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Phytochemical and Antioxidant Profiling of Different Solvent Extractions of Calabash (*Crescentia cujete* L.)

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Crescentia cujete L., a tropical tree, is often used in traditional medicine since ancient times. All parts of the plant seem to have variety of functionalities and exhibit biological activities. The fruit, particularly the pulp, has been mostly used for medicinal purposes. This study aimed to provide phytochemical profiling of the constituents present in the fruit pulp from the extraction using a variety of organic solvents and by performing household preparation like decoction of the pulp. A gas chromatograph-electron ionization - mass spectrometer (GC-EI-MS) was used for the analyses of the extracts. A total of 18 identified compounds were revealed by the GC-EI-MS analyses of all the prepared fruit extracts of *C. cujete* L. The following are prominent identified compounds *viz*. benzoic acid, phenol, 2,2′-methylene*bis*[6-(1,1-dimethylethyl)-4-methyl-, hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester, 4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 5-hydroxymethylfurfural, α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl, D-fructose, diethyl mercaptal, pentaacetate, 2-propenoic acid, 3-phenyl-, dodecanoic acid, oleic acid, dodecanoic acid, 1,2,3-propanetriyl ester, octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, ascaridole epoxide, phenol, 2,4-*bis*(1,1-dimethylethyl)-, *n*-hexadecanoic acid, hexadecanoic acid, methyl ester and methyl salicylate. An antioxidant assay using a 2,2-diphenyl-1-picrylhydrazyl [DPPH] protocol was performed on boiled and raw *C. cujete* fruit with ascorbic acid as positive control. Results verified that the boiled sample had higher antioxidant activity than the raw fruit. The boiled preparation yielded roughly 80% DPPH % inhibition or around 1000 mg of vitamin C per 100 g serving of the pulp. This is the first comprehensive work targeting constituents in *C. cujete* from a range of relatively polar to non-polar solvent systems as well as the antioxidant properties of the selected preparations.

Keywords: *Crescentia cujete* L., Gas chromatograph-electron ionization-mass spectrometry, Solvent systems, Decoction.

INTRODUCTION

Medicinal plants played a significant role in human societies particularly in providing the needs for human health. These medicinal plants served as a medical resource in almost every country and culture [1] and can be potentially used in drug development and synthesis [2]. Medicinal plants have been recognized as alternative sources of medicines used in the treatment of various diseases such as cancer, cardiovascular diseases, diabetes, hypertension and many others [3-6].

Among several plants considered for medicinal purposes, *Crescentia cujete L.* is one promising source as an alternative medicine. It is a tropical tree belonging to family Bignoniaceae that originates from tropical America and tropics of the old World but is now considered as a common element of home gardens in Mexico, Central and South America, Africa and Asia [7,8]. While it is commonly known as güira in Cuba, totumo in Colombia and jicaro in Panama [8], *C. cujete* L. is known as the "miracle tree" in the Philippines [9]. This tree grows

about 6 to 10 m in height, with clusters of bright green tripinnate leaves. The flowers are pollinated by bats and bloom at night. The fruit immediately develops after pollination and can grow 10-30 cm in diameter that takes about 6 to 7 months to reach maturity. It is characterized as large, green, globular fruit with hard shell which directly hangs from the branches of the tree. The white pulp is embedded with several small flat brown seed and is reportedly to have variety of medicinal applications [10-13].

Different parts of *C. cujete L.* have been already studied for their biological activities. Leaves were analyzed for their cytotoxic and mutagenic potentials. Secondary metabolites including flavonoids, tannins and steroids were observed from the qualitative screening of phytochemical constituents. Brine shrimp lethality test (BSLT) revealed cytotoxic effect of the extracts of the leaves with median lethal concentration (LC_{50}) of 572 ppm, 3048 ppm and 220 ppm for hexane, aqueous and crude ethanolic extract, respectively, after 6 h of treatment exposure [14]. The bark, together with the leaves extract, was studied for its antioxidant activity. Both bark and leaves extract exhibited antioxidant activities, but the ethyl acetate fraction of leaves showed highest antioxidant activity. Moreover, the leaves extract showed significant DPPH scavenging activity with IC_{50} of 8.78 µg/mL while standard ascorbic acid had IC_{50} of 7.68 µg/mL [15]. The fruit was also screened for its cytotoxic properties, antioxidant and anthelmintic properties. Presence of alkaloids, flavonoids, cardiac glycosides, reducing sugars, saponins, tannins, phytosterols and terpenoids were observed in the phytochemical screening of the fresh *C. cujete* fruit [16].

However, in all these studies, the extracts used were only visually screened for the presence or absence of the classes of active chemical constituents and did not identify the actual compounds. One study revealed the metabolite profile of the fresh fruit extract using gas chromatography-mass spectrometry (GC-MS) analysis and compared it with the metabolite profile of the processed commercially available juice from *C. cujete* fruit using untargeted liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Hexane was used as the extracting solvent for the fresh fruit extract while ethanol was used for the commercially available fruit juice. Analyses revealed volatile compounds such as methyl salicylate, (+)-δ-cadinene, benzene and benzene derivatives such as toluene and *o*-xylene [17]. We have also previously conducted a phytochemical analysis of the crude methanol extract of *C. cujete* fruit using GC-MS [18]. However, complete profile of the constituents of the fruit was deemed necessary in order to fully study the potential of this miracle tree. Thus, this study aimed to identify the phytochemical constituents present from the fruit pulp of *C. cujete* L. from the different crude extracts using different organic solvents and extract produced by decoction process normally done in household preparations.

EXPERIMENTAL

Sample preparation for gas chromatography-mass spectrometry (GC-MS) trials: *Crescentia cujete* L. fruits were collected from Bocaue, Bulacan, Philippines. The identification of the plant was confirmed and authenticated at De La Salle University Herbarium (DLSUH). The seeds were removed, utilizing only the fruit pulp. Six extracts were prepared for the analyses *viz*. boiled after subsequent extraction with DMSO and solvent extractions using dichloromethane (DCM), ethyl acetate (EA), ethanol (EtOH), methanol (MeOH) and petroleum ether (PE).

The boiled sample was prepared by boiling 50 g of fresh pulp and then filtered right after boiling followed by cooling. The filtered sample was mixed with 10 mL DCM. It was filtered again and dried over nitrogen gas. The extract was diluted in DCM and filtered into a vial using 0.45 µm syringe filter with a final volume of 1 mL.

The crude extracts were prepared by incubating 20 g of fresh pulp in the above-mentioned solvents for 3 h. All samples were filtered each using Whatman filter paper No. 1 (11 µm pore size) and dried over nitrogen gas. All dried extracts were diluted again with their corresponding extracting solvent. Each were filtered into a vial using a 0.45 µm syringe filter with a final volume of 1 mL.

Sample preparation for antioxidant activity: The diced calabash fruit (40 g) was added with 100 mL of water and boiled for 30 min until the fruit darkened. The sample was then filtered and 2.00 g of filtrate were soaked in 50 mL of extracting solution for 3 h. The extracting solution consisted of a ratio (volume per volume) of 7.00 of MeOH:2.95 of distilled water:0.05 of conc. HCl. Five concentrations of ascorbic acid, as positive control, were prepared specifically 6 ppm, 7 ppm, 8 ppm, 9 ppm and 10 ppm. These reference solutions were prepared *via* dilution from 50 ppm stock solution.

Antioxidant activity: The DPPH assay was conducted following the reported method [19] utilized to assess the free radical scavenging activity of methanolic leaf extracts. In a volumetric flask, 3.94 mg of DPPH was diluted in methanol (100 mL) and then the solution was incubated at room temperature for 2 h. A 4 mL of methanol was used as blank. The analytical sample was prepared by obtaining 2 mL of extracted sample solution by mixing with 2 mL of DPPH solution. The DPPH, which served as negative control, was prepared by adding 2 mL of DPPH with 2 mL of methanol. Triplicate trials were made for both the raw and boiled pulp. The samples were then incubated at room temperature for 0.5 h and the absorbance of each trial was monitored at 515 nm (UV-Vis Hitachi spectrophotometer 2900). The percent inhibition was deducted through linear interpolation. Inhibition of 50% of DPPH free radicals in solution was established by the following equation:

DPPH scavenging effect (%) or

$$\text{Percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 = absorbance of negative control; A_1 = absorbance of trial/sample.

Gas chromatography-mass spectrometry: All samples were analyzed and characterized using a gas chromatograph-electron ionization-mass spectrometer (GC-EI-MS) using an Agilent GC MS7890B. A HP-5ms (5%-phenyl)methylpolysiloxane Ultra Inert column (30 m × 250 mm × 0.25 mm) was utilized for the analyses. The mobile phase used was an ultra-

pure helium gas delivered at 1 mL/min, with maintained pressure at 8.2 psi, rate of 36.62 cm/s and holdup interval of 1.37 min. The splitless inlet was maintained at 250 ºC, at 8.2 psi and overall stream of 24 mL/min and a septum purge stream velocity of 3 mL/min. The temperature for the injector was set at 250 ºC. Temperature started at 70 ºC with a programmed linear ramp of 2 ºC/min until 135 ºC and was maintained for 10 min, increased to 220 ºC at 4 ºC/min and held for 10 min and finally increased to 270 ºC at 3.5 ºC/min, maintained at 37 min. Compound identification was employed using the NIST Archive 2.0. Percent peak area average was computed from the subsequent total ion chromatograms. The comparison of the constituents based on their elution succession with their relative retention indices on an intermediate polar gas chromatograph column verified the identification of the compounds. The retention indices were computed for all the volatile constituents using a homologous series of *n*-alkanes. All tests were done and analyzed in triplicates.

RESULTS AND DISCUSSION

Phytochemical profiling: Six extraction procedures were performed for the phytochemical profiling of the fruit pulp of *C. cujete* L. Decoction or boiling the pulp, which is a household preparation for extracting the fruit juice of herbal plants, was performed together with extracting the fruit pulp using five different organic solvents, including dichloromethane (DCM), ethyl acetate (EA), ethanol (EtOH), methanol (MeOH) and petroleum ether (PE). Gas chromatography-mass spectrometry was employed to analyze all the extracts of the fresh fruit pulp of *C. cujete* L. A total of 18 identified compounds were revealed by the GC-MS analyses of all the six fruit extracts of *C. cujete* L. A summary list of the volatile compounds is presented in Table-1. Three volatile compounds were eluted from the GC-MS analysis of boiled fruit pulp. They were composed of hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester (79.23%), phenol, 2,2′-methylene*bis*[6-(1,1-dimethylethyl)-4-methyl- (1.98%) and benzoic acid (1.37%) as shown in Table-2. Total ion chromatogram of the boiled fruit analysis is shown in Fig. 1.

Meanwhile, eleven eluents were present from the DCM crude extract of *C. cujete* L. fruit. Ten compounds were identified, which were mostly composed of esters, carboxylic acid and furan. Table-3 listed the volatile constituents identified by NIST Archive 2.0. Fig. 2 presents the chromatogram of DCM crude extract. Two volatile compounds were identified from the ethyl acetate crude extract of *C. cujete* L. fruit (Table-4 and Fig. 3). They were composed of benzoic acid (5.16%), an aromatic carboxylic acid and dodecanoic acid, 1,2,3-propanetriyl ester (52.15%), an ester. The GC-MS analysis (Fig. 4) of the ethanolic crude extract eluted thirteen volatile compounds (Table-5). They were mostly composed of furanones, pyranone derivative, carboxylic acids, furan, phenol, fatty acid and ester. Eight eluents were identified from the methanolic crude extract, composed primarily of furan (9.6%), carboxylic acid (7.25%) and pyranone derivative (5.74%). Other volatile compounds such as furanone and ester were also present (Table-6 and Fig.

TABLE-1
SUMMARY OF THE VOLATILE COMPOUNDS IDENTIFIED FROM *C. cujete* L. FRUIT

S. No.	Compounds	B	DCM	EA	EtOH	MeOH	PE
1	Benzoic acid	+	+	+	+	+	–
2	Phenol, 2,2'-methylene*bis*[6-(1,1-dimethylethyl)-4-methyl-	+	–	–	–	–	–
3	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	+	–	–	–	–	–
4	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	–	+	–	+	+	–
5	5-Hydroxymethylfurfural	–	+	–	–	–	+
6	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	–	+	–	+	+	–
7	D-Fructose, diethyl mercaptal, pentaacetate	–	+	–	–	+	–
8	2-Propenoic acid, 3-phenyl-	–	+	–	+	+	–
9	Dodecanoic acid	–	+	–	–	–	–
10	Oleic acid	–	+	–	–	–	–
11	Dodecanoic acid, 1,2,3-propanetriyl ester	–	+	+	–	–	–
12	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester	–	+	–	–	–	–
13	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	–	–	–	+	+	–
14	Ascaridole epoxide	–	–	–	+	–	–
15	Phenol, 2,4-*bis*(1,1-dimethylethyl)-	–	–	–	+	–	–
16	*n*-Hexadecanoic acid	–	–	–	+	–	–
17	Hexadecanoic acid, methyl ester	–	–	–	–	+	–
18	Methyl salicylate	–	–	–	–	–	+

*B = boiled fruit pulp; DCM = dichloromethane crude extract; EA = ethyl acetate crude extract; EtOH = ethanolic crude extract; MeOH = methanolic crude extract; PE = petroleum ether crude extract; + = present; – = absent

TABLE-2
VOLATILE CONSTITUENTS OF BOILED FRUIT PULP OF *C. cujete* L.

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	Benzoic acid	13.49	1195	1.37	Aromatic carboxylic acid
2	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	68.89	2418	1.98	Phenol
3	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	104.02	3454	79.23	Ester

[a]Retention index (HP 5 ms column)

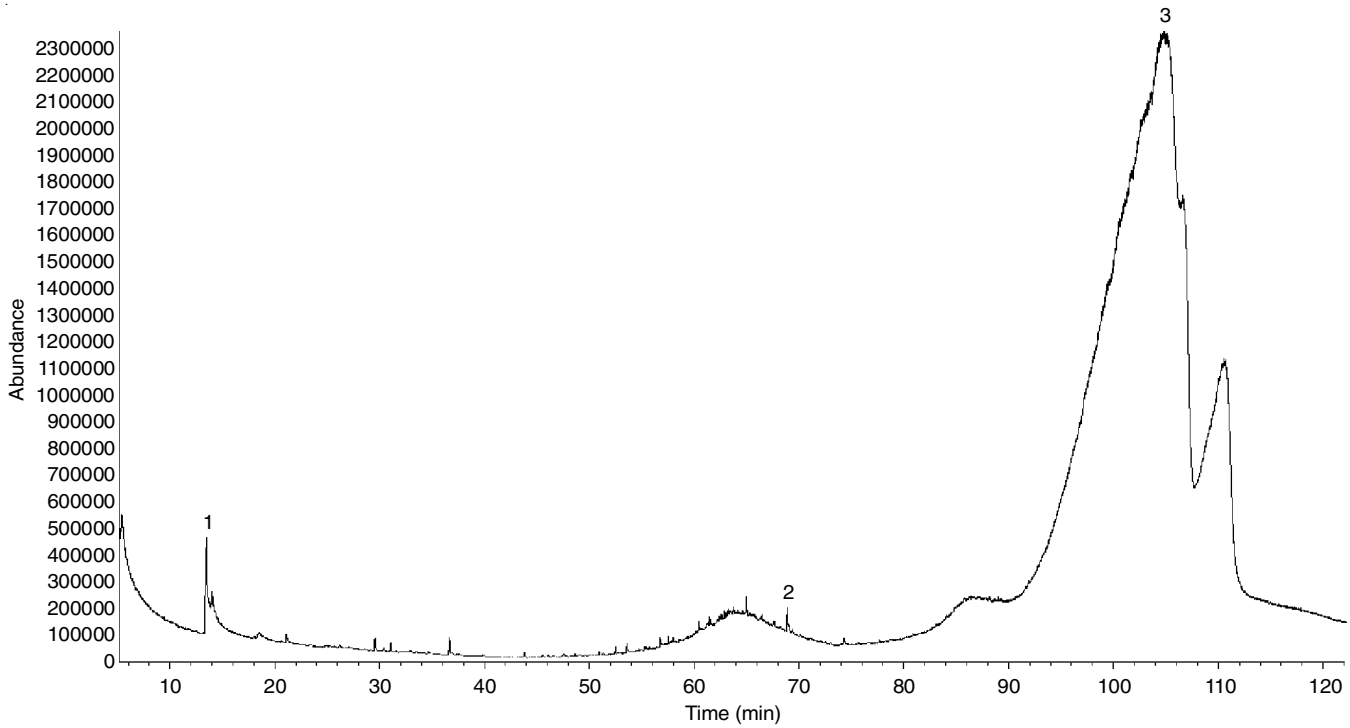

Fig. 1. Total ion chromatogram of boiled fruit pulp of *C. cujete* L.

TABLE-3
VOLATILE CONSTITUENTS OF DICHLOROMETHANE CRUDE EXTRACT OF *C. cujete* L. FRUIT

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.07	1164	3.09	Pyranone derivative
2	Benzoic acid	14.24	1210	3.88	Aromatic carboxylic acid
3	5-Hydroxymethylfurfural	16.76	1256	4.52	Furan
4	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	21.62	1344	0.92	Diverse functional group
5	D-Fructose, diethyl mercaptal, pentaacetate	25.98	1420	0.37	Diverse functional group
6	2-Propenoic acid, 3-phenyl-	28.06	1456	0.58	Carboxylic acid
7	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	33.31	1543	1.46	Diverse functional group
8	Dodecanoic acid	35.11	1572	0.92	Saturated fatty acid
9	Oleic acid	61.55	2146	2.49	Fatty acid
10	Dodecanoic acid, 1,2,3-propanetriyl ester	105.75	3463	66.11	Ester
11	Octadecanoic acid, 3-[(1-oxododecyl)oxy]-1,2-propanediyl ester	111.41		10.78	Ester

[a]Retention index (HP 5 ms column)

TABLE-4
VOLATILE CONSTITUENTS OF ETHYL ACETATE CRUDE EXTRACT OF *C. cujete* L. FRUIT

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	Benzoic acid	13.91	1203	5.16	Aromatic carboxylic acid
2	Dodecanoic acid, 1,2,3-propanetriyl ester	103.25	3440	52.15	Ester

[a]Retention index (HP 5 ms column)

5). Meanwhile, two compounds were only identified from the petroleum ether crude extract (Table-7 and Fig. 6). They were ethyl salicylate (1.07%) and hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester (88.86%), both an ester.

Benzoic acid was mostly found to be present from all extracts except from petroleum ether extract. It inhibits the growth of yeast and bacteria that induces food spoilage and increases the shelf life of food products and beverages. However, excessive amount of benzoic acid may cause diarrhea, abdominal pain and could even interfere with the metabolic processes of the human body, hence there is a restriction for the maximum allowed concentrations of addition of benzoic acid in every food. The US Food and Drug Administration restricts the addition of benzoic acid to 1000 mg kg^{-1} in general types of food [20]. The presence of benzoic acid could be an indication that continuous intake of *C. cujete* fruit extracts may induce harmful effects to the body. Thus, moderation in taking *C. cujete* fruit juice is necessary.

Another notable compound present in DCM, EtOH and MeOH extracts, is the 2-propenoic acid, 3-phenyl- (*trans*-

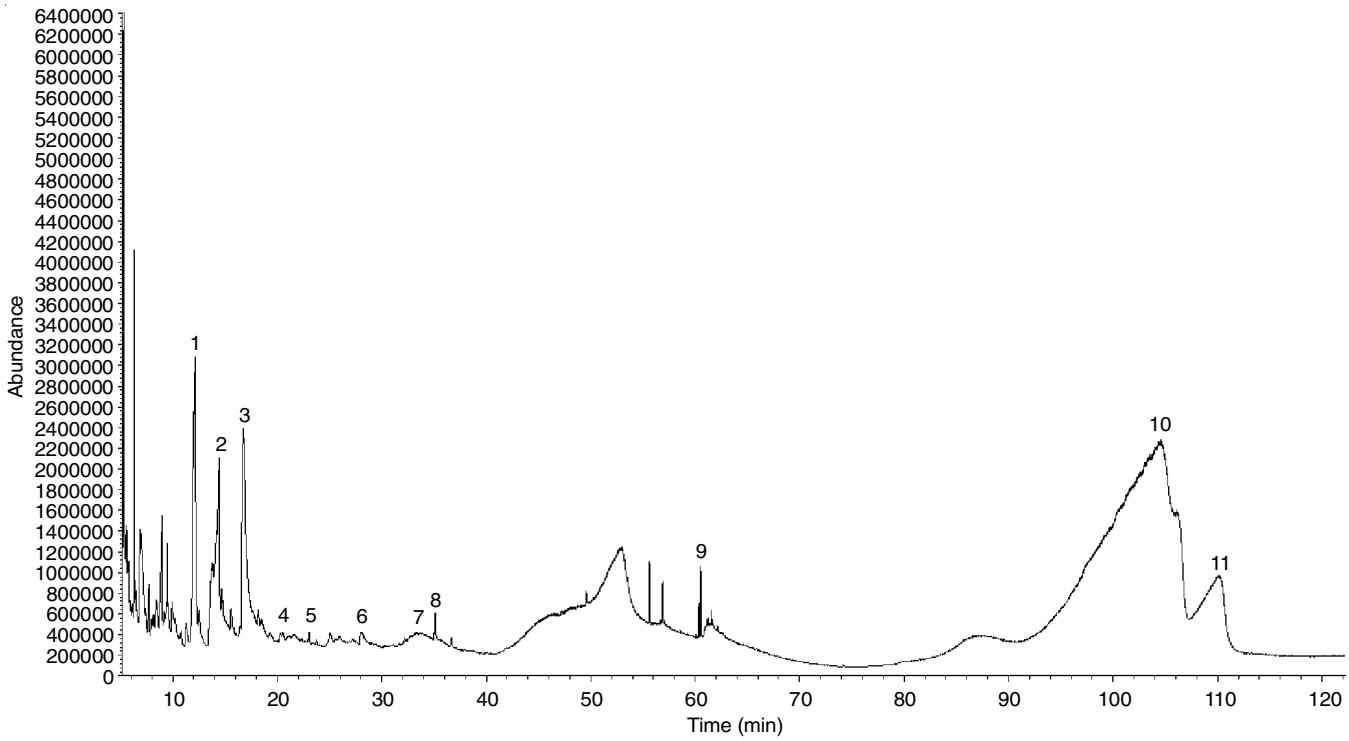

Fig. 2. Total ion chromatogram of crude dichloromethane extract of *C. cujete* L. fruit

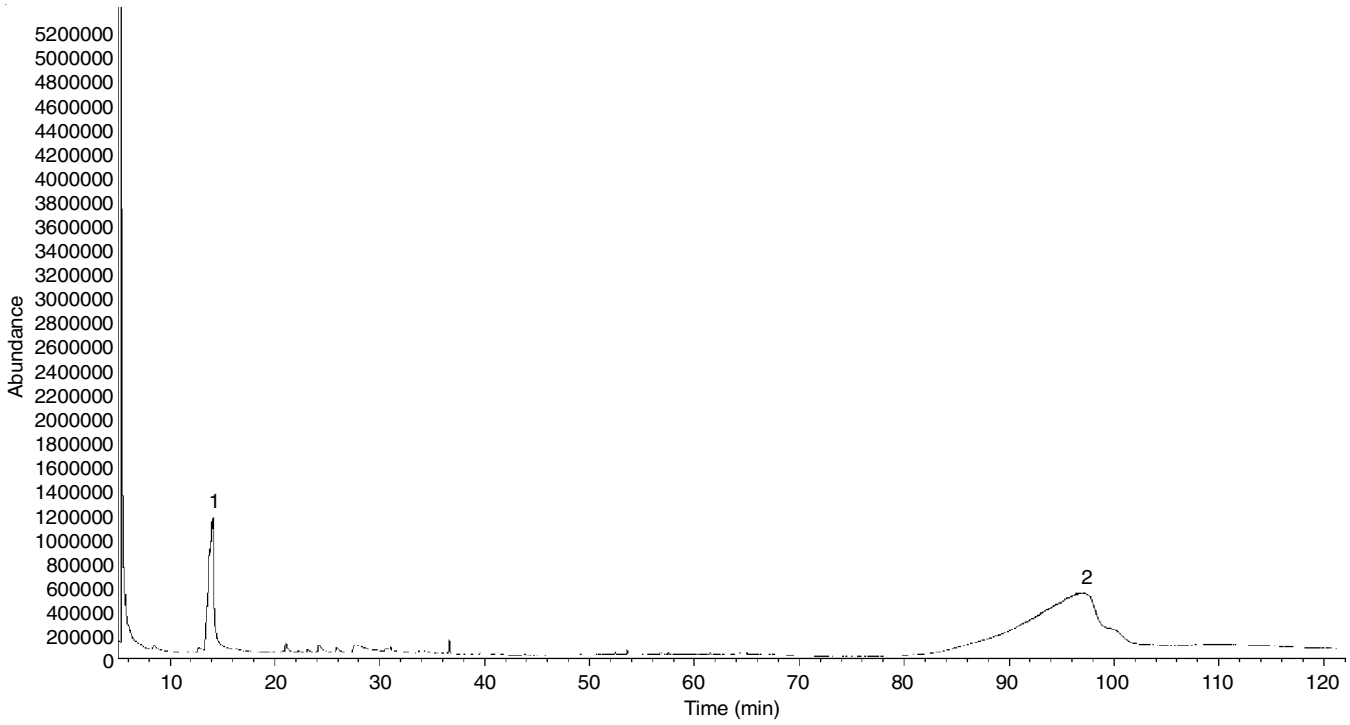

Fig. 3. Total ion chromatogram of crude ethyl acetate extract of *C. cujete* L. fruit

cinnamic acid). We have previously emphasized and isolated this compound from the MeOH extract of *C. cujete* fruit [18]. Cinnamic acids are auxins, which is a class of plant hormones that regulates cellular growth and differentiation in plants. Chemically, cinnamic acids exist in two configurations, *cis*- and *trans*-, but they commonly occur in nature in *trans* configuration [21]. Generally, cinnamic acids are known for the production of dyes, flavourings and in cosmetics [22,23]. Cinnamic acid has a number of biological activities and has potential in treatment of various diseases. It has been reported as potential anticancer agent [22], hypoglycemic agent [24], anti-inflammatory [25], antioxidant and antimicrobial [26] agent. Significant reduction of cell proliferation by 50% (IC_{50}) was observed in glioblastoma, melanoma, prostate and lung carcinoma cells

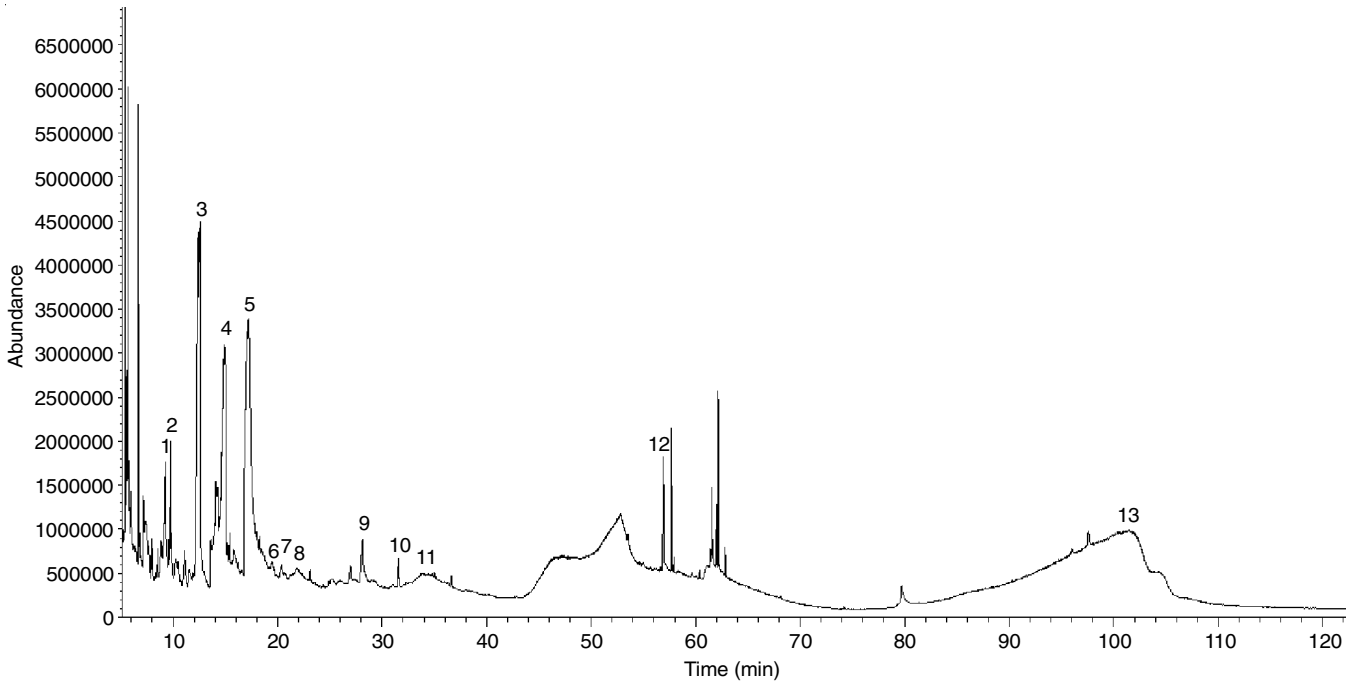

Fig. 4. Total ion chromatogram of crude ethanol extract of *C. cujete* L. fruit

TABLE-5
VOLATILE CONSTITUENTS OF ETHANOLIC CRUDE EXTRACT OF *C. cujete* L. FRUIT

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	9.22	1101	1.31	Furanone
2	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	9.73	1113	1.07	Furanone
3	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.41	1171	4.92	Pyranone derivative
4	Benzoic acid	14.44	1213	3.83	Aromatic carboxylic acid
5	5-Hydroxymethylfurfural	17.13	1263	7.79	Furan
6	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	19.42	1305	0.56	Diverse functional group
7	Ascaridole epoxide	20.38	1322	0.48	Monoterpenoid
8	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	21.81	1347	1.04	Diverse functional group
9	2-Propenoic acid, 3-phenyl-	28.10	1456	1.09	Carboxylic acid
10	Phenol, 2,4-*bis*(1,1-dimethylethyl)-	21.32	1511	1.21	Phenol
11	α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	40.82	1646	1.52	Diverse functional group
12	*n*-Hexadecanoic acid	56.94	1973	0.63	Fatty acid
13	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	101.41	3405	21.93	Ester

[a]Retention Index (HP 5 ms column)

TABLE-6
VOLATILE CONSTITUENTS OF METHANOLIC CRUDE EXTRACT OF *C. cujete* L. FRUIT

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	8.81	1090	2.04	Furanone
2	4*H*-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.08	1164	5.74	Pyranone derivative
3	Benzoic acid	14.45	1214	7.25	Aromatic carboxylic acid
4	5-Hydroxymethylfurfural	16.81	1257	9.60	Furan
5	D-Fructose, diethyl mercaptal, pentaacetate	26.01	1420	0.77	Diverse functional group
6	2-Propenoic acid, 3-phenyl-	28.01	1455	0.92	Carboxylic acid
7	α-D-glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	32.40	1528	3.31	Diverse functional group
8	Hexadecanoic acid, methyl ester	55.62	1930	1.00	Ester

[a]Retention index (HP 5 ms column)

using 1-4.5 mM concentration range of cinnamic acid [22]. Being the most abundant phenolics, cinnamic acid and its derivatives are being studied as potential hypoglycemic agents [24]. Ferulic acid (100 μM), with the presence of *p*-hydroxy and *m*-methoxy groups in the structure of cinnamic acid, exhibited the highest insulin secreting activity and started

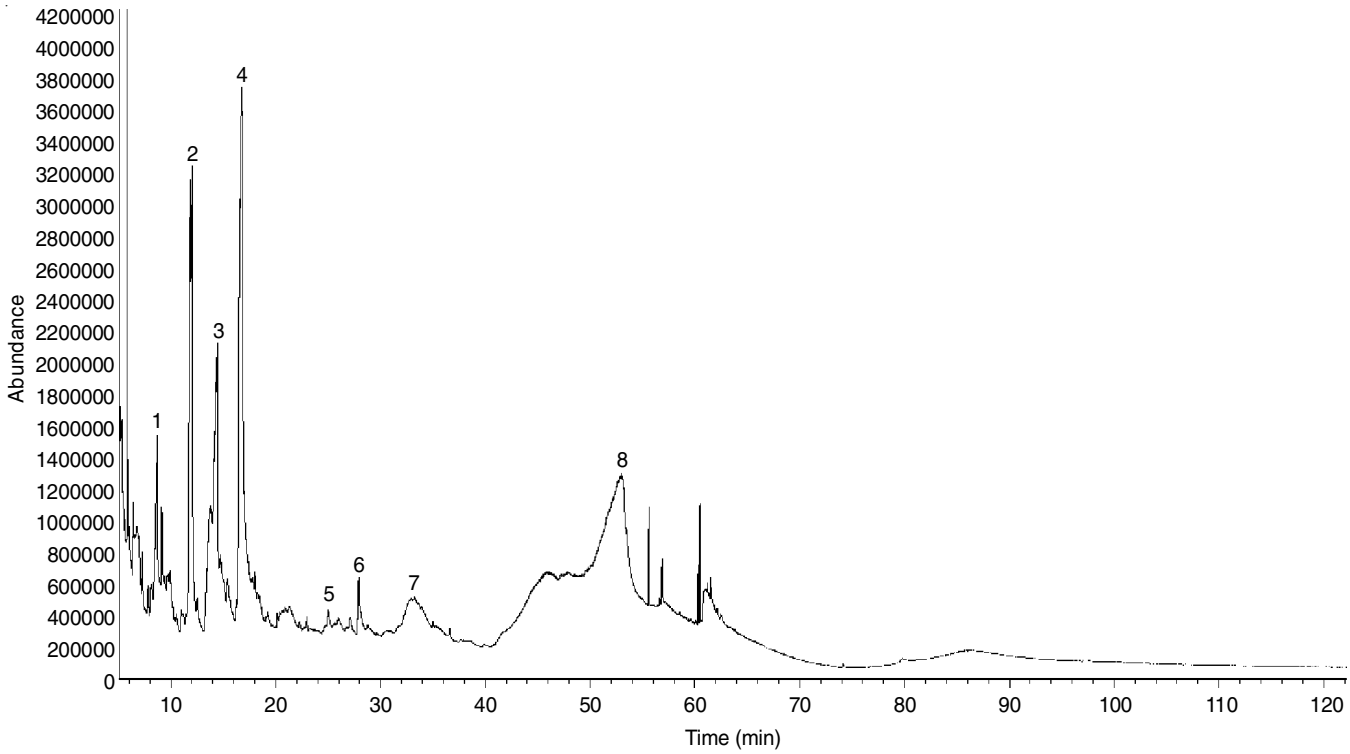

Fig. 5. Total ion chromatogram of crude methanol extract of *C. cujete* L. fruit

TABLE-7
VOLATILE CONSTITUENTS OF PETROLEUM ETHER CRUDE EXTRACT OF *C. cujete* L. FRUIT

S. No.	Compound	RT (min)	RI[a]	Peak area (%)	Functionality
1	Methyl salicylate	13.80	1202	1.07	Ester
2	Hexadecanoic acid, 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester	101.85	3414	88.86	Ester

[a]Retention index (HP 5 ms column)

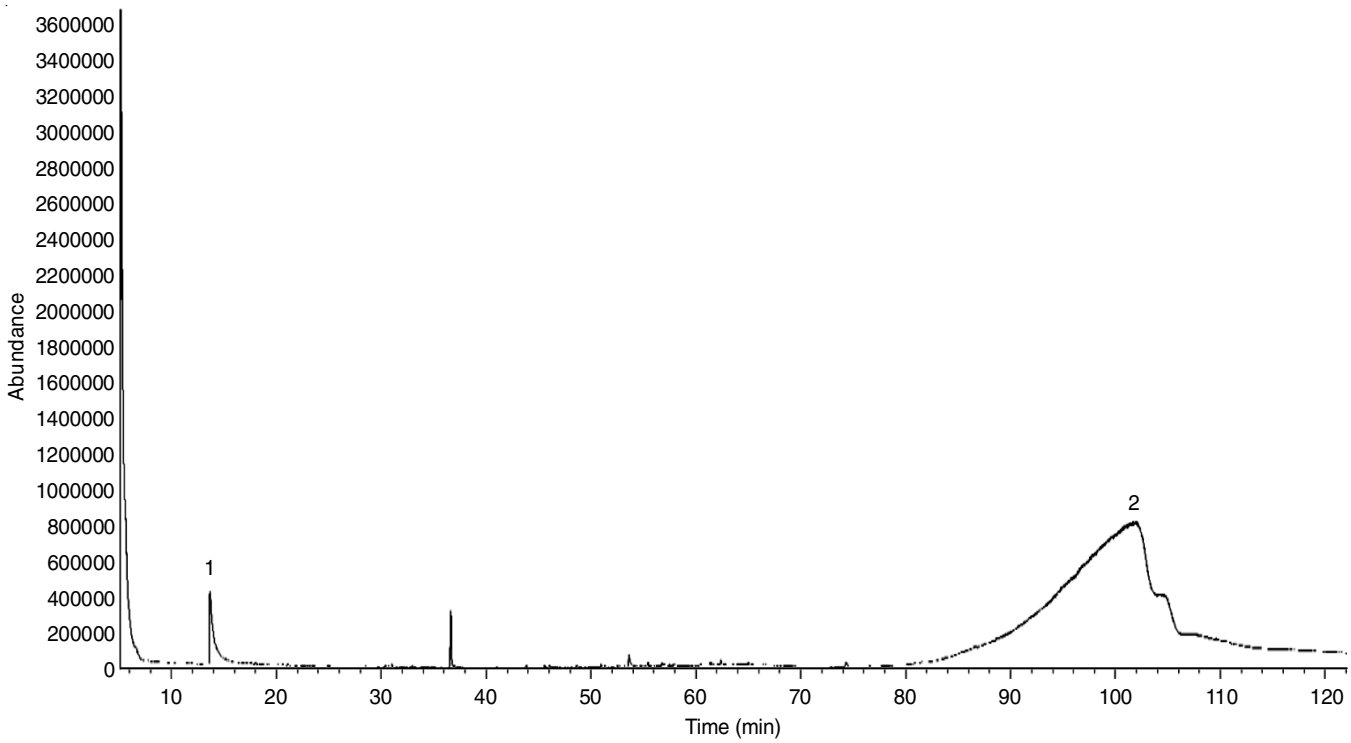

Fig. 6. Total ion chromatogram of crude petroleum ether extract of *C. cujete* L. fruit

insulin secretion at less than 1 min reaching the peak at 4 min resulting at about 3.4-fold increase from the baseline level [27]. Meanwhile, *p*-methoxycinnamic acid stimulated insulin release with concentrations ranging from 10-100 µM [28]. Anti-inflammatory effect of cinnamic acid derivatives based on 2′-hydroxycinnamaldehyde isolated from *Cinnamomum cassia* showed an IC_{50} value of 8 mM in inhibiting lipopolysaccharide-induced NO production and IC_{50} value of 22 mM in inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) transcriptional activity in murine macrophage-like RAW 264.7 cells [29].

Furan and pyranone were identified as well in DCM, EtOH and MeOH extracts. 5-Hydroxymethylfurfural (5-HMF) is a furan produced from the Maillard reaction. 5-HMF was investigated for its anti-inflammatory effect in RAW 264.7 cells. It proved its anti-inflammatory effect by suppressing the production of pro-inflammatory cytokines and significantly down-regulated mRNA expression of major inflammatory mediators [30]. Moreover, this compound is easily absorbed from food by the body through the gastrointestinal tract, metabolized into different derivatives and then excreted through the urine output. Humans may consume 5-HMF daily between 30 and 150 mg from various food products. However, the limit for 5-HMF consumption is not yet well clarified and studied. It has been reported that 5-HMF's metabolism, biotransformation and excretion depends on the organ function of an individual and thus, vary from one another [31]. Meanwhile, 4*H*-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) is a pyranone derivative, which is identified to have a strong antioxidant activity among Maillard reaction products with concentrations ranging from 50 µg/mL to 400 µg/mL [32].

On the other hand, methyl salicylate was only found to be present from the petroleum ether extract. Methyl salicylate is a topical agent, applied on the skin, used for treatment of minor aches and pains of the body's muscles and joints caused by strains, sprains, arthritis or backaches [33]. Methyl salicylate was historically extracted from the small wintergreen plant (*Gaultheria procumbens* L.) and from birch trees (*Betula lenta* L.). The widely used of methyl salicylate for pain reliefs also poses damage to human health. Freshly distilled form of wintergreen oils contains over 98% of methyl salicylate. It has been established that ingestion of a single teaspoon (about 5 mL) of this oil can be equivalent to 22 conventional aspirin tablets, which could lead to potentially acute toxic dose of salicylate [34]. Over-application or accidental ingestion which result to salicylate poisoning can be fatal in severe cases [34,35].

Boiled calabash exhibited a maximum 80% inhibition of the free radicals produced by DPPH (Table-8). The percent inhibition of raw *C. cujete* resulted to be 40.5% inhibition. The average of the antioxidant concentrations of the boiled and raw calabash were 7.940 ppm and 5.093 ppm per 40.00 mg sample, respectively. One serving of boiled and raw calabash which is approximately 100 g, contains an equivalent amount of antioxidant equal to 992 mg and 636.36 mg of vitamin C, respectively [36].

C. cujete fruit extracts have a variety of components that have several medicinal purposes. In the Philippines, *C. cujete* is known as the miracle tree and these compounds could be the reason behind its popularity. Several chemical constituents present various biological activities according to literatures. However, since toxic agents such as benzoic acid were prevalent in most of the solvent extractions, the use of this as a traditional herbal preparation must be heavily weighed. The benefits accorded to the fruit pulp and leaf concoctions or formulations to address respiratory, diabetes, internal abscesses and snake envenomation [37]. Moreover, to truly test the miracle claims of this fruit, further study and clinical trials must be done on the extracts of *C. cujete*.

TABLE-8
ANTIOXIDANT ACTIVITY DATA OF PROCESSED *C. cujete* L. FRUIT

Boiled calabash		Raw calabash	
Absorbance (mAU)	Correlation to vitamin C activity concentration (ppm)	Absorbance (mAU)	Correlation to vitamin C activity concentration (ppm)
0.096	7.82	0.207	5.473
0.093	7.88	0.225	5.093
0.082	8.11	0.243	4.713
Mean ± SD			
0.0903±7.37E-3	7.94 ± 0.156	0.225 ± 0.0180	5.09 ± 0.380

Conclusion

A total of 18 compounds were identified from *C. cujete* fruit from the six extraction procedures performed using different organic solvents. Several components present biological activities that have potentials for medicinal purposes. The boiled pulp extracts was seen to have higher antioxidant activity than the raw fruit possibly due to the presence of benzoic acid, phenol, 2,2′-methylene*bis*[6-(1,1-dimethylethyl)-4-methyl-hexadecanoic acid and 2-[(1-oxododecyl)oxy]-1,3-propanediyl ester. However, there are some components that might present toxicity to human health when taken and used excessively. Further studies are needed to fully utilize the health benefits of the miracle tree.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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