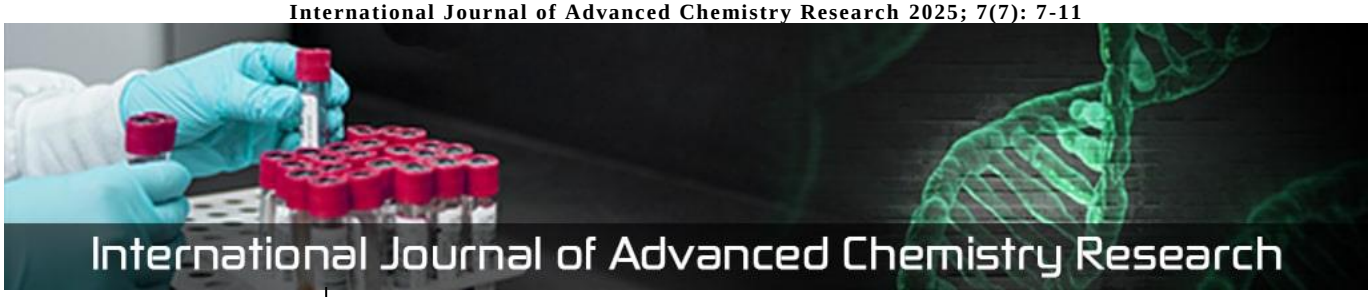

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Structural determination of an isolated vasodilatory molecule from *Passiflora edulis* Sims (Passifloraceae)

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Abstract
This study aimed to isolate and characterize a vasodilatory compound from *Passiflora edulis* Sims using bioassay-guided fractionation, chromatographic and spectroscopic techniques. The bioactive molecule was isolated from the ethyl acetate fraction using preparative thin-layer chromatography and structurally elucidated via spectroscopic methods (UV, 1D- and 2D NMR, MS). The vasodilatory activity of the isolate was investigated on isolated rat aorta. Thin Layer Chromatography revealed two principal compounds in the ethyl acetate fraction with retention factors (R_f) of 0.65 and 0.54. The compound with R_f 0.65 demonstrated significant vasorelaxant effects on norepinephrine-precontracted aorta with an EC_{50} of 3.64×10^{-5} M. Comparison of chemical shifts (δ_H) and spin-spin coupling constants (J_{HH}) with literature data confirmed the identity of the isolate (D65) as 8-methylheptadecane. This discovery bridges traditional knowledge with modern pharmacological research, offering new perspectives for cardiovascular drug development from natural sources.

Keywords: 8-methylheptadecane, *Passiflora edulis*, vasodilatory activity

Introduction
Cardiovascular disorders represent the leading cause of morbidity and mortality worldwide, with arterial hypertension serving as a primary risk factor for cardiovascular disease development [1,2]. Despite the availability of conventional antihypertensive medications including calcium channel blockers, diuretics, angiotensin II receptor antagonists, angiotensin-converting enzyme inhibitors, and beta-blockers [3,4,5,6] Stephen *et al*., 2024), the search for novel therapeutic agents remains crucial.
Traditional medicine systems, founded on indigenous knowledge and cultural practices, continue to provide valuable resources for hypertension management. *Passiflora edulis* leaves have been documented for their antihypertensive properties in traditional medicine across several regions, including Madagascar [7,8]. Our previous investigations have demonstrated the plant's antihypertensive, diuretic, and vasodilatory activities [9,10].
Elucidating the mechanism of action of ethnomedicinal preparations is essential to understand their pharmacological effects and predict potential adverse reactions [11,12]. Systematic investigation is necessary to establish both safety and efficacy profiles, thereby enabling evidence-based utilization with consequent social, health, and economic benefits. In this study, we report the isolation and structural characterization of a vasodilatory compound from *P. edulis* with potential applications in hypertension management. This approach aligns with the historical contribution of plant-derived compounds to pharmaceutical development, where natural products have provided starting points for over 50% of currently used drugs [13].

Materials and Methods
Chemicals
All solvents (hexane, chloroform, ethanol, methanol, and ethyl acetate) of analytical grade were obtained from Labosi. Deuterated methanol (MeOD), deuterated chloroform ($CDCl_3$), and components for Krebs-Henseleit solution preparation were purchased from Sigma-

Aldrich Inc. (St. Louis, MO, USA). Standard 5 mm, 7″ NMR tubes (XR-55 series) were sourced from Norell Inc. (Landisville, NJ, USA).

Animal handling

Male Wistar rats (200-250 g) were maintained at the Laboratory of Pharmacology General, Pharmacokinetics and Cosmetology (LPGPC) animal facility under standard conditions (12/12 h light/dark cycle, 22 °C ambient temperature). Animal care and experimental protocols were approved by the bioethics committee of the Sciences Faculty, University of Antananarivo, Madagascar (approval number: CBQ20/029).

Sample preparation

Passiflora edulis leaves were collected from Antsirabe, Vakinakaratra region, Madagascar. The plant material was transported to the laboratory and maintained at ambient temperature until processing. A voucher specimen was authenticated and deposited at the herbarium of Parc Botanique et Zoologique de Tsimbazaza (PBZT), Antananarivo, Madagascar.

The leaves were thoroughly washed with tap water to remove extraneous matter and shade-dried at room temperature for 30 days. The dried material (200 g) was pulverized using a Brook Crompton© série 2000 grinder.

Bioassay fractioning

The powdered material underwent sequential extraction with hexane, dichloromethane, ethyl acetate, and methanol using a Soxhlet apparatus for 72 h per solvent. After extraction, solvents were removed under reduced pressure using an Evapotec® rotary evaporator to yield dry extracts. The percentage yield of extracts was then determined.

All crude extracts were evaluated for vasodilatory activity on isolated thoracic aorta pre-contracted with norepinephrine. The extract demonstrating the highest activity was selected for further isolation procedures.

Isolation of the vasodilatory molecule

The bioactive compound was isolated using precoated preparative silica gel (GF_{254}) glass base plates (Merck, 107747) with 1 mm thickness. The ethyl acetate fraction was deposited on preparative chromatographic plates and developed using an ethyl acetate-methanol (95:5) (v/v) solvent system. After development, plates were dried, and separated compounds were visualized under UV light (254/365 nm). The retention factor (R_f) was calculated for each band, and compounds were recovered by scraping. Purity assessment was conducted using two-dimensional TLC on aluminum base precoated silica gel GF_{254} plates (Merck) developed with ethyl acetate-methanol (70:30) (v/v) and ethyl acetate-methanol (50:50) solvent systems.

Vasodilatory investigation

Vasodilatory activity of the isolated compound was investigated on isolated aorta contracted with norepinephrine. The rats were sacrificed under thiopental sodium at the dose of 50 mg/kg (Pentothal Sodium, Abbott, Turkey) and the thoracic aorta was removed. Ring segments (4 mm long) were prepared in Krebs-Henseleit solution (composition in mM: NaCl: 118, KCl: 4.7, $MgSO_4$: 1.2, KH_2PO_4: 1.2, $NaHCO_3$: 25, $CaCl_2$: 2.5, and D-Glucose: 10.6). The aortic rings were mounted between two wire hooks and suspended in organ baths containing 10 mL Krebs-Henseleit solution, maintained at 37 °C, and bubbled with 95% CO_2 and 5% O_2. The isolated aorta was washed at 15-minute (min) intervals. After 60 min of equilibration under the resting tension of 1 g, the organ was subjected to a viability test and sensibilisation by exposure to norepinephrine 10^{-4}M (dissolved in distilled water) prior to the execution of the experimental protocol. After this test, the preparation was rinsed and left to stabilise for 30 minutes, during which it was rinsed every 15 minutes. Afterwards, the organ was contracted with norepinephrine injected in the bath in a cumulative manner until maximum contraction, then the isolate (D65) was injected in the bath in a cumulative manner to get concentration from 10^{-10} M in the bath until complete relaxation. Isometric contraction and relaxation were recorded using force displacement transducer. The responses were expressed as percentage relaxation from maximal norepinephrine precontraction.

Data analysis

Vasorelaxation induced by D65 was expressed as percentage relaxation relative to norepinephrine-induced contraction. Results are presented as mean ± standard error of the mean (SEM), where n represents the number of aortic preparations. Statistical analysis was performed using one-way ANOVA followed by Student's *t*-test to evaluate significant differences between relaxation responses at various concentrations of D65. Differences were considered statistically significant at $p<0.05$.

Structural determination of the bioactive isolate

The chemical structure of the isolated compound was elucidated using spectroscopic methods including UV spectroscopy, 1D- and 2D- Nuclear Magnetic Resonance (NMR) spectroscopy (Bruker FT-NMR Avance-500 spectrometer, Ettlingen, Germany), and mass spectrometry (Q-TOF) at the Department of Pharmacognosy and Herbal Medicine, School of Pharmacy, University of Ghana, Legon, Accra, Ghana.

All NMR experiments were conducted at a temperature of 300 K to ensure consistency and accuracy throughout the analysis. The ^{1}H NMR spectra were acquired at 500 MHz using the "zg30" pulse sequence optimized for proton spectroscopy. Measurements were performed using a 500 MHz Bruker spectrometer with AVANCE console equipped with 5 mm TXI and TCI triple resonance inverse detection cryoprobes with z-axis pulse field gradient. Spectral data processing was conducted using Bruker Topspin software (version 3.6.3).

Results and discussion

Determination of the active fraction

This study aimed to isolate and characterize a vasodilatory compound from *Passiflora edulis* leaves, traditionally used for hypertension management in rural Madagascar. Our previous studies demonstrated that the hydroalcoholic extract of these leaves reduces hyper-sodium diet-induced hypertension through diuretic and vasodilatory mechanisms [14,15].

To identify the bioactive principles responsible for the vasodilatory effect, bioassay-guided fractionation was undertaken. Comparative evaluation of different fractions (hexane, ethyl acetate, and methanol) revealed that while the hexane fraction exhibited no vasodilatory activity, the ethyl

acetate fraction demonstrated superior potency compared to the methanolic fraction, with EC_{50} values of 0.99 and 2.49 mg/ml, respectively (p<0.05) (Fig 1). Consequently, the ethyl acetate fraction was selected for further isolation procedures.

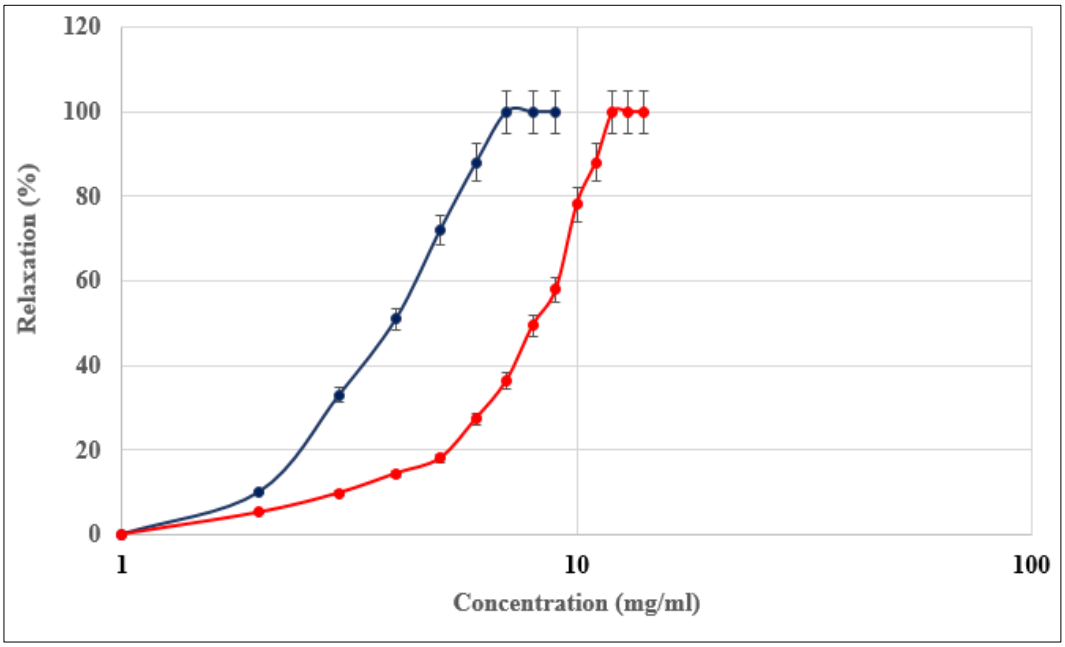

Fig 1: Relaxation of guinea pig isolated aorta pre-contracted with norepinephrine in the presence of ethyl acetate and methanolic extracts, injected in the bath in a cumulative manner ($\bar{x} \pm \bar{\sigma}$; n = 6; p<0.05)

Identification of an active isolate

Separation of the ethyl acetate fraction by preparative chromatography using ethyl acetate-methanol (70:30) (v/v) as the mobile phase revealed three distinct spots with R_f values of 0.91, 0.6, and 0.08 when visualized under UV light at 254 and 365 nm.

Pharmacological evaluation on norepinephrine (10^{-5} M) pre-contracted isolated aorta demonstrated that the isolate with R_f 0.6, designated as D65, exhibited the most potent vasorelaxant activity. When administered cumulatively to the organ bath, D65 induced concentration-dependent relaxation with an EC_{50} of 3.64×10^{-5} M (Fig 2). These findings indicate that D65 represents one of the principal compounds responsible for the vasodilatory and consequent antihypertensive effects of *P. edulis* leaves.

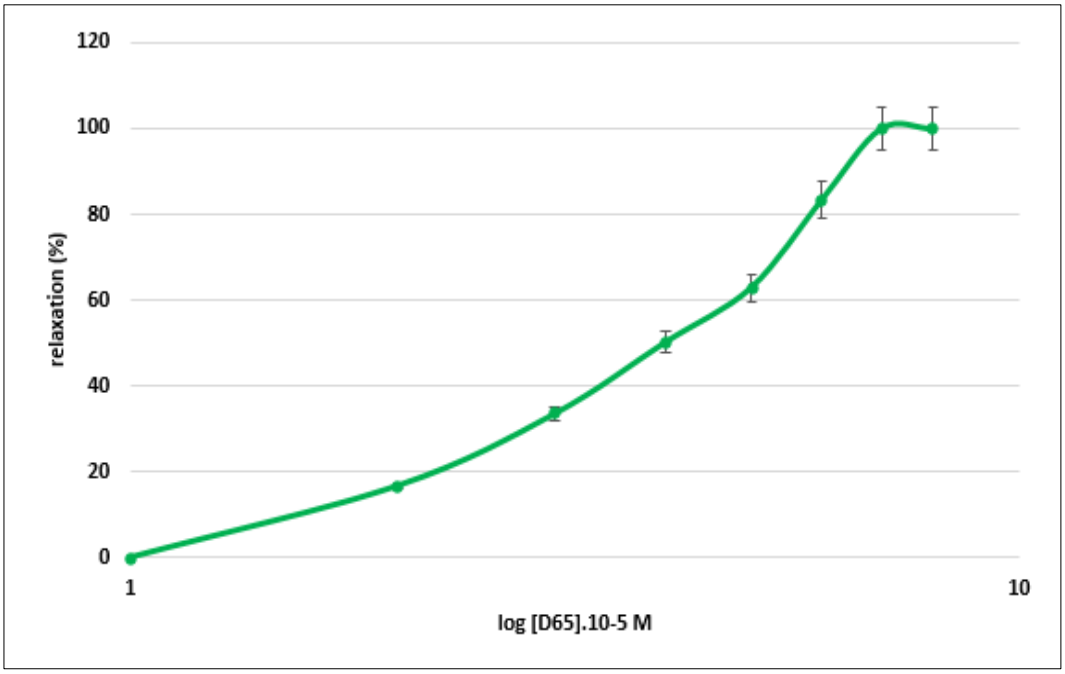

Fig 2: Relaxation of rat isolated aorta pre-contracted with norepinephrine in the presence of D65, injected in the bath in a cumulative manner ($\bar{x} \pm \bar{\sigma}$; n = 6; p<0.05)

Structural determination of D65

Spectroscopic analysis identified compound D65 as 8-methylheptadecane (Fig 3). The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data for D65 ($CDCl_3$, δ in ppm, J in Hz) are presented in Table 1.

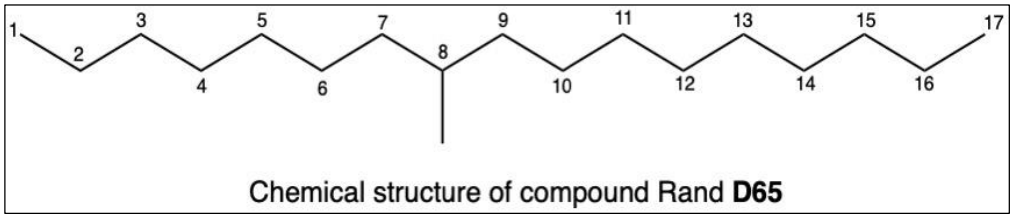

Fig 3: Chemical structure of 8-methylheptadecane

Table 1: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data for compound D65 ($CDCl_3$, δ in ppm, *J* in Hz)

No.	^{13}C-NMR	^{1}H-NMR
1	11.10	
2	19.95	0.87 m
3	22.88	
4	32.97	
5	33.44	1.28 br, s
6	37.32	
7	39.09	
8 (CH)	29.89	
9	32.15	1.28 br, s
10	30.4	
11	30.26	1.28 br, s
12	29.93	
13	22.92	1.31 br, s
14	26.97	
15	27.31	1.27 br, s
16	29.59	
17	11.63	0.82
Me-	14.34	0.90 m

The ^{13}C-NMR and ^{1}H-NMR data were consistent with those reported for 8-methylheptadecane in the literature [16].

Conclusion

This study demonstrates the significant vasorelaxant effect of 8-methylheptadecane (D65) isolated from *Passiflora edulis* leaves on thoracic aorta isolated from rats. The ability of this aliphatic hydrocarbon to induce aortic relaxation provides scientific validation for the traditional use of *P. edulis* leaves in hypertension management. These findings suggest that D65 could represent a promising lead compound for the development of novel antihypertensive agents. Further investigation, including comprehensive *in vivo* pharmacological evaluation, pharmacokinetic profiling, and toxicological assessment, is warranted to fully characterize the therapeutic potential of this compound.

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