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Vasodilator effects of *Cymbopogon pruinosus* (Poaceae) from Madagascar on isolated rat thoracic aorta and structural elucidation of its two bioactive compounds

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Abstract
The aerial part of *Cymbopogon pruinosus* is widely used in the Southern part of Madagascar for the treatment of hypertension. The aims of the present study were to analyze the vasorelaxant properties of different extracts from *C. pruinosus* and to isolate and characterize bioactive secondary metabolites.
An ethno-pharmacological survey was conducted in the south of Madagascar about medicinal plants used in folk medicine to treat hypertension. The vasorelaxant activity of various extracts (n-Hexane, Dichloromethane, Ethyl acetate and Ethanol) from the most cited plant *C. pruinosus* was carried out on rat aorta ring. The chemical structures of the pure compounds were determined by LC/MS/NMR.
The ethyl acetate extract was the most effective. The ethyl acetate extract inhibited phenylephrine contraction in isolated rat thoracic aorta. Bioassay-guided fractionation of this extract led to the isolation and structural characterization of two bioactive pure compounds (named PY-1 and PY-2) which exhibited very good vasorelaxant activities with the EC_{50} values of 0.0125 ± 0.006 $mgml^{-1}$ and 0.00731 ± 0.0018 $mgml^{-1}$ respectively. The bioactive compounds were attributed respectively to Scopoletin (PY-1) and Bis(2-ethyl hexyl)phthalate (DEHP). The vasorelaxant potency of the bioactive extract was diminished in the absence of endothelium and by a pre-treatment with propranolol, a β_2 adrenergic receptor blocker, which was however not affected by indomethacin pre-treatment. These findings indicated that the vasorelaxant effect of *Cymbopogon pruinosus* may be partially endothelium dependent, mediated by nitric oxide and that vasoactive prostanoids might not be contributing to the vasorelaxation effect.
C. pruinosus possess vasorelaxant activity on isolated organs. The ability of plant extracts and its isolates in this study to cause relaxation of the aortic rings pre-contracted with phenylephrine may represent a rational explanation for the use of the plant species to treat hypertension by Malagasy traditional healers.

Keywords: Traditional medicine, Cymbopogon *pruinosus*, hypertension, bioactivity validation, Madagascar.

1. Introduction
Recent reports indicate all over the word that hypertension is a major cardiovascular disease with the high epidemiological impact and represent risk factor for developing other diseases such as endothelial dysfunction, metabolic syndrome, etc. [1, 2]. In Africa, the prevalence of hypertension is between 31.1 and 32.5% [3]. In Madagascar, 28.05% of adult populations suffer from this disease with an average mean age population of 49 years [4]. Several plants in Malagasy were reported to have pharmacological relevance for the local communities [5-8]. It is also known that in Africa, the first line of treatment for poor people is the use of herbal medicine at home [9-14].
During an ethno-botanical survey, 80% of traditional practitioners interviewed reported that the aerial part of *Cymbopogon pruinosus,* known as Ahibero in Malagasy language, is used by the local communities to treat conditions assumed to be hypertension. This plant could be promising source of vasorelaxant secondary metabolites. The aims of this study were to evaluate the vasorelaxant properties of some extracts obtained from the aerial part the *Cymbopogon pruinosus* using rat aorta ring as model and to elucidate the chemical structures of bioactive compounds.

2. Materials and Methods

2.1. Ethno-botanical Survey

Ethno-botanical information about the plant species selected for this study was obtained from 20 traditional healers during a field work in the South of Madagascar. Informants were selected for their authentic knowledge on the utilization of medicinal plants Malagasy, the national language of Madagascar was used during anthropological interviews. Traditional healers were, interviewed on a voluntary basis. The study followed principles laid out in the declaration of Helsinki as previously reported [5, 10]. Informed consent was obtained from both the Government of Madagascar to collect plant samples and to conduct non-commercial research on Malagasy medicinal plants and the respondents to divulge their knowledge.

2.2. Selection and collection of plant material

The plant species *Cymbopogon pruinosus* (Nees ex Steud.) Chiov (*syn. Cymbopogon giganteus Subsp. Madagascariensis* Chiov., family Poaceae) was selected based on its relative citation frequency (use values 0.61) and the information consensus factor value (0.317). The aerial part of *C. pruinosus* was collected in Befoly village, district of Toliara-II (Southern part of Madagascar) on May 2013. 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 DUEL-01 was deposited at the herbarium of Laboratory of Applied Chemistry, Lay Flaylle Street University of Toliara-Madagascar.

2.3. Phytochemical studies

2.3.1 Extraction and chemical screening

The plant material (6 Kg of *C. pruinosus*) was kept at room temperature (25 to 30 °C) for air drying (two week). The air-dried and powdered material (2 kg) was extracted by repeated maceration with ethanol 90° (3x4 hrs, 5l) at room temperature. After filtering the mixture, the aqueous-ethanol filtrates were pooled, dried over Na_2SO_4 and evaporated to dryness under reduced pressure using a rotary evaporator to yield crude ethanolic extract (30.11 g). Twenty-six grams (26 g) of the crude ethanolic extract were suspended in water and sequentially partitioned with n-hexane, dichloromethane, and ethyl acetate (1:1, v/v) to yield the corresponding extract fractions. The different extracts were evaporated to dryness on an evaporator apparatus and were evaluated for their pharmacological properties to verify and to localize the active fraction. All extracts were stored at +4 °C. Chemical screening was done in aqueous and organic extracts according to a well-known protocol [15].

2.3.2 Bio-guided isolation

Bioassay-guided extraction revealed interesting activity only with the ethyl acetate extract fraction. This fraction displayed a good vasorelaxant effect on both phenylephrine pre-contracted rat aortic ring with intact endothelium and on endothelium-denuded aortic ring. Ten grams (10 g) of the ethyl acetate crude extract was first subjected to fractionation, using a silica gel column chromatography eluted with n-hexane and gradient of ethyl acetate (9:1 to 1:9) resulting into eight fraction (F_1-F_8). The fraction F_5 showed strong vasorelaxant activity on both phenylephrine pre-contracted rat aorta ring. Then 700 mg of the fraction F_5 was resubmitted to separation by silica gel column chromatography eluting with a mixture of n-hexane/dichloromethane/acetone (2:7:1), the column was in isocratic regime and at the end, it resulted into seven fractions. Two fractions, F_{54} andF_{55} showed strong vasorelaxant activities. These fractions were checked for purity by analytical TLC, and the zone was detected with a UV lamp 254 and 365 nm and spraying with sulfuric vanillin acid, followed by heating at 120 °C for 1-5 min.F_{54} andF_{55} 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 (50:50), and the column was in isocratic regime at the end, it resulted into five fractions. Each fraction was tested at 1µg/ml for its vasorelaxant activity on rat aorta contracted by phenylephrine. The fraction F_{553} showed the highest vasorelaxant activity which was further investigated. Then 20 mg of the fraction was subjected to further purification by preparative TLC using n-hexane/ethyl acetate/acetone (2.5:6:1.5) as the solvent affording compounds PY-1 (6.28 mg) and PY-2 (7.01 mg).

The two pure compounds showed strong vasorelaxant activities on both phenylephrine pre-contracted rat aorta thoracic ring, with the EC_{50} values were 0.0125 ± 0.006 $mgml^{-1}$ and 0.00731 ± 0.0018 $mgml^{-1}$ respectively.

2.4. Pharmacological studies

2.4.1 Animals

Wistar rats weighing between (250 ± 25) g were used for all experiments. Animals were kept under conditions of controlled temperature (24 ± 1) °C and a 12 hrs light/dark cycle. They freely access to food and tap water. All experimental procedures were approved by the Malagasy Institute of Applied Research (IMRA) Avarabohitra- Itaosy lot AVB 77, P.O. Box 3833, 102 Antananarivo- Madagascar.

2.4.2 Drugs/Reagents

The fallowing drugs were used: ethanolic crude extract from the aerial part of *Cymbopogon pruinosus*, their fractions (n-hexane, dichloromethane, ethyl acetate), two pure compounds (PY-1 and PY-2), phenylephrine (PE), Indomethacin (IND), Propranolol, Dimethyl sulfoxid (DMSO) and ethyleneglycol-bis-N,N'-tetra acetic acid (EGTA), were purchased from sigma chemical company (St. Louis, MO,USA). Ethanol, ethyl acetate, dichloromethane and n-hexane were purchased from Scharlab (Barcelona-Spain). For all experiments, the different extracts were diluted dimethyl sulfoxid/deionized water (DMSO/dH_2O) mixture.

2.4.3 Preparation of the Krebs-Henseleit Solution

The composition of Krebs-Henseleit solution was (in mM): KCl: 4.75; NaCl: 118.5; $NaHCO_3$: 25; Glucose: 11.1; $MgSO_4$: 1.2; KH_2PO_4: 1.2; $CaCl_2$:1.36. The K^+ depolarizing solution (KCl: 60 and 80mM) were prepared by replacing 60 or 80 mM KCl in the Krebs solution with equimolar NaCl. In nominally zero Ca^{2+} solution, $CaCl_2$ was omitted and 0.5 mM EGTA was added as previously reported [5].

2.4.4 Preparation of isolated aortic rings from Wistar rat and experimental conditions

Vascular isometric tension was evaluated by organ bath technic as previously described [5, 16-18] with minor modification. Briefly, their animals (Wistar rats of either sex weighing between 250 ± 25 g) were sacrificed, the thoracic aorta isolated and cut into rings of 5mm in length. The rings were mounted under a tension of 1 g in organ bath containing Krebs-Henseleit solution. When required, the endothelium was removed from the rat aorta rings by gently rubbing the luminal

surface with a cotton rod before mounting the aorta ring in the organ bath.

The organ bath was filled with a Krebs-Henseleit solution maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2.

The rings were allowed to equilibrate for 1h under a resting tension of1g using micromanipulator until a constant base force was established. During this time, the bathing medium was measured after every 15 min. The isometric tension was measured using a force transducer and recorded digitally using a data acquisition system and the stored and analyzed with computer program.

After the equilibration period, all time were exposed repeatedly to 1 µM phenylephrine solution in order to test their contractile capacity. Once the response to phenylephrine had reached a stable plateau, the aortic rings were acetylcholine (1 µM). Two series of experiments were carried out in order to assess the vasorelaxant activity of plant extract. The first ones used isolated rat aorta with the intact endothelium and second series on endothelium-denuded aortic tissues. The presence of functional endothelium was confirmed by the ability of acetylcholine to induce superior 50% relaxation of ring pre-contracted with phenylephrine. In experiment involved denuded aortic rings, a relaxation ≤ 10% by acetylcholine indicated satisfactory removal of endothelium and only such tissues were used for experiments.

2.4.5 Effect of extract on the contraction induced by phenylephrine

Two tonic responses to PE (0.001 $moll^{-1}$) which stabilized in 10 min were registered. After a third response, different concentrations of various extracts were added cumulatively to isolated aortic preparations. Some experiments were also conducted in which PE was added to the tissue and left for at least 30 min to observe whether the tension was maintained during the period. The activity of extracts on resting tone of aorta was also studies. The relaxations were measured by comparing the developed tension before and after addition of extracts and curves were constructed.

2.4.6 Effect of inhibitors on the vasorelaxant activity of ethyl acetate extract.

- **Effect of propranolol (β_2 adrenergic receptor blocker)**

In order to investigate the role of cAMP mediated biochemical signaling pathway in the vasorelaxant activity of ethyl acetate, aorta ring were pre-treated for 30min with propranolol (10^{-5} $moll^{-1}$) prior to PE contraction. After that, all the isolated rat thoracic aortic rings have been contracted with PE (10^{-6} $moll^{-1}$). After pre-treatment of Wistar rat aorta rings by propranolol, ethyl acetate extract cumulative concentrations, ranging from 0.03 to 1 mg/ml, were evaluated for their vasorelaxant effects. The effect of plant extract on contraction induced by PE was compared in the absence and presence of propranolol.

- **Effect of indomethacin (non-selective cyclooxygenase inhibitor)**

In order to investigate the role of prostacyclin (PGI_2) on the vasorelaxant activity of ethyl acetate, aorta ring were pre-treated for 30 min with indomethacin (10^{-5} $moll^{-1}$) prior to PE contraction. After that, all the isolated rat thoracic aortic rings have been contracted with PE (10^{-6} $moll^{-1}$). After pre-treatment of Wistar rat aorta rings by indomethacin, ethyl acetate extract cumulative concentrations, ranging from 0.03 to 1 mg/ml, were evaluated for their vasorelaxant effects. The effect of plant extract on contraction induced by PE was compared in the absence and presence of indomethacin.

2.5. Statistical analysis

The relaxant effect of the tested products (extracts and pure compounds) was expressed as percent of the steady-state contraction induced by the agonist and antagonist. The log values of EC_{50} which is defined as the concentration producing 50% of the maximum response, or the IC_{50} (inhibition concentration by 50%) were determined from the non-linear regression of the experimental data using Prism or Graph Pad software package. The results were expressed as the mean ± SEM of six determinations. The difference between the mean values was tested for statistical significance using Student's paired t-test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Ethno-botanical and chemical screening

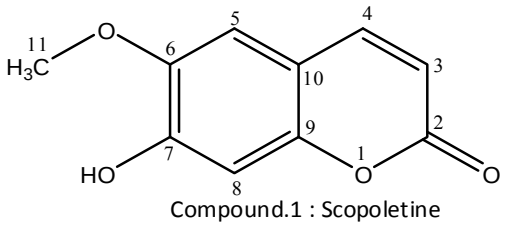
During ethno-botanical survey, twenty (20) traditional healers were interviewed about medicinal plants of ethno-pharmacological relevance in Malagasy folk medicine for the treatment of hypertension. The most cited plant *Cymbopogon pruinosus* (Poaceae) has the use value and informant consensus factor of 0.613 and 0.371 respectively.

The results of chemical screening of *Cymbopogon pruinosus* (aerial part) revealed the presence of coumarins, flavonoids, steroids, terpenoids, polyphenols, and tannins. However, chemical groups such as alkaloids, anthocyanins, leuco-anthocyanins, anthraquinones, and saponins were not found in the investigated plant material. The presence of various secondary metabolites in this plant species could justify it ethno-medical use.

3.2. Isolation and structural elucidation

The ethanolic crude extract from the aerial part of *Cymbopogon pruinosus* was suspended in water and was partitioned successively with different organic solvent of increasing polarity (n-hexane, dichloromethane, ethyl acetate). The vasorelaxant 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 bioactive compounds named PY-1 and PY-2, as evidenced by analytical TLC and at the end, in pure forms as proved by HPLC analysis.

The isolated compound 1 (PY-1, fig. 1), showed a quasi-molecular ion at m/z = 193.04776 $[M+H]^+$ calculated, observed in the High-Resolution EIS-MS spectrum which corresponds to the molecular formula $C_{10}H_8O_4$. The ^{1}H-NMR spectrum showed characteristic singlet attributed to methoxy group δ3.63, and four protonsδ5.44 (d), δ5.90 (s), δ6.58 (s), and δ7.52 (d) typical for benzo-pyron skeleton, and at the end one proton acid attributed to hydroxyl proton of phenol at δ10.03.



Compound.1 : Scopoletine

Fig 1: Structure of compound 1 (PY-1)

Examination of the ^{13}C-NMR (Broad Band and DEPT), and the HSQC spectra data of the pure compound 1 revealed the presence of one carbonyl carbon at δ163.2 (C-2), and eight (8) alcens carbons (C=C) double bound typical for benzenes skeleton at δ98.1 (C-3), δ101.1 (C-8), δ105.2 (C-5),δ143.3 (C-4), δ148.7 (C-7), δ149.9 (C-6), δ151.1 (C-10), and δ154.4 (C-9), and at the end, one methoxy group (OCH_3) at δ53.6 (C-11). The ^{1}H and ^{13}C chemical shift values of individual spin systems were determined by correlation in the 2D HSQC spectrum.

The individual ^{1}H and ^{13}C chemical shift assigned by the ^{1}H-^{1}H COSY spectrum and 2D HSQC and HMBC correlation spectra, are presented in table 1. The UPLC-MS-UV analysis (fig 2) showed that at retention time of Tr = 3.1 min, Compound 1 (fig. 2a) was identical to Scopoletin (reference molecule, fig.2b) confirming that the chemical structure of the pure compound PY-1 corresponds to Scopoletin.

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

Position	1D-NMR experiments		1D-NMR experiments	
			Homonuclear correlation	Heteronuclear correlation
	δ ^{1}H	δ ^{13}C	COSY	HMBC
1				
2		163.2		
3	5.44 d	98.1	H-4	C-2 and C-9
4	7.52 d	143.3	H-3	C-2, C-5 and C-10
5	6.58 s	105.2		C-7 and C-10
6		149.9		
7	10.03 (OH)	148.7		C-6 and C-8
8	5.90 s	101.1		C-6 and C-9
9		154.4		
10		151.1		
11	3.63 s	53.6		C-5

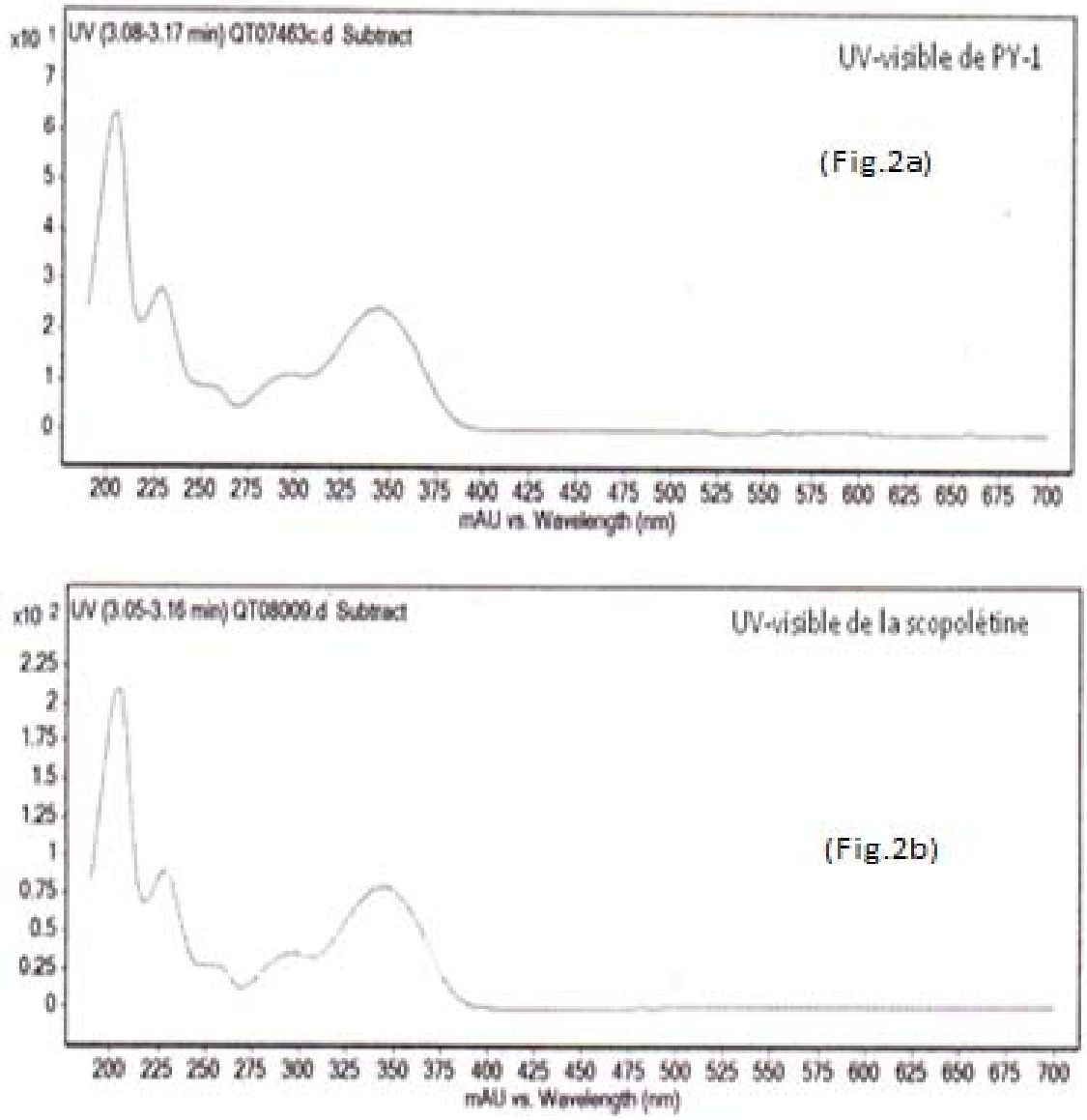


Fig 2: UPLC-MS-UV- analysis spectrum

In addition to fragments of molecular ion identified by MS (fig.3), data about four peaks of molecular ion parents respectively at m/z = 178.02493 indicated to the methyl parted of the phenol méthoxy group, the peak at m/z = 150.03138 attributed at 2 hydroxy-5-vinylbenzene-1,4-bis(olate), indicated to the carbonyl function (C=O) parted, the peak m/z = 133.02764 attributed at 2- hydroxyl-5-vinylphenolate observed at decarboxylation of reaction on benzo-pyron, and at the end, the peak at m/z = attributed at 2-hydroxybenzaldehyde. The fragments molecular mechanism of compound 1 is given in figure 4.

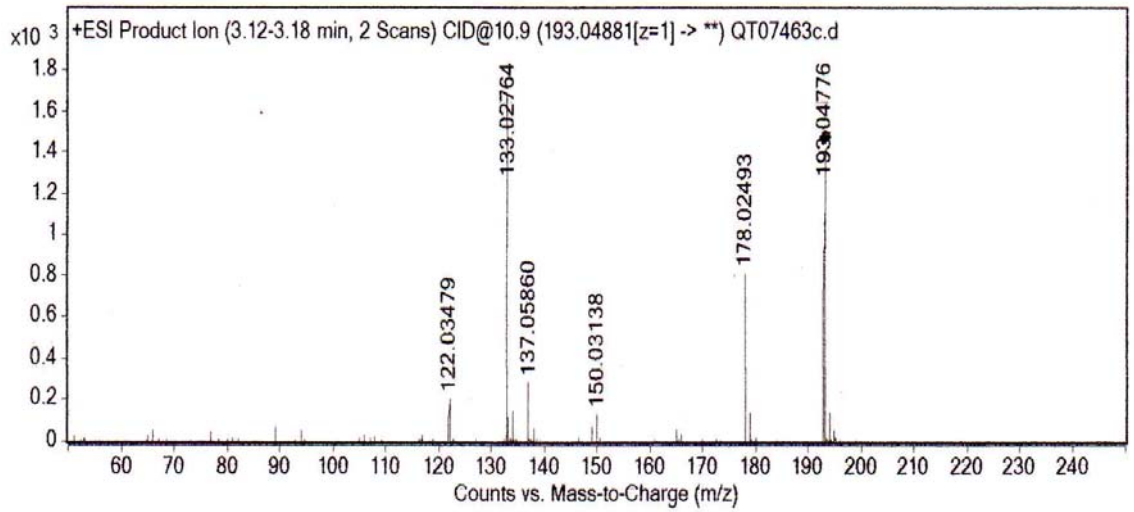


Fig 3: Mass spectrum of PY-1

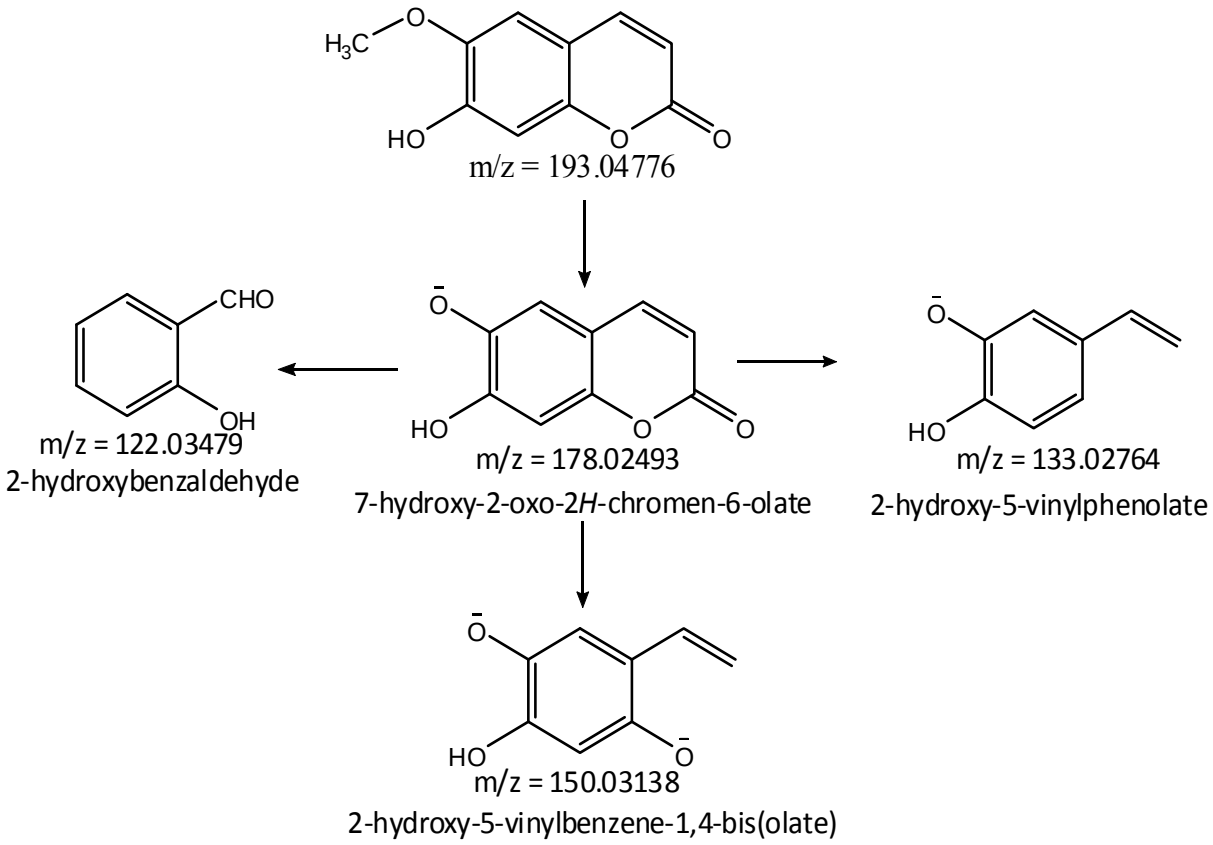


Fig 4: The fragments important for the structure elucidation of PY-1

The molecular formula of the pure compound-2 (PY-2) was determined to be $C_{24}H_{38}O_4$ by ESI-TOF-SM (m/z = 803.54397 $[2M + Na]^+$; m/z = 413.26691 $[M + Na]^+$ and m/z = 391.28312 $[M + H]^+$) (fig. 5) and 1D, 2D-NMR experiments. UV analysis result (fig. 6) shown that the absorption band was at λ_{max} = 225 nm with a shouldering at λ = 275 nm, a characteristic indicating the presence of benzenes skeleton with linked to the carbonyls functions. Examination of the 1D 1H-NMR, spectra data of the compound PY-2 revealed of the presence seven signals at δ0.86 (t, J= 7.5), δ0.89 (t, J=7.5), δ1.28 (m),δ1.29 (m), δ1.32 (m), δ1.36 (m), and δ1.63 (m), characteristic attributed to the linear chain typical of alkyl group, two doublet-doublet between δ4.13 and δ4.16, characteristic attributed to the signals of geminate protons of different chemical environments of the methylene group with linked to the oxygen atom (O-CH_2-). Two signals alcens protons between δ7.68 (m) and δ7.72 (d), attributed to the characteristic of alcens signals typical for benzene skeleton. The 1D ^{13}C broad band NMR spectrum contained 12 signals of the carbons, including one the carbonyl group between δ_C167.4 (C-7). Examination of the 1D ^{13}C (DEPT), and the 2D HSQC spectra data of the PY-2 revealed the presence of about free alcens carbons(C=C) double bound typical for benzenes skeleton at δ_C129.1 (C-6), δ_C132.1 (C-5) and δ_C132.2 (C-1), and seven signals carbons at δ_C11.3 (C-8'), δ_C14.4 (C-6'), δ_C22.9 (C-4'),δ_C23.7 (C-7'), δ_C28.8 (C-5'), δ_C30.3 (C-3') and δ_C38.6(C-2'), a characteristic attributed to linear chain of the alkyl group, and at the end, one signal carbon attributed to the characteristic of méthoxy group between δ_C67,9 (C-1').

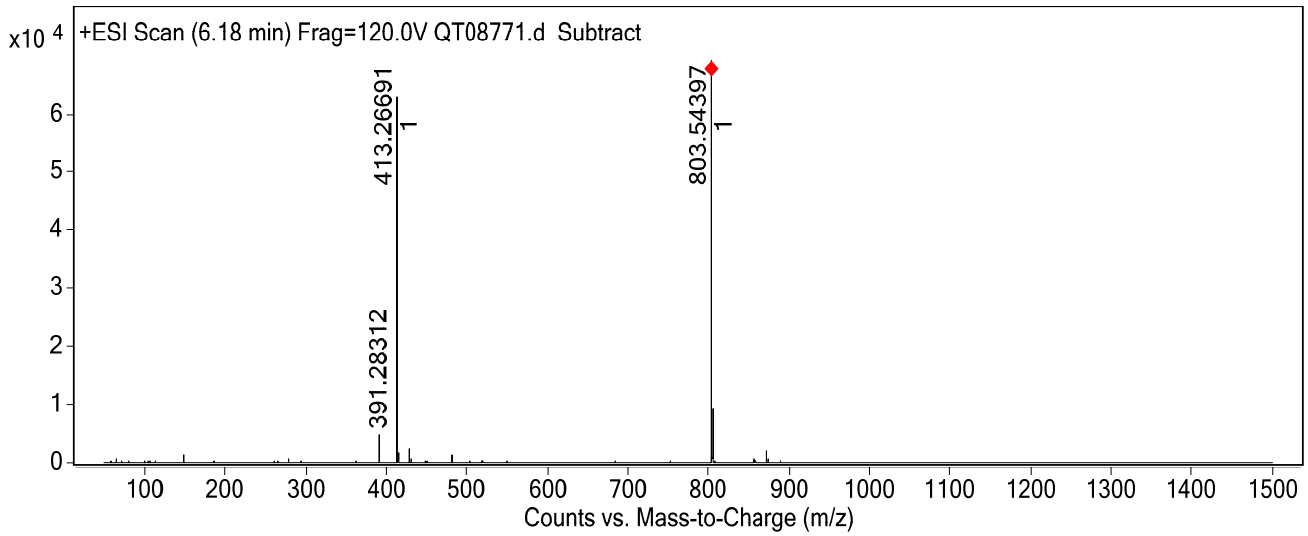


Fig 5: Mass spectrum of PY-2

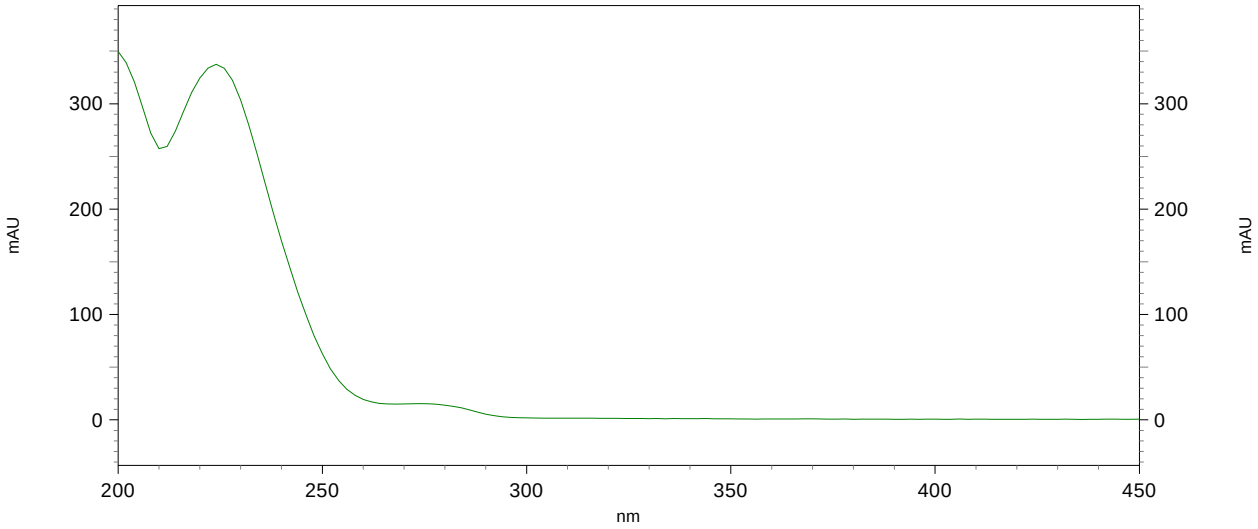
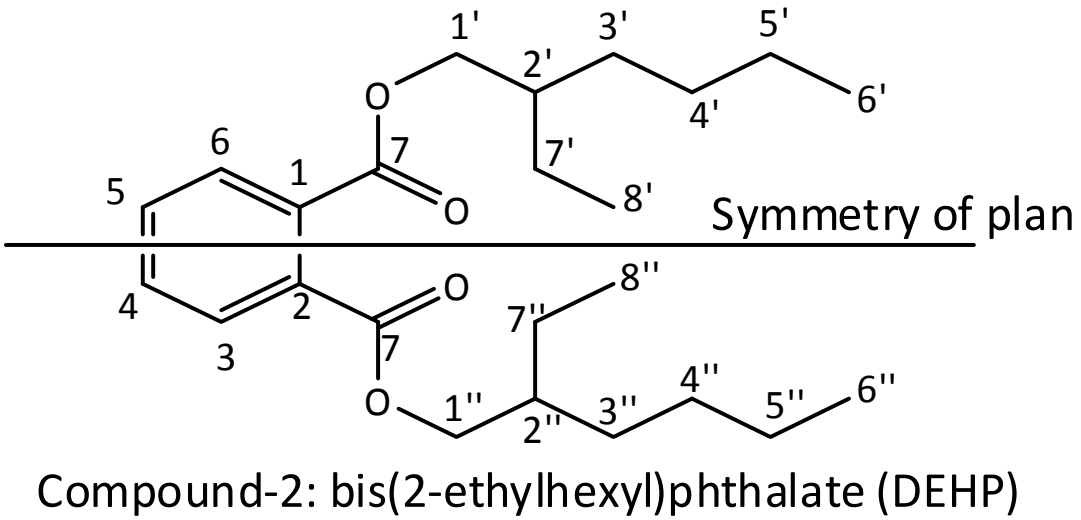


Fig 6: UPLC-MS-UV- spectrum analysis of PY-2

According to mass spectrometry analysis, compound PY-2 is constituted of 24 carbons. However the examination of the 1D, 2D-NMR show the presence 12 signals carbons, whereas the compound PY-2 presented to the symmetry plan (fig 7). The ^{1}H and ^{13}C chemical shift values of individual spin systems were determined by correlation with the 2D HSQC spectrum. The individual ^{1}H and ^{13}C chemical shift assigned by the ^{1}H-^{1}H COSY spectrum and 2D HSQC and HMBC correlation spectra respectively (table 2).



Compound-2: bis(2-ethylhexyl)phthalate (DEHP)

Fig 7: Structure of compound 2 (PY-2)

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

Position		1D-NMR experiments		2D-NMR experiments	
	Types	δ ^{1}H	δ ^{13}C	**COSY**	**HMBC**
1	Cq	-	132,2		
2	Cq	-	132,2		
3	CH	7,72 d	129,1	H-4	C-1, C-5 and C-7
4	CH	7.68 m	132,1	H-3 and H-5	C-2 and C-6
5	CH	7.68 m	132,1	H-4 and H-6	C-1 and C-3
6	CH	7,72 d	129,1	H-5	C-2,C-4 and C-7
7	CO	-	167,4	-	-
1'H_a	CH_2	4,13 dd (J=11Hz)	67,9	1'H_b and H-2'	C-7,C-2',C-3' and C-7'
1'H_b		4,16 dd (J=5.5Hz)		1'H_a and H-2'	C-7,C-2',C-3' and C-7'
2'	CH	1,63 m	38,6	H-1a',H-1b, H-3'and H-7'	C-1',C-3',C-7' and C-8'
3'	CH_2	1,32 m	30,3	H-2'and H-4'	C-5'
4'	CH_2	1,29 m	22,9	H-3' and H-5'	C-5'
5'	CH_2	1,29 m	28,8	H-4' and H-6'	C-6'
6'	CH_3	0,86 t(J=7.5Hz)	14,4	H-5'	C-4' and C-5'
7'	CH_2	1,36 m	23,7	H-2' and H-8'	C-1',C-2',C-3'and C-8'
8'	CH_3	0,89 t (J=7.5Hz)	11,3	H-7'	C-2' and C-7'
1''H_a	CH_2	4,13 dd (J=11Hz)	67,9	1''H_b and H-2''	C-7, C-2'',C-3'' and C-7''
1''H_b		4,16 dd (J=5.5Hz)		1''H_a and H-2''	C-7, C-2'',C-3'' and C-7''
2''	CH	1,63 m	38,6	H-1''a,H-1''b, H-3''and H-7''	C-1'',C-3'', C-7'' and C-8''
3''	CH_2	1,32 m	30,3	H-2''and H-4''	C-5''
4''	CH_2	1,29 m	22,9	H-3'' and H-5''	C-5''
5''	CH_2	1,29 m	28,8	H-4'' and H-6''	C-6''
6''	CH_3	0,86 t(J=7.5Hz)	14,4	H-5''	C-4''and C-5''
7''	CH_2	1,36 m	23,7	H-2'' and H-8''	C-1'',C-2'',C-3''and C-8''
8''	CH_3	0,89 t(J=7.5Hz)	11,3	H-7''	C-2'' and C-7''

3.3. Pharmacological *studies*

3.3.1. Vasorelaxant effects of *C. pruinosus* extracts on rat aortic rings

The vasorelaxant activities of *C. pruinosus* ethanolic extract and its fractions (hexane, dichloromethane, and ethyl acetate) were studied on phenylephrine (10^{-6} M) pre-contracted rat aortic rings with intact endothelium and on endothelium-denuded aortic rings. The addition of the ethanolic extract and fractions different to the incubation medium resulted in a concentration-dependent relaxation. The vasorelaxant responses expressed as EC_{50} are given in table 3. From this table, it can be noticed that ethyl acetate extract was the most potent extract. This fraction displayed a good vasorelaxant activity on both phenylephrine pre-contacted rat aortic rings with intact endothelium and on endothelium aortic rings. In addition, the contribution of endothelium in the vasorelaxant effect of DEL-ACoET was remarkable. These results conducted to undertake further studies for elucidating the mechanisms of action of the most bioactive fraction.

Table 3: Relaxant effects induced by the aerial part of on the aortic rings with endothelium or without endothelium contracted with the Phenylephrine (10^{-6}M, n= 6).

Extracts	Aorta with intact endothelium		Aorta without endothelium	
	CE_{50} (mgml^{-1})	**E_{max} (%)**	**CE_{50} (mgml^{-1})**	**E_{max} (%)**
DEL	0.313 ± 1.800	81.280 ± 0.400	0.383 ± 1.800	80.68 ± 0.400
DEL-Hex	1.570 ± 0.090	40.000 ± 0.006	1.470 ± 0.090	41.31 ± 0.006
DEL-DCM	0.801 ± 1.200	58.670 ± 1.300	0.861 ± 1.200	51.47 ± 1.300
DEL-ACoET	0.215 ± 1.500	84.010 ± 0.060	0.201 ± 1.500	85.31 ± 0.060

(Legend: DEL ethanolic extract, DEL-Hex n-hexane soluble extract, DEL-DCM dichloromethane soluble extract, DEL-ACoET ethyl acetate soluble extract).

3.3.2. Elucidation of mechanisms of action of ethyl acetate soluble extracts (DEL-ACoET)

- **Involvement of nitric oxide (NO) in the vasorelaxant effect of *the ethyl acetate extract***

Figure 8 gives the concentration-response curves of the aerial part of *Cymbopogon pruinosus* ethyl acetate fraction on the rat aortic rings with endothelium or without endothelium contracted with the phenylephrine 10^{-6} M. This figure indicates that DEL-ACoET has bioactivity on both pre-contracted aortic ring with and without endothelium. However, this vasorelaxant effects is marked by the presence of the endothelium suggesting a probable implication of NO and others pathways. The mean values of the EC_{50} of relaxation of DEL-ACoET on rat isolated aorta with [(+) E] endothelium and without [(-) E] endothelium pre-contracted with the phenylephrine (10^{-6} M) were 2.85 ± 0.05 µgml^{-1}(E_{max} = 57.36 ± 2.4%) and 3.08 ± 0.04 gml^{-1} (E_{max} = 37.61 ± 1.91%) respectively.

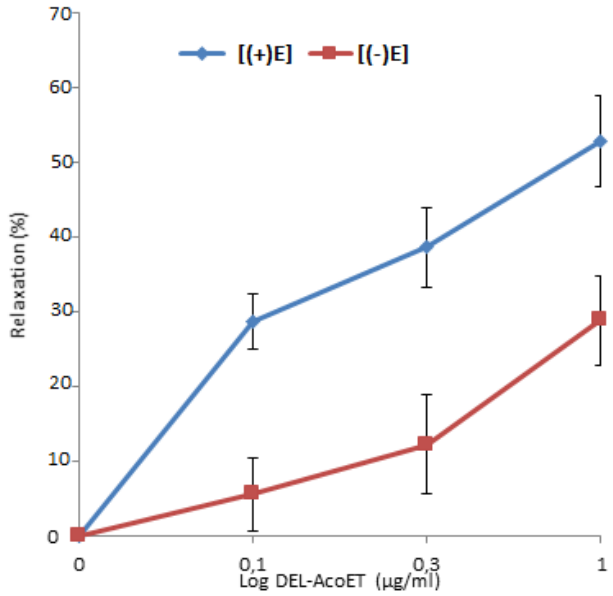


Fig 8: Concentration-response curves of ethyl acetate soluble extract from of the aerial part of *Cymbopogon pruinosus* on the aortic rings with endothelium or without endothelium contracted with the phenylephrine (10^{-6}M, n = 6). Log [DEL-AcoET] is the logarithm of the concentration of DEL-AcoET (ethyl acetate soluble extracts) expressed in µg/ml.

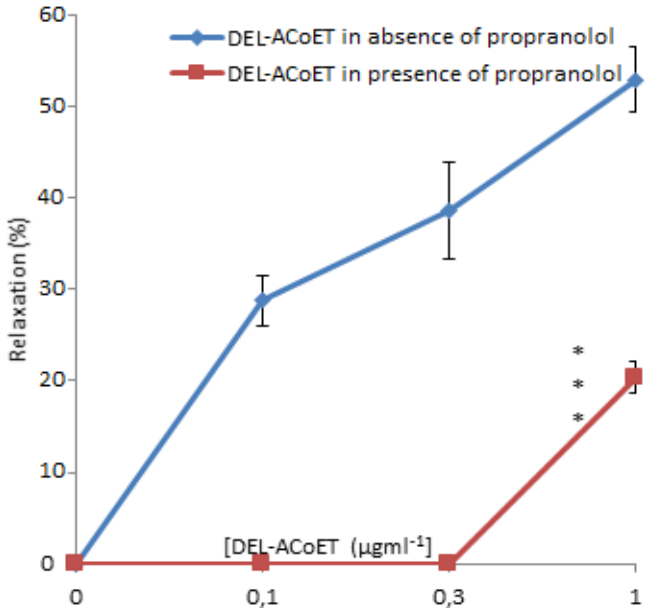


Fig 9: Concentration-response curves of ethyl acetate soluble extract from of the aerial part of *Cymbopogon pruinosus* the aortic rings contracted with the phenylephrine (10^{-6} M), in the presence or absence of 10^{-5} M propranolol (n = 6, $^{**}p<0.01$).

- **Effect of propranolol on the bioactivity of DEL-ACoET**

Figure 9 gives the effect of propranolol on the vasorelaxant activity of the ethyl acetate soluble extract. This figure indicated that the vasorelaxing effect caused by DEL-ACoET was significantly reduced with pre-incubation of propranolol. The mean values of the EC_{50} of relaxation of DEL-ACoET on rat isolated aorta treated with or without propranolol (10^{-5} M) were 2.85 ± 0.05 µgml^{-1} (E_{max} = 57.36 ± 2.4%) and 4.48 ± 0.01 µgml^{-1} (E_{max} = 21.18 ± 0.91%) respectively. Propranolol is an inhibitor of β_2 adrenergic receptor blocker. So DEL-ACoET could act through the interferences with the β_2 adrenergic receptor blocker.

- **Effect of indomethacin on the bioactivity of DEL-ACoET**

Figure 10 gives the effect of indomethacin on the vasorelaxant activity of ethyl acetate soluble extract from of the aerial part of *Cymbopogon pruinosus*.

This figure indicated that the vasorelaxing effect caused by DEL-ACoET was not significantly reduced with pre-incubation of indomethacin. The mean values of the EC_{50} of relaxation of DEL-ACoET on rat isolated aorta treated with or without indomethacin (10^{-5}M) were 2.85 ± 0.05 µgml^{-1} (E_{max} = 56.26 ± 1.85%) and 2.15 ± 0.03 µgml^{-1} (E_{max} = 55.08 ± 2.91%) respectively. Thus, it could be postulated that prostacycline (PGI_2) is not implicated in the vasorelaxant activity of DEL-ACoET.

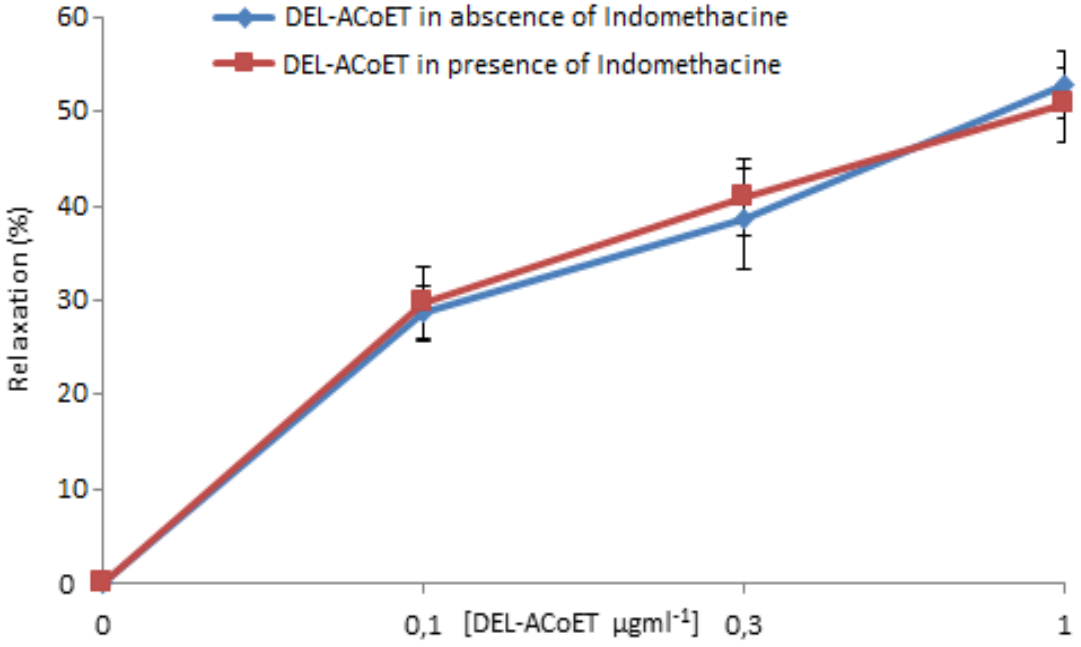


Fig 10: Concentration-response curves of ethyl acetate soluble extract from of the aerial part of *Cymbopogon pruinosus* the aortic rings contracted with the phenylephrine (10^{-6} M), in the presence or absence of 10^{-5} M indomethacin (n = 6)

4. Discussion

Nowadays, hypertension represents the first cause mortality among all cardiovascular diseases [19]. Our ethnobotanical investigation conducted in the south of Madagascar revealed that the aerial part of the plant known under the vernacular name of Ahibero (Malagasy name) and scientifically named as *Cymbopogon pruinosus* is used by the local communities to fight against this illness. This plant is well-known and very important recipe in this region because of its therapeutic values in the Malagasy traditional medicine. In addition, the majority of people in the south part of Madagascar rely on traditional medicine for the health care needs, because the cost of conventional drugs is unaffordable for them [6, 7, 14]. We showed here that ethanolic crude extracts of its aerial part exhibited significant concentration-dependent relaxation on rat aorta pre-contracted by phenylephrine (10^{-6} M) (table 3), and the result of phytochemical screening of the plant extract revealed the presence of many secondary metabolites such as coumarins, flavonoids, steroids, terpenoids, polyphenols, and tannins thus confirming/validating the ethno-medical use of this plant species in traditional pharmacopoeia of Madagascar.

The ethyl acetate extract fraction displayed a good vasorelaxant effect on phenylephrine pre-contracted rat aortic ring. The vasorelaxant induced by ethyl acetate extract was weak on endothelium-denuded rings indicating the role of Endothelium-Derived-Relaxing Factor (EDRF), in the vasorelaxant activity of DEL-ACoET. The results of the present study revealed also that pretreatment of aorta with propranolol significantly reduce the vasorelaxant activity of DEL-ACoET (fig. 9) providing strong evidence that this plant extract could act by interfering with β2-adrenergic-receptor. The vasorelaxant activity of DEL-ACoET was not modified in aortic rings pre-treated with indomethacin. Phytochemical screening revealed the presence of the shikimic acid biogenetic pathway derived compounds such as phenolics (coumarins, tannins, flavonoids), and terpenoids and steroids, which are well-known for the treatment of cardiovascular affections [20-22].

Bioassay-guided fractionation of the active fraction led to the isolation and structural elucidation of two pure compounds PY-1 and PY-2 which exhibited very good vasorelaxant activities.

Chemically, the two bioactive compounds derived from the same biogenetic pathway with dihydroxycinnamic acid as precursor as shown in figure 11. The difference in the bioactivity of these two molecules is due to the difference in their chemical structures. Coumarins based compounds such as Scopoletin were reported to possess vasorelaxant effects [23] while DEHP is reported for the first time through this work to have such property.

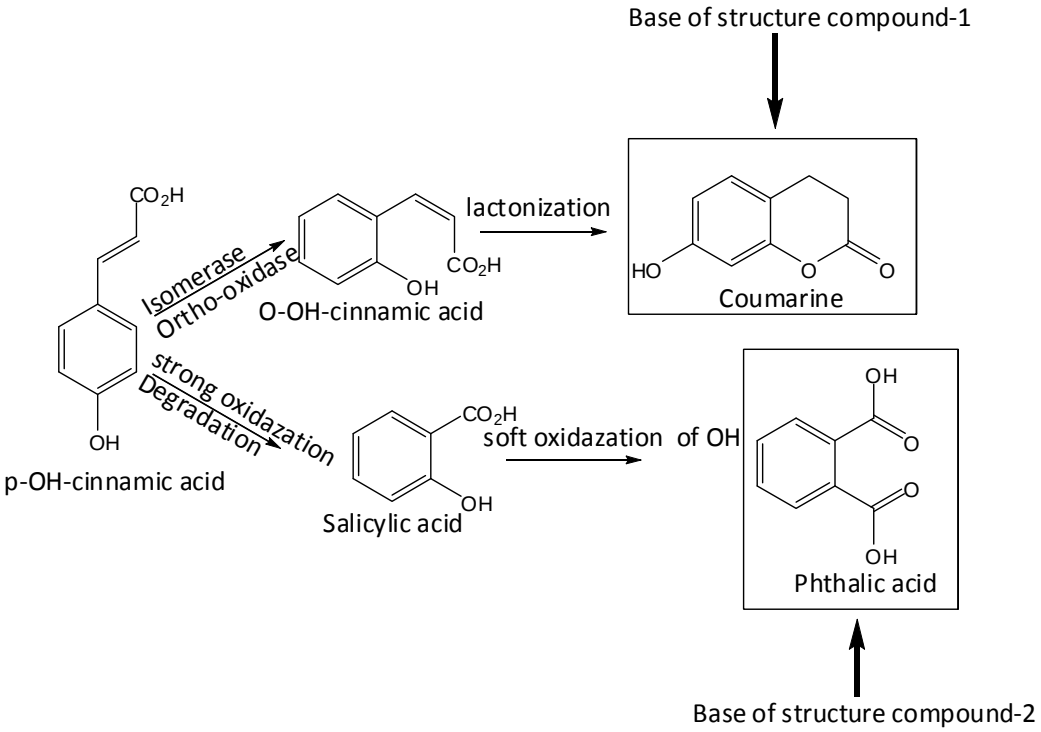


Fig 11: Possible biogenesis pathways of the two pure compounds

5. Conclusions

The present study evaluated the vasorelaxant effects of *Cymbopogon pruinosus*. This plant species and its isolates displayed promising bioactivity. Ethyl acetate extract inhibited Phenylephrine induced contraction in isolated rat thoracic aorta. The vasorelaxant potency of *Cymbopogon pruinosus* was diminished in the absence of endothelium and by a pre-treatment with propranolol, which was however not affected by indomethacin pre-treatment. These findings indicated that the vasorelaxant effect of *Cymbopogon pruinosus* may be partially endothelium dependent, mediated by nitric oxide and that vasoactive prostanoids might not be contributing to the vasorelaxation effect. The ability of this plant species to display such pharmacological property represents a rational explanation for the use of this medicinal plant as valuable source of vasodilator agents.

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