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Identification of a vasodilator compound isolated from the fruit of *Sechium edule* (Jacq.) Swartz (Cucurbitaceae)

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Abstract

The present paper aimed to identify a vasodilatory isolate from *Sechium edule* fruit. The hydroalcoholic extract of *Sechium edule* fruit has been proven to have vasodilatory activity. After a bio guided fractionation, ethyl acetate fraction exhibited the highest activity. Preparative chromatography was used to isolate a bioactive compound from this fraction. The structure was determined using spectroscopy (UV, NMR 1 and 2D, MS). Its vasodilation activity was assessed on rat isolated aorta precontracted with norepinephrine. Analytical chromatography shows 3 spots at R_f 0.27, 0.36 and 0.9. Our results show that the spot with R_f 0.36 possesses a vasodilator activity. It relaxes isolated aorta pre contracted with norepinephrine with EC₅₀ equal to 37.16 µg/ml. Chemical shifts (δH) and spin-spin coupling constants (JHH) data were compared with literature to confirm the isolate as 24-hydroxymethyl-23,26,27-trihydroxy-cucurbit-5-ene-3-yl-acetate, a cucurbitane derivate.

Keywords: Cucurbitane, isolate, *Sechium edule*, vasodilation

Introduction

According to the World Health Organization (WHO), more than 80% of the world's population rely on traditional medicine for their primary healthcare needs. The use of herbal medicines represents a long history of human interactions with the environment ^[1, 2]. In many cases the people claim the good benefit of certain natural or herbal products, and scientific investigations have reported the efficacy of medicinal plants, justifying their folklore use. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. Since plants used in traditional medicine contain a wide range of substances, identification of bioactive molecules is necessary ^[3].

Sechium edule (Jacq.) Sw., a member of the Cucurbitaceae family, has been the subject of some phytochemical and pharmacological investigations. Previous studies on its fruits have reported the presence of diverse secondary metabolites, including phenolic compounds, flavonoids, and cucurbitane-type triterpenoids, together with notable antioxidant, antidiabetic, antihypertensive, and vasorelaxant activities. Hydroalcoholic extract of the fruit has demonstrated significant biological effects *in vitro* and *in vivo*, supporting its traditional use in metabolic and cardiovascular disorders ^[4, 5, 6]. Our previous studies demonstrate its antihypertensive activity with diuretic and vasorelaxation properties of its hydro alcoholic extract ^[7, 8]. Despite these findings, the specific compounds responsible for the reported bioactivities remain incompletely characterized, and bioactivity-guided isolation studies are still limited. Therefore, the isolation and structural elucidation of active principles from *S. edule* fruits are warranted to better define the chemical entities underlying its pharmacological properties and to further expand the phytochemical knowledge of this species. The aim of the current study was to isolate a vasorelaxant molecule from ethyl acetate fraction of *S. edule* fruits.

Materials and Methods

Drugs and reagents: All the drugs and reagents used were with the required standard and analytical grade: hexane, chloroform, ethanol, methanol and ethyl acetate were purchased

from LABOSI, 141 Rue de JAVEL, Paris 75. Deuterated methanol (MeOD), deuterated chloroform (CDCl₃) and ingredients for making Krebs-Henseleit solution were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). Standard 5 mm, 7" NMR tubes (XR-55 series) were purchased from Norell Inc. (Landisville, NJ, USA). Chromatographic preparative plates (Ref 4856-840) were purchased from MERK (39 Rte Industrielle de la Hardt, 67120 Molsheim, France).

Animal handling: Wistar rats, weighing between 200 and 250 g were used in this work. They were bred at the animal house of Laboratory of General Pharmacology, Pharmacokinetic and Cosmetology of Sciences Faculty, University of Antananarivo, in standard conditions: 12/12 hours cycle of light and darkness and at room temperature of 22 °C. Care and use of the animals were approved by the bioethics committee of the Sciences Faculty, University of Antananarivo, Madagascar, registered as CEA/FS-UA/Registre n° 12/2023.

Bioassays fractionation: *Sechium edule* fruits were collected from Antanamitarana and Andranomanitra, two localities in the DIANA region, Madagascar. A voucher specimen was identified at the herbarium of Parc Botanique et Zoologique de Tsimbazaza (PBZT), Antananarivo, Madagascar. The collected samples were dried under shade, in aerated area at room temperature for 30 days; 200 g of dry materials were ground to powder using a grinder (BROOK CROMPTON® series 2000).

The powder obtained was subjected to successive Soxhlet extraction using hexane, dichloromethane, ethyl acetate, and methanol until exhaustive extraction was achieved. The extracts were evaporated to dryness under reduced pressure using a rotary evaporator (Evapotec®). Each fraction was evaluated for vasodilating activity on isolated thoracic aorta precontracted with norepinephrine, and EC₅₀ values were determined. The bioactive compound was subsequently isolated from the fraction exhibiting the highest activity [9].

Isolation of the vasodilatory molecule: The active compound was isolated from the ethyl acetate fraction by preparative chromatography using precoated silica gel GF₂₅₄ glass plates (Merck, 107747; 1 mm thickness). The sample was deposited on the preparative plates and developed with a dichloromethane-methanol solvent system (95:5, v/v). After development, the plates were air-dried, and separated bands were visualized under UV light at 254 nm. Retention factor (Rf) values were calculated, and individual bands were scraped and eluted. The purity of the isolated compound was confirmed by two-dimensional TLC using plastic-backed precoated silica gel GF₂₅₄ plates (Merck), developed with dichloromethane-methanol 95:5 and 92:8 (v/v) [10]. The compounds obtained from the different bands were evaluated for vasodilatory activity on isolated thoracic aorta precontracted with norepinephrine to identify an active compound.

Vasodilatory investigation

Vasodilatory activity of the different isolates was assessed on isolated thoracic aorta of rat according to the methods described by Kitchen (1984) [11]. The rat was anesthetized with thiopental sodium, injected intramuscularly at the dose of 50 mg/kg (Pentothal Sodium, Abbott, Turkey). The animal was exsanguinated by cutting the two aortas, and a thoracotomy was carried out. The aorta was identified, and

the descending thoracic aorta was immediately removed and freed from connective tissues. It was cut into small pieces of 3 mm length, and immediately mounted between two S-shaped stainless-steel hooks, under resting tension of 1 g, in an organ bath containing 20 mL Krebs-Henseleit physiological solution at 37 °C. The composition of the Krebs-Henseleit solution was as follows (in mmol/L): Na⁺ 137.4, K⁺ 5.9, Ca²⁺ 2.5, Mg²⁺ 1.2, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134 and glucose 11.5. The organ bath was continuously aerated with carbogen (95% O₂ + 5% CO₂ mixture) at a pH of 7.4. Experiments were performed in preparations with intact endothelium. The tissue was allowed to stabilize for about 45 min during which the bath was renewed every 15 min and the tension readjusted. After stabilization, the aorta was contracted with norepinephrine at a concentration of 10⁻⁴ M in the bath. Once a contraction plateau was achieved, the isolate was injected into the bath in a cumulative manner until complete relaxation of the precontracted organ, and tension changes of the tissue were recorded.

The effect of the different isolates was tested with isometric sensors (Model Gould - Statam). The vasorelaxation due to the different isolates from the ethyl acetate fraction is expressed as a percentage relaxation in response to contraction induced by norepinephrine. A concentration-effect curve of each isolate was traced on semilogarithmic scale and EC₅₀ was determined by linear regression. The isolate that exhibited the lowest EC₅₀ was considered as the most active compound and was the subject of structural determination [12].

Data analysis

Results were expressed as mean ± SEM and were subjected to biostatistical analysis. One-way and two-way ANOVA followed by Student's t-test were used to compare the different means. A value of *p* < 0.05 was considered as the level of significance.

Structural determination of the most active isolate

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

To ensure consistency and accuracy throughout the analysis, the experiment was conducted at a temperature of 300 K. The ¹H NMR spectrum was recorded at a frequency of 400 MHz, using the "zg30" pulse sequence optimized for proton spectroscopy.

Deuterated methanol (MeOD) from Sigma-Aldrich Inc. (St. Louis, MO, USA) and standard 5 mm, 7" NMR tube (XR-55 series) from Norell Inc. (Landisville, NJ, USA) were used. Measurement was taken on a 500 MHz Bruker spectrometer with AVANCE console and equipped with 5 mm TXI and TCI triple resonance inverse detection cryoprobes with z-axis pulse field gradient.

The structure of the isolate was identified by concerted analysis of the ¹H, ¹³C, HSQC, NOESY, COSY NMR spectra and infrared spectroscopy and by comparison with data from the literature [13].

Results and Discussion

Extraction yields: Evaporation of the solvents under reduced pressure using a rotary evaporator yielded a hexane fraction (3.1%), a dichloromethane fraction (2.18%), an

ethyl acetate fraction (1.03%), and a methanolic fraction (4.52%) from *Sechium edule* fruit.

Principal compounds in the different fractions

The ethyl acetate fraction of *Sechium edule* fruit contains a moderate content of phenolic compounds, steroids and triterpenes, alkaloids, flavonoids and polysaccharides. The methanolic fraction contains a moderate content of flavonoids and a low quantity of steroids, triterpenes and alkaloids. Both hexane and dichloromethane contain low quantities of steroids and triterpenes.

Vasodilator effect of the different fractions

Cumulative addition of norepinephrine to the organ bath induced a concentration-dependent contraction of the isolated aorta, with maximal contraction (100%) achieved at 10^{-4} M. The hexane and dichloromethane fractions of *Sechium edule* fruit did not relax the precontracted organ. The ethyl acetate and methanolic fractions produced a concentration-dependent relaxation of the norepinephrine-precontracted aorta, with EC_{50} values of 1.79 and 5.32 mg/mL, respectively (Fig 1).

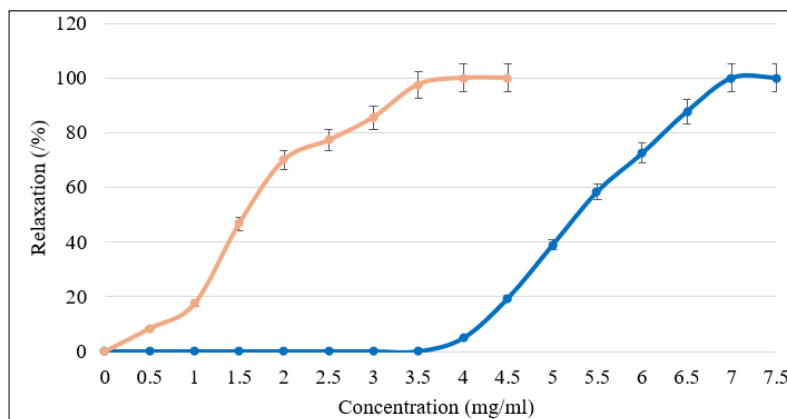


Fig 1: Relaxation of the isolated aorta precontracted with norepinephrine at the concentration of 10^{-4} M in the bath, in the presence of ethyl acetate (orange) and methanolic (blue) fractions injected in a cumulative manner in the bath ($\bar{x} \pm \text{sem}$; $n = 10$; $p < 0.05$)

These results demonstrate that the ethyl acetate and methanolic fractions of *Sechium edule* fruit exert significant vasorelaxant effects on norepinephrine-precontracted aortic rings. Based on EC_{50} values, the ethyl acetate fraction exhibits greater potency than the methanolic fraction. These results suggest that the bioactive constituents responsible for the vascular effects of *Sechium edule* fruit are predominantly enriched in the ethyl acetate fraction. The lack of vasorelaxant activity observed in the hexane and dichloromethane fractions further supports a polarity-dependent distribution of the active compounds [14, 15]. Collectively, these findings highlight the pharmacological potential of *Sechium edule* fruit as a source of natural antihypertensive agents and emphasize the need for further studies to isolate and structurally characterize the active constituents in this fruit, as well as to elucidate their mechanisms of action. Given the superior vasorelaxant

activity of the ethyl acetate fraction, subsequent isolation of the active compound was therefore performed from this fraction.

Identification of an active compound from ethyl acetate fraction:

Separation of the ethyl acetate fraction by preparative chromatography using dichloromethane-methanol (95: 5) (v/v) as the mobile phase revealed three distinct spots with R_f values of 0.27, 0.36 and 0.9 when visualized under UV light at 254 nm. After testing each isolate on pre contracted isolated thoracic aorta, the spot at R_f 0.36 exhibited the highest vasorelaxant activity. When injected cumulatively into the organ bath, it induced concentration-dependent relaxation with an EC_{50} of 37, 16 $\mu\text{g/ml}$ (Fig 2). These findings indicate that the molecule with R_f 0.36 is one of the principal compounds responsible for the vasodilatory action of *Sechium edule* fruit.

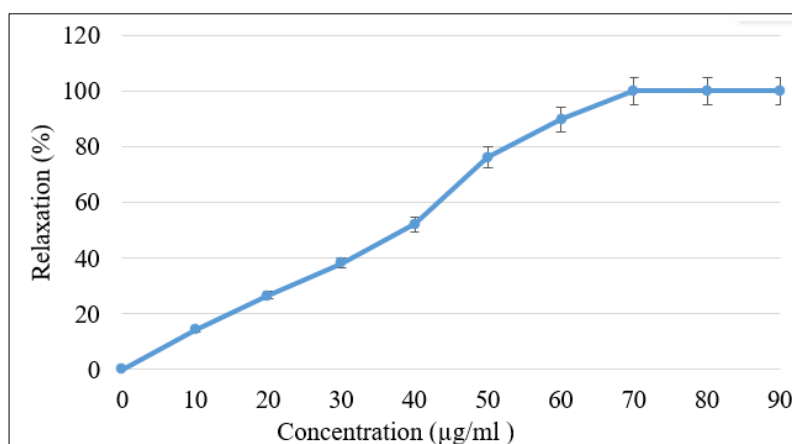


Fig 2: Relaxation of the isolated aorta precontracted with norepinephrine at the concentration of 10^{-4} M in the bath, in the presence of the isolate injected in a cumulative manner in the bath ($\bar{x} \pm \text{sem}$; $n = 10$; $p < 0.05$)

This active isolate was identified by concerted analysis of the recorded NMR spectra and by comparison with data in the literature (Table I, II) [16].

Table 1: ^1H (500 MHz) and ^{13}C (125 MHz) NMR chemical shifts (δ , ppm) data of compound NC36 (MeOD).

Position		NC36 δ_{H}	Literature δ_{H}	NC36 δ_{C}	Literature δ_{C}
1		1.00/1.80	1.36/1.57	31.63	23.1
2		1.25/1.50	1.41/2.06	29.12	29.1
3		3.51	3.49	78.12 (O-Ac)	87.9 (O-All)
4		-	-	38.59	42.7
5		-	-	132.66	150.0
6		5.35	5.76	129.40	120.0
7		1.70/1.98	3.15	29.19	79.0
8		1.40	2.18	47.14	48.9
9		-	-	47.50	33.9
10		1.90	2.27	49.05	40.2
11		1.44/1.50	1.35/1.57	35.50	31.3
12		1.10	1.31/1.69	30.20	30.7
13		-	-	42.10	47.4
14		-	-	47.48	48.7
15		1.85	1.41	29.30	35.1
16		1.58	1.37	30.32	28.7
17		1.02	1.59	57.50	51.4
18		0.98 s	1.00	12.61	15.9
19		0.90 s	0.97	13.05	29.5
20			1.61	26.03	37.9
21		2.05 s	0.96	19.57	19.3
22		1.40	1.89/2/35	31.50	40.8
23		4.12	5.68	64.57	130.2 (CH=)
24		1.62	5.95	26.68	135.5 (CH=)
25		3.00	-	49.05	143.0
26		4.50	4.91/4.95	65.71	114.5
27		4.08	1.84	64.48	18.9
28		3.62	1.07 s	67.55	28.7
29		1.38 s	1.27 s	22.30	26.0
30		1.38 s	0.78 s	22.30	18.7
31		1.48 s		18.74	
			7-OMe 3.37 s		56.6
		3-O-Ac	Sugar 3-O-All		
		-	4.68		104.0
		2.00	3.32	169.04	72.8
			4.05	22.27	73.1
			3.47		69.1
			3.65		75.1

Ac: acetate; All: allopnyranosyl

Table 2: Correlations ^1H - ^1H COSY of NC36

^1H ^1H	5.35	4.12	3.51	2.30	1.98	1.80	1.50	1.38	0.90
5.35					X				
3.51						X	X		
1.80							X		X
1.62		X							

The structural elucidation of the active isolate NC36, performed using spectroscopic techniques including ^1H NMR, ^{13}C NMR, and mass spectrometry, indicates the presence of multiple methyl groups and hydroxyl functions consistent with known cucurbitane derivatives. The molecular framework and functional group pattern closely resemble those of other bioactive cucurbitane triterpenoids commonly found in *Sechium edule* and related Cucurbitaceae species, confirming its classification as a cucurbitane derivative. Based on its distinct tetracyclic cyclopenta[a]phenanthrene core structure, which is characteristic of the cucurbitane skeleton, the isolate was identified as a derivative of cucurbitane-type triterpenoids.

Comparison of the observed chemical shifts of NC36 with published data for cucurbitane revealed a close correspondence, suggesting that the isolate is a tetracyclic cucurbitane-type triterpenoid, identified as 24-hydroxymethyl-23,26,27-trihydroxy-cucurbit-5-ene-3-yl-acetate (Fig 3).

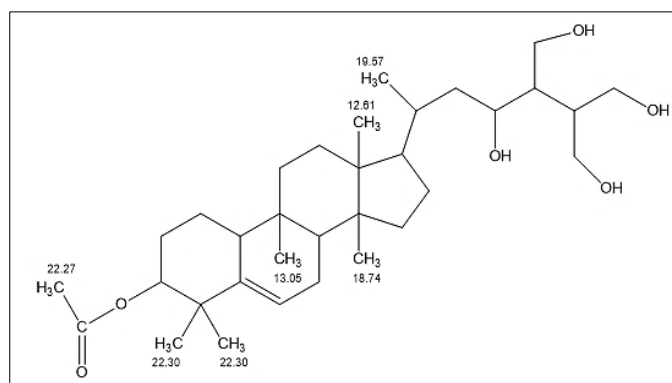


Fig 3: Structure of the active isolate (24-hydroxymethyl-23,26,27-trihydroxy-cucurbit-5-ene-3-yl-acetate)

The present study reports the identification of a cucurbitane-type triterpenoid, 24-hydroxymethyl-23,26,27-trihydroxy-cucurbit-5-ene-3-yl-acetate, from *Sechium edule* fruit. To the best of our knowledge, this specific structure has not previously been described from *S. edule* to date. Phytochemical literature and compound database research that we conducted did not report the presence of 24-hydroxymethyl-23, 26, 27-trihydroxy-cucurbit-5-ene-3-yl-acetate from *Sechium edule* fruit. However, cucurbitane-type triterpenoids, including cucurbitacins and related derivatives, are well documented in members of the Cucurbitaceae. In addition to its structural characterization, the compound demonstrated significant vasodilatory activity, indicating a potential contribution to the cardiovascular-related biological properties attributed to *S. edule*. The presence of a cucurbitane scaffold combined with multiple hydroxyl functionalities may be relevant to its observed bioactivity [17, 18, 19]. These findings expand the chemical profile of *S. edule* and identify this triterpenoid as a promising lead for further pharmacological investigation. Future studies should aim to elucidate its mechanism of action, assess its structure-activity relationships, and evaluate its efficacy and safety in *in vivo* models to better define its therapeutic potential in vascular disorders.

Conclusions

The present finding extends the current knowledge of the triterpenoid constituents of *S. edule* fruit. Overall, this discovery highlights *S. edule* as a promising source of bioactive triterpenoid derivatives, supporting its potential as a source of natural antihypertensive agents and motivating further phytochemical and pharmacological investigations

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