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Chemical constituents of *Sechium edule* (Jacq.) Swartz

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ABSTRACT

*Chemical investigation of the dichloromethane extract of the leaves of Sechium edule (Jacq.) Swartz led to the isolation of a mixture of trans-cinnamic acid (**1a**) and phenylacetic acid (**1b**) in 3:2 ratio, 3-octadecenoic acid (**2**), trilinolenin (**3**), and α-linolenic acid (**4**). The structures of **1a**, **1b** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **3** and **4** were identified by comparison of their ^{1}H and ^{13}C NMR data with those reported in the literature.*

Keywords: *Sechium edule*, Cucurbitaceae, sayote, *trans*-cinnamic acid, phenylacetic acid, 3-octadecenoic acid, trilinolenin, α-linolenic acid

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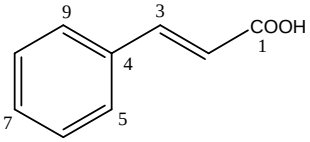
INTRODUCTION

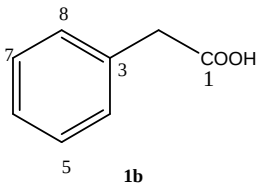
Sechium edule (Jacq.) Swartz, locally known as sayote is commonly cultivated and used as a vegetable in the Philippines. In traditional folk medicine, the fruit is used as laxative and the leaves are employed in suppuration of boils. It exhibits antiulcer, laxative and diuretic properties [1]. Several studies have been conducted on the biological activities of *S. edule*. The aqueous extract of the fruit of *S. edule* exhibited hypotensive effect [2], while the ethanol extract showed anti-ulcer property [3]. Another study reported that *S. edule* alcoholic extract and tincture showed very good antimicrobial efficacy against all tested strains of multiresistant staphylococci and enterococci [4]. Furthermore, the chloroform and methanolic extracts of *S. edule* fruits exhibited antibacterial activity against most of the Gram negative bacteria tested (*Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri*) [5]. A recent study reported that the ethanol extract of *S. edule* fruits at 200 mg/kg body weight significantly reduced the duration of various phases of convulsions in both MES-induced seizures and in PTZ-induced convulsion. In the CNS depressant model, the locomotor activity was also decreased in a dose dependent manner [6]. The ethanolic extract of the fruits of *S. edule* and its different fractions at 100 and 200 mg/kg exhibited significant hepatoprotective activity against CCl_4 induced hepatotoxicity in rats [7]. Leaf ethanolic extracts and leaf and seed water extracts were reported to exhibit antioxidant activities [8]. *S. edule* fruit juice reduced oxidative stress and the development of hyperglycemia and hyperglycemia–induced complications [9]. The aqueous extract of leaves of *S. edule* exhibited protective effect against gentamicin– and potassium dichromate–induced nephrotoxicity and streptozotocin–induced diabetic nephropathy in experimental animals [10]. *S. edule* shoots decrease serum lipids and cholesterol and prevent atherosclerosis and fatty liver [11]. A recent study reported that hydroalcoholic extracts from the roots of *S. edule* showed an antihypertensive activity. Cinnamic acid derivatives like cinnamic acid methyl ester were identified

by MS-PDA-HPLC from the extract, fraction, and active subfraction [12]. Eight flavonoids were characterized by NMR spectroscopic data, and quantified in roots, leaves, stems, and fruits of *S. edule* by LC-photodiode array–MS. The aglycone moieties were represented by apigenin and luteolin, while the sugar units were glucose, apiose, and rhamnose [13]. The leaves of *S. edule* were reported to contain 2.32% lipids, composed mainly of linolenic and palmitic acid [14].

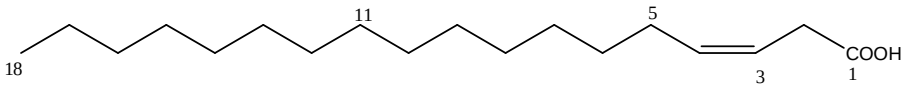
We report herein the isolation and structure elucidation of *trans*-cinnamic acid (**1a**), phenylacetic acid (**1b**) and 3–octadecenoic acid (**2**), and the identification of trilinolenin (**3**) and α–linolenic acid (**4**) from *S. edule* leaves. To the best of our knowledge this is the first report on the isolation of **1**-**3** from the plant.



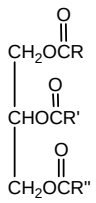
1a



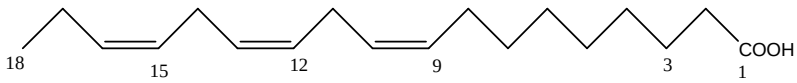
1b



2



3 R = R' = R" = linolenic acid



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MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. The ESIMS spectrum was run on a Finnigan LCQ mass spectrometer. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

General Isolation Procedure

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and

rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Sample Collection

Samples of the leaves (1.45 kg) of *Sechium edule* (Jacq.) Swartz were collected from San Joaquin Market, Pasig City, Philippines in January 2012. The samples were authenticated at the Bureau of Plant Industry-San Andres, Manila, Philippines.

Isolation

Leaf samples of *Sechium edule* (Jacq.) Swartz were air-dried for about one week. The air-dried leaves (300 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (14.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using 2.5% EtOAc in petroleum ether, followed by rechromatography using 5% EtOAc in petroleum ether to afford **3** (12 mg). The 40% acetone in CH_2Cl_2 was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to yield **2** (8 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed in 12% EtOAc in petroleum ether, followed by rechromatography in 15% EtOAc in petroleum ether to afford **4** (7 mg). The more polar fractions were combined and rechromatographed (2 ×) in 15% EtOAc in petroleum ether to afford a mixture of **1a** and **1b** (10 mg) after trituration with petroleum ether.

***Trans*-Cinnamic Acid** (**1a**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.42 (d, *J* = 16.2 Hz, H-2), 7.76 (d, *J* = 16.2 Hz, H-3), 7.53 (H-9), 7.40 (m, H-6, H-7, H-8); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.98 (C-1), 116.94 (C-2), 147.08 (C-3), 134.01 (C-4), 128.34 (C-5, C-9), 128.96 (C-6, C-8), 130.75 (C-7).

Phenylacetic Acid (**1b**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.66 (s, H_2-2), 7.32 (d, *J* = 6.6 Hz, H-4, H-8), 7.28 (dd, *J* = 6.6, 8.4 Hz, H-5, H-7), 7.29 (m, H-6); ^{13}C NMR (150 MHz, $CDCl_3$): δ 176.07 (C-1), 40.81 (C-2), 133.31 (C-3), 128.67 (C-4, C-8), 129.38 (C-5, C-7), 127.35 (C-6).

3-Octadecenoic Acid (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.06 (dd, *J* = 6.6, 1.2 Hz, H_2-2), 5.50 (H-3), 5.58 (H-4), 2.00 (H_2-5), 1.28 (H_2-6), 1.25-1.35 (H_2-7–H_2-15), 1.25 (H_2-16), 1.28 (H_2-17), 0.85 (t, *J* = 6.6, H_3-18); ^{13}C NMR (150 MHz, $CDCl_3$): δ 176.12 (C-1), 37.41 (C-2), 120.63 (C-3), 135.64 (C-4), 32.47 (C-5), 29.70 (C-6), 29.36–29.70 (C-7–C-15), 31.91 (C-16), 22.69 (C-17), 14.12 (C-18).

Trilinolenin (**3**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.28 (dd, *J* = 4.2, 12.0 Hz), 4.12 (dd, *J* = 6.0, 12.0 Hz, glyceryl CH_2O), 5.30 (glyceryl CHO), 2.30 (α-CH_2), 5.33 (olefinic H), 2.78 (double allylic CH_2), 2.05 (allylic, CH_2), 1.25-1.35 (CH_2), 0.96 (t, *J* = 7.2, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.08 (glyceryl CH_2), 68.86 (glyceryl CH), 173.25 (C=O α), 172.84 (C=O β), 34.01 (C-2α), 34.18 (C-2β), 24.82 (C-3α), 24.86 (C-3β), 29.08 (C-4α), 29.04 (C-4β), 29.19 (C-5α), 29.26 (C-5β), 29.11 (C-6α), 29.17 (C-6β), 29.60 (C-7α), 29.70 (C-7β), 29.19 (both C-8), 130.01 (C-9α), 129.9 (C-9β), 128.06 (C-10α), 128.07 (C-10β), 25.62 (both C-11), 127.88 (C-12α), 127.74 (C-12β), 130.21 (both C-13), 27.20 (both C-14), 29.36 (both C-15), 31.52 (both C-16), 22.54 (both C-17), 14.27 (both C-18).

α-Linolenic Acid (**4**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.33 (t, *J* = 7.2 Hz, H_2-2), 1.61 (H_2-3), 1.23-1.33 (H_2-4–H_2-7), 2.04 (H_2-8, H_2-17), 5.34 (m, H-9, H-10, H-12, H-13, H-15, H-16,), 2.79 (t, *J* = 6.6 Hz, H_2-11, H_2-14), 0.96 (t, *J* = 7.2, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 33.26 (C-2), 24.72 (C-3), 29.12, 29.23 (C-4–C-6), 29.66 (C-7), 29.18 (C-8), 130.24 (C-9), 127.74 (C-10), 25.61 (C-11), 128.28 (C-12, C-13), 25.52 (C-14), 127.11 (C-15), 131.96 (C-16), 20.54 (C-17), 14.27 (C-18).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of *S. edule* afforded a mixture of *trans*-cinnamic acid (**1a**) and phenylacetic acid (**1b**), 3-octadecenoic acid (**2**), trilinolenin (**3**), and α-linolenic acid (**4**). The structures of **1a**, **1b** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy. The structure of **2** was supported by ESI-MS which gave a pseudomolecular ion at *m/z* 307.52 $[M+Na]^+$ corresponding to a molecular formula of $C_{18}H_{36}O_2Na$. Compounds **3** and **4** were identified by comparison of their ^{1}H NMR and ^{13}C NMR data with those reported in the literature for trilinolenin [14] and α-linolenic acid [15], respectively.

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on the biological activities of *trans*-cinnamic acid (**1a**) and linolenic acid (**4**) as follows.

Trans-cinnamic acid (**1a**) showed *in-vitro* antioxidative effects [16, 17], high antifungal and antibacterial activities [18], antihyperglycemic activity [19], and antifertility effect [20]. The antihyperglycemic activity of cinnamic acid may be attributed to its activation of glucose transport by a PI3-K-independent pathway [21]. Eugenol and cinnamic acid exhibited antioxidant activity *in vitro* and showed protective effect against gastric damage *in vivo* through stimulation of mucus secretion [22]. A recent study reported that although both *cis*-cinnamic acid and **1a** decreased the viability of MDR-TB bacilli in a dose-dependent manner, the antituberculosis activity of *cis*-cinnamic acid was approximately 120 × the activity of **1a**. *Cis*-cinnamic acid also exhibited higher synergistic effect with INH or RIF against tuberculosis [23]. In another study, cinnamic acid was reported to inhibit the proliferation of human lung adenocarcinoma (A549) cells and the telomerase activity [24]. Furthermore, cinnamic acid at 2.5-8.0 mM prolonged the doubling time and inhibited the DNA synthesis of growing human colon adenocarcinoma cells Caco-2. The antiproliferative effects occurred after 2 h of treatment with 8.0 mM cinnamic acid and reached maximum values after 8 h of treatment [25]. Cinnamic acid and some of its derivatives also exhibited larvicidal activity against dog roundworm 2nd stage larvae [26].

Oils rich in α-linolenic acid (**4**) protect against the characteristics of fatty liver disease in the Δ6-desaturase null mouse [27]. Another study reported that the larval viability fifty (LV_{50}) values of linolenic acid and linoleic acid were 0.849×10^3 ppm and 0.857×10^3 ppm, respectively. This indicates that both fatty acids possess insectistatic and insecticidal activities against *S. frugiperda* [28]. Moreover, combined application of **4** and vitamins A, C and E could enhance the activity of telomerase, prevent the shortening of telomere and protect cells from aging [29]. The bioassay-guided fractionation of rose hip afforded linoleic acid which gave IC_{50} values of 85 and 0.6 μM for COX-1 and COX-2, respectively and **4** which gave IC_{50} values of 52 and 12 μM for COX-1 and COX-2, respectively. Thus, linoleic acid and **4** contribute to the COX-1 and COX-2 inhibitory activity of rose hip [30]. Furthermore, **4** may prevent calcium oxalate urolithiasis formation by increasing urinary fibrinolytic activity [31]. It was also reported to downregulate inflammatory iNOS, COX-2, and TNF-α gene expressions through the blocking of NF-κB and MAPKs activations in LPS-stimulated RAW 264.7 cells, which may be the mechanistic basis for the anti-inflammatory effect of **4** [32]. In another study, dietary supplement of plant-derived **4** for four weeks showed cardioprotective effects similar to the effects of fish oil [33]. Furthermore, n-3 fatty acid containing spray-dried milk formulation would bring about the hypocholesterolemic effect by lowering HMG Co A reductase activity in liver and by increasing the secretion of bile constituents [34]. Phenylheptatriyne, linoleic acid and **4** were shown to exhibit antimicrobial properties [35]. The omega-3 polyunsaturated fatty acids (PUFA) which include **4** are known to possess the most potent immunomodulatory activities. Some of the effects of omega-3 PUFA include actions on intracellular signaling pathways, transcription factor activity and gene expression [36]. Flaxseed oil is the most abundant plant source of **4**. Ingestion of flaxseed oil may exert antiallergic, antiatherosclerotic and antiarrhythmic effects, and prevent and manage cardiovascular disease. A review on the biological effects of dietary **4** was provided [37]. Recently, another review on mechanisms of action of (n-3) fatty acids which includes **4** has been provided [38]. The very long-chain (n-3) PUFA have a range of physiological roles that relate to optimal cell membrane structure and optimal cell function and responses. Thus, (n-3) PUFA play a key role in preventing, and perhaps treating, many conditions of poor health and well-being [38].

CONCLUSION

Sechium edule was reported to exhibit antihypertensive, antimicrobial, antihyperglycemic, anti-ulcer, antioxidant, and hepatoprotective effects. The dichloromethane extracts of *S. edule* leaves yielded cinnamic acid and α-linolenic acid which were reported to exhibit antimicrobial properties. *Trans*-cinnamic acid was also reported to exhibit hypoglycemic, anti-ulcer and antioxidant properties, while α-linolenic acid was also reported to possess hypotensive, cardioprotective, and hepatoprotective effects. Thus, some of the biological activities of *S. edule* may be attributed to two of the isolated compounds, *trans*-cinnamic acid (**1**) and α-linolenic acid (**4**), among other chemical constituents from the leaves of the plant.

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