhibited, their two singlets at δ2.15 and δ2.24, characteristic attributed to two methyl groups and one peak singlet between δ3.83, characteristic attributed to the signal of the methoxy group. Five signals alcens protons between δ6.41(s), δ7.07(s), δ7.38 (.s 2H resonance) and δ7.56 (s), attributed to the characteristic of signals alcens proton typical for benzene skeleton. Regarding the signals of alcens protons between δ7.38 (.s 2H resonance) and δ7.56 (s) indicate that these alcens protons signals are characteristic the presence typical for isobenzofuro skeleton in the compound-1, and at the end, in the presence of the four hydroxyl protons between δ9.61, δ12.04, δ13.04 and δ14.35 typical for phenolic at the end implying that the compound 1 is di-O-Substituted Xanthone.



(E)-5, 6', 7-trihydroxy -10-(2-hydroxyprop-1-enyl)-4-methoxy-8-methyl-6*H*-
Isobenzofuro [4, 5-c] Xanthen-6-one

Figure 1: Structure of compound-1 (CG-1).

The 1D ^{13}C broad band-NMR spectrum contained 24 signals of the carbons indicating 13 signals correspond to the carbons of typical Xanthone[30-33] skeleton including the carbonyl group between δ183.1, is not symmetry in the molecule, six signals carbons attributed to the typical for isobenzofuro skeleton and three signals carbons attributed to the linear chain of the 2-hydroxyprop-1-enyl are present in the compound-1, and at the end in the presence of the two carbons characteristic to the signals of the methyl and methoxy groups.

Examination of 1D ^{13}C and the 2D HSQC spectrum data of the compound 1 revealed that of about twenty alkene carbons (C=C) double bonds indicating one (1) shielded aromatic methine group at δ131.4 (C-9), eleven (11) quaternary carbons of which the characteristic are attributed to the typical carbons of Xanthone skeleton at δ110.3 (C-10), δ111.1 (C-7a), δ118.8 (C-8), δ125.0 (C-2), δ127.1 (C-5), δ127.2 (C-3), δ129.0 (C-5a), δ142.9 (C-4), δ145.6 (C-2a), δ151.7 (C-10a) and δ160.8 (C-7), six carbons characteristic attributed to the typical carbons for the isobenzofuro skeleton between one methine group at δ103.8 (C-7'), five quaternary carbons at δ120.1 (C-5'), δ121.2 (C-1'), δ134.2 x2 (C-2' and C-4') and δ158.4 (C-6') and two carbons characteristic attributed to linear chain of alkene groups between one methine groups at δ114.3 (C-1") and one quaternary carbon at δ156.9 (C-2").

In addition to the examination of the 1D ^{13}C and the 2D HSQC spectrum that permitted to reveal the presence of the two methyl groups at δ2.15 (8-CH_3) and δ15.9 (C-3") at the end one carbon of methoxy group δ60.6 (4-OCH_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 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.

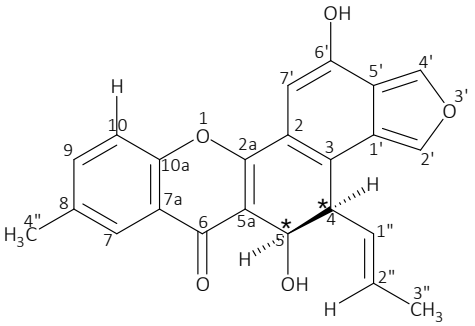
Table 1: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H (COSY) and important HMBC correlation of compound-1.

Position		1D-NMR experiments		2D-NMR experiments	
N°	Types	δH (ppm) and multiplicity	δC (ppm)	COSY	HMBC
1	-O-	-	-	-	-
2	Cq	-	125.0	-	-
2a	Cq	-	145.6	-	-
3	Cq	-	127.8	-	-
4	Cq	-	142.9	-	-
5	C-OH	13.04 (s)	127.1	-	C-4 and C-5a
5a	Cq	-	109.0	-	-
6	C=O	-	182.2	-	-
7	C-OH	12.04 (s)	160.8	-	C-7a and C-8
7a	Cq	-	111.1	-	-
8	Cq	-	118.6	-	-
9	CH	7.07 (s)	131.4	-	C-10a, C-7, C-1" and C-4"
10	Cq	-	110.3	-	-
10a	Cq	-	151.7	-	-
1'	Cq	-	121.2	-	-
2'	CH	7.38 (s)	134.2	-	C-1', C-5' and C-6'
3'	-O-	-	-	-	-
4'	CH	7.38 (s)	134.2	-	C-1', C-3 and C-5'
5'	Cq	-	120.0	-	-
6'	C-OH	9.61	158.4	-	C-5' and C-7'
7'	CH	7.56 (s)	103.8	-	C-2, C-2a, C-3 and C-5'
1"	CH	6.24 (s)	125.5	-	C-9, C-10, C-10a and C-2" and C-3"
2"	C-OH	5.79	156.9	-	C-1' and C-3'
3"	CH_3	2.14 (s)	12.8	-	C-2"
4"	CH_3	2.25 (s)	15.5	-	C-7 and C-8
5"	O-CH_3	3.83 (s)	60.6	-	-

The compound-2 (fig.2) had a molecular formula of $C_{23}H_{18}O_5$ determined by the positive-ion high resolution electrospray ionization (HRESI) mass spectrum (m/z = 375.01724 $[M+H]^+$). Its 1H-NMR-spectrum showed signal for two methyl groups (δH 2.05(s) and δH 2.34 (s)), eight alcens protons between at δH 7.34x2 (s) and δH 6.84 (s) attributed to alcens protons of the isobenzofuron skeleton, δH 5.41(d), δH 6.08 (dd), δH 6.88(d), δH 7.43(s) and δH 7.61 (d) attributed to the to the characteristic of the alcens protons typical of the

benzene skeleton, and two signal at δH 3.75 (dd) and δH 4.37(d) attributed to the signal of cyclic group and at the end two signal of the hydroxyls proton between at δH 9.61 (OH) attributed to the phenol group and δH 4.17(OH) attributed to the secondary group alcohol in the cycle. Regarding the range of multiplicity, the two signals of alcens protons between δH 5.41(d), δH 6.08 (dd) (region of the linear alcens groups) indicate that these alcens protons signals are characteristic the presence of linear chain alkene proton in the compound-2 was indicated by the peaks in this region and their information of range multiplicity.

The 1D ^{13}C broad band-NMR spectrum contained 23 signals of the carbons indicating 13 signals correspond to the carbons of typical Xanthone skeleton including the carbonyl group between δ183.0, six signals carbons attributed to the typical for isobenzofuro skeleton and three signals carbons attributed to the linear chain of the prop-1-enyl are present in the compound-2, and at the end in the presence of the one carbon characteristic to the signal of the methyl groups.



(4R, 5R)-5, 13-dihydroxy-8-methyl-4-((E) prop-1-enyl)-4H-
Isobenzofuro [4, 5-c]-Xanthen-6(5H)-one
Ion moléculaire à m/z = 375.01724 $[M+H]^+$
Formula brute: $C_{23}H_{18}O_5$

Figure 2: Structure of compound-2 (CG-2).

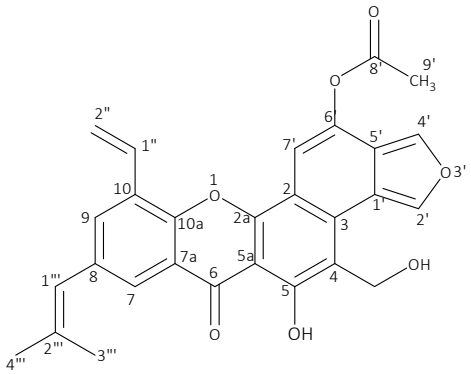
Table 2: ^1H and ^{13}C chemical shift, the correlation ^1H-^1H (COSY) and important HMBC correlation of compound-2.

Position		1D-NMR experiments		2D-NMR experiments	
N°	Types	δH (ppm) and multiplicity	δC (ppm)	COSY	HMBC
1	-O-	-	-	-	-
2	Cq	-	128.3	-	-
2a	Cq	-	159.3	-	-
3	Cq	-	130.5	-	-
4	CH	3.57 (m)	42.1	H-5 and H-1’	C-3, C-5, and C-1’
5	HC-OH	4.37 (H, d)	68.2	H-4	C-2a, C-4, C-6 and C-5a
		4.17 (OH)		-	C-4 and C-5a
5a	Cq	-	109.1	-	-
6	C=O	-	183.0	-	-
7	CH	7.43(s)	124.6	-	C-10a, C-9, C-6, and C-4”
7a	Cq	-	123.8	-	-
8	Cq	-	133.1	-	-
9	CH	6.68 (d)	138.9	H-10	C-4”, C-10a and C-7
10	CH	7.61 (d)	120.8	H-9	C-8 and C-7a -
10a	Cq	-	153.2	-	-
1’	Cq	-	109.9	-	-
2’	CH	7.38 (s)	134.2	-	C-1’, C-3 and C-5’
3’	-O-	-	-	-	-
4’	CH	7.38 (s)	134.2	-	C-1’, C-5’ and C-6’
5’	Cq	-	110.9	-	-
6’	C-OH	9.68	153.8	-	C-5’ and C-6’
7’	CH	6.84 (s)	112.6	-	C-2, C-2a, C-3, C-5’ and C-6’
1”	CH	6.08 (dd)	135.3	H-2’ and H-4	C-3, C-4, C-5, C-2” and C-3”
2”	CH	5.41 (d)	127.7	H-1’	C-1”, C-3” and C-4

3”	CH_3	2.05 (s)	17.9	-	C-2”’
4”	CH_3	2.34 (s)	21.3	-	C-8

The isolated compound 3 (CG-3 fig.3) showed a quasi-molecular ion at m/z = 471.01821 $[M+ H]^+$ calculated, observed in the High-Resolution EIS-MS spectrum, which correspond to the molecular formula $C_{28}H_{22}O_7$, whit eighteen (18) of the insaturation degree. Examination of the 1D, ^{1}H-NMR spectra data of the compound 3 (CG-3) revealed of the presence three singlets peaks between at δ1,82, δ1,84 and δ2,28 characteristic attributed to three methyl groups. Nine alkenes protons between at δ7.34 x2 (s), and δ8.04 (s), attributed to the characteristic of signals alcens protons typical of isobenzofuron skeleton and δ6.90 (m), δ6.13 (s), δ5.44 (dd), δ5.34 (dd), δ6.83 (s) and δ6.92 (s) attributed to the characteristic of signals alcens proton typical for benzene skeleton. Regarding the range of multiplicity, the four signals of alcens protons between δ6.90 (m), δ6.13 (s), δ5.44 (dd) and δ5.34 (dd) (region of the linear alcens groups) indicate that these alcens protons signals are characteristic the presence of linear chain alkene proton in the compound 3 was indicated by the peaks in this region and their information of range multiplicity., and in the presence of the two hydroxyl protons between δ12.04 typical for phenolic and at δ5.27 attributed to the characteristic of signal hydroxyl proton of the linear chain alkyl (secondary alcohol group) and at the end, one signal at δ5.05 attributed to the signal of the methylene groups.

The ^{13}C 1D NMR spectrum of compound CG-03 in DEPT 135° mode revealed three methyl (CH_3), two methylene (CH_2), and seven methine (CH) carbons. In broad-band mode, the ^{13}C-1D NMR spectrum identified 28 carbons as vertical lines, including three characteristic signals at δ 179.3, 169.0, and 160.9. The first two signals are attributed to carbonyl carbons and the last signal is attributed to the quaternary alkenic carbon of the benzene ring carrying a labile proton with acidic properties as well as a hydroxyl proton (OH). Three thick peaks emerging at 19.2, 20.3, and 25.2 ppm, respectively, are unambiguously attributed to methyl group carbons. Next, a series of peaks corresponding to the alkenic carbons of the xanthone ring are observed, exiting at δC 108.7, 110.1, 117.7, 123.2, 125.6, 127.3, 130.6, 134.2, 152.5, and 154.9 ppm. Another series emerges at δC 114.1, 122.3, 124.5 (2C), and 148.0, which is attributed to the benzofuran ring carbons. The outgoing carbon signals at 114.3, 125.8, 134.5, and 137.5 ppm are attributed to linear alkenic carbons. The peak at 56.3 ppm is attributed to the methylene carbon.



5-hydroxy-4-(hydroxymethyl)-8-(2-methylprop-1-enyl)-6-oxo-10-
6H-isobenzofuro [4, 5-c] Xanthen-13yl-acétate

Figure 3: Structure of compound-3 (CG-03).

Table 3: ^{1}H and ^{13}C chemical shift, the correlation ^{1}H-^{1}H (COSY) and important HMBC correlation of compound-3.

Position		1D-NMR experiments		2D-NMR experiments	
N°	Types	δH (ppm) and multiplicity	δC (ppm)	COSY	HMBC
1	-O-	-	-	-	-
2	Cq	-	123.4	-	-
2a	Cq	-	154.9	-	-
3	Cq	-	134.2	-	-
4	Cq	-	110.1	-	-
5	C-OH	12.04 (s)	160.9	-	C-4 and C-5a
5a	Cq	-	108.7	-	-

6	C=O	-	179.9	-	-
7	CH	6.92 (s)	125.6	-	C-10a, C-9, C-6, and C-3”
7a	Cq	-	123.2	-	-
8	Cq	-	127.9	-	-
9	CH	6.83 (s)	130.6	-	C-10a, C-7, C-1” and C-3”
10	Cq	-	117.7	-	-
10a	Cq	-	152.5	-	-
1’	Cq	-	122.3	-	-
2’	CH	7.38 (s)	134.2	-	C-1’, C-3 and C-5’
3’	-O-	-	-	-	-
4’	CH	7.38 (s)	134.2	-	C-1’, C-5’ and C-6’
5’	Cq	-	124.5	-	-
6’	Cq	-	148.0	-	-
7’	CH	8.04 (s)	144.1	-	C-2, C-2a, C-3 and C-6’
8’	C=O	-	169.0	-	-
9’	CH_3	2.28 (s)	20.3	-	C-8’
1”	CH	6.90 (m)	134.5	H-2’a and H-2’b	C-9, C-10, C-10a and C-2”
2”	CH_2	5.34 (dd)	114.3	H-1’ and H-2’b	C-1” and C-10
		5.44 (dd)		H-1’ and H-2’a	
3”	CH	6.13	125.8	-	C-7, C-8, C-9, C-4” and C-5”
4”	Cq	-	137.5	-	-
5”	CH_3	1.82 (s)	19.2	-	C-4”
6”	CH_3	1.84 (s)	25.2	-	C-4”
7”	CH_2OH	5.02 (2H, s)	56.3	-	C-3, C-4 and C-5
		5.27 (OH, s)			C-4

3.2. Antimicrobial activity

The antimicrobial activity of the three pures compounds from the dichloromethane extract against microorganisms was determined. The results are shown in Tables 4 and 5. All bacteria demonstrated some degree of sensitivity to the purse principles within the concentrations tested. The compound-1 (CG-01) displayed antimicrobial activity against all bacterial strains tested with the inhibition zones varying from 7.50 to 17.50 mm (Table 5). B. cereus, S. aureus, E. coli, P. aeruginosa and C. albicane are the most sensitive strains while B. subtilis, E. cloacae and S. typhii are the least sensitive. The compound-3 (CG-03) displayed interesting bioactivity on all tested germs showing the inhibition zones from 11 to 20 mm. All the germs were very sensitive. The compound-2 (CG-02) was inactive on B. subtilis, E. cloacae and S. typhii; but it was bioactive on B. cereus, S. aureus, E. coli, P. aeruginosa and C. albicane. The inhibition zones varied from 13 to 16 mm. The minimum inhibitory concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values ranged from 0.035 to 10 µg/mL (Table 5).

Table 5: Inhibitory effect of the three purse compounds against bacteria (expressed as the inhibition zones of bacterial growth).

Bacterial strains	Diameter of inhibition zones (mm)							
	Concentration 2.5µg/ml (n=6)				Concentration 5µg/ml (n=6)			
	CG-1	CG-2	CG-3	E.$CHCl_3$	CG-1	CG-2	CG-3	E.$CHCl_3$
B. cereus	17,50	16,20	20,30	16,42	30,20	31,25	33,63	22,5
B. subtilis	7,50	1.23	11,04	5,50	12.90	4,01	16,45	6,50
S. aureus	15,30	14,18	16,04	13,28	25,20	26,13	28,01	17,01
E. colis	16,50	13,26	12,50	10,21	23,21	25,10	21,40	16,20
E. cloacae	8.25	1.04	11,98	4,10	13,22	2,77	16,42	7,11
P. aeruginosa	17,13	14,02	17,03	11,50	28,00	28,10	29,53	19,30
S. Typhii	8.01	1.75	11,53	5,00	14,11	4,31	17,33	6,80
C. albicane	15,43	16,02	15,74	11,01	26,32	23,07	21,06	17,05

Table 6: Inhibitory effect of the three purse compounds against bacteria (expressed as the Minimum Inhibitory Concentration MIC and the Minimum Bactericidal Concentration MBC).

Souche Microbiennes	CMI (µg/ml)				CMB (µg/ml)			
	CG-1	CG-2	CG-3	E.$CHCl_3$	CG-1	CG-2	CG-3	E.$CHCl_3$
B. cereus	0,035	0,035	0,035	0,250	0,035	0,035	0,035	0,250
B. subtilis	5,00	3,50	1,250	2,250	5,00	3,50	1,250	2,250

S. aureus	0,125	0,125	0,125	0,312	0,125	0,125	0,125	0,312
E. colis	0,312	0,312	0,312	1,250	0,312	0,312	0,312	1,250
E. cloacae	7,25	7,50	1,250	3,750	7,25	7,50	1,250	3,750
P. aeruginosa	0,065	0,065	0,065	0,614	0,065	0,065	0,065	0,614
S. Typhii	6,25	10,00	2,250	5,000	6,25	10,00	2,250	5,000
C. albicans	0,125	0,125	0,125	0,750	0,125	0,125	0,125	0,750

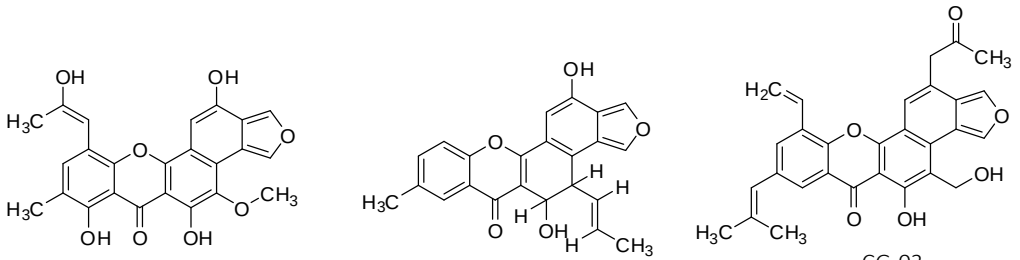
DISCUSSION

Madagascar is scientifically recognized in terms of biodiversity due to its endemicity[5], which reaches almost 80% of the plants listed[4], and the majority of these plants are used in traditional medicine.[11] Moreover, in Madagascar, traditional medicine holds an important place in society[20] because of its customs and the inadequacy of medical infrastructures.[21] Ethnobotanical information gathered from populations in the south and south-west of the Big Island led to the selection of *Cymbopogon giganteus Chiov var. madagascariensis* (Poaceae), known by its vernacular name of "Ahibero" because it has been used in this region to treat fever, general fatigue, bacterial inflictions and coughs. The literature mentions that the aerial part of *C. giganteus Chiov variety madagascariensis* has a β2-adrenergic vasorelaxant activity[17], and the vast majority of species in the Cymbopogon genus are aromatic plants.[23] Many authors have studied the chemical composition and some of the biological activities of essential oils from these species.[35-37] Some of these species also contribute to biodiversity conservation worldwide.

Because of its very pleasant scent, *Cymbopogon giganteus* is used in traditional African medicine, most often on its own, sometimes in association with other plants, with uses varying from country to country.[27]

The results of biological studies carried out on the different extracts of the aerial part of *C. giganteus Chiov var. madagascariensis* collected at Ankidona-Manja in the western part of Madagascar show that the dichloromethane extract has antimicrobial activity and the ethyl acetate extract has an antioxidant effect. All these results of the biological studies have helped to justify the ethnobotanical data concerning the empirical uses of this plant.

Application of the bioguided fractionation method to the dichloromethane extract resulted in the isolation of three pure antibacterial products, and their chemical structures were elucidated using spectral analysis techniques (fig.6).



Structural analysis has shown that these three compounds share a common basic structure called isobenzofurano-Xanthone (Fig.7). This basic structure is due to the association of two functions: isobenzofurone and Xanthone include in the phenolic compound chemical family. Phenolic compounds are known for their antioxidant and antibacterial activities.[34]

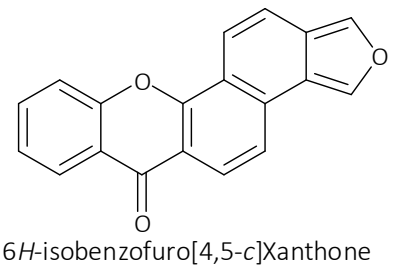


Figure 7: Basic structure of isolated active principle.

Bibliographic research carried out on these three molecules revealed that they were not described in the literature, nor were their biological activities. Hence they are all new molecules, and this is the first time they have been isolated from the Cymbopogon genus.

CONCLUSION

Ethnobotanical surveys carried out in south-west Madagascar led to the selection of a plant known by the Sakalava name Verompoly (Cymbopogon giganteus Chiov. var. madagascariensis Poaceae). This plant is very important in the region due to its therapeutic properties.

Biological screening tests on the extract of this plant's aerial parts have confirmed its traditional uses. Applying bioguided fractionation techniques to the dichloromethane extract of the plant's aerial parts enabled us to isolate three pure active principles, and the chemical structures of which were elucidated using NMR

(1D and 2D) and HR-MS spectral analysis methods. These are all derivatives of complex phenolic Isobenzofurano [4,5c] Xanthone compounds. Bibliographical studies on these three molecules show that they are new, and these are the first to be isolated from *Cymbopogon giganteus Chiov. var. madagascariensis* (Poaceae). All of our study results can be used to further the chemotaxonomy of Madagascan plants.

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