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Research Article

Exploring the Potential Health Benefits of Plants and Fruits Traditionally Consumed in the Yucatan Peninsula

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Abstract

In addition to the great biodiversity of flora and fauna, Mexico has a wide cultural diversity with more than fifty ethnic groups, which include the Mayans in the Yucatan peninsula. Presently, many species of the Yucatecan flora are consumed by the people living in the Yucatan peninsula, as part of their everyday diet. However, to date, little is known about the health benefits of these species and the bioactive secondary metabolites responsible for these effects. This investigation evaluated the antioxidant, antidiabetic, anticancer, and anti-AGEs activities, as well as the phytochemical composition, of fifteen fruits and one plant traditionally consumed in the Yucatan peninsula, to explore their potential as functional foods. The results showed that the high antioxidant activity of the fruits of *Byrsonima bucidifolia* Standl, *Melicoccus bijugatus* (Jacq) and *Phyllanthus acidus* (L) Skeels positively correlated with their total polyphenol content. Alternatively, testing of all the extracts for their potential antidiabetic activity showed that only the fruits of *Cucurbita moschata* (Duchesne ex Lam.) and *Cordia dodecandra* (A.DC.) significantly inhibited the α -glucosidase activity. Finally, UPLC-MS analyses allowed the identification of a number of polyphenols, including quercetin, phenolic acids, and epicatechin, in the different extracts. Given that functional foods demonstrate beneficial biological functions beyond basic nutrients, the results of this investigation suggest that the fruits of *Byrsonima bucidifolia* Standl, *M. bijugatus* (Jacq), *C. moschata* (Duchesne ex Lam.) and *C. dodecandra* (A.DC.) could be considered as potential functional foods because of their antioxidant capacity and potential antidiabetic properties.

Keywords: Antioxidant activity; α -glucosidase inhibition; Polyphenols; Bioactive metabolites; Yucatecan cuisine.

Introduction

About two-thirds of the world's biodiversity is located in about a dozen countries known as mega-diverse countries, with Mexico occupying the fourth place [1]. The biodiversity of Mexico is a result of its geographic position, its complex geological history, its rugged topography, and its extensive coastline along two oceans [2]. These features have allowed the development of a wide diversity of terrestrial ecosystems harboring thousands of species belonging to all taxonomic groups, with close to 50% of these species being endemic [2,3].

In addition to its biodiversity, Mexico has a rich and unique cultural diversity with more than 50 ethnic groups, which include the Mayan people living in the southeastern states of Yucatan, Quintana Roo and Campeche, located in the Yucatan peninsula [4]. The biodiversity of the region includes over 3,000 plant species; of these, close to 175 are considered to be endemic and many, both introduced and endemic, are part of the diet of the Mayan people living in the Yucatan peninsula and are not commonly consumed in other parts of Mexico.

Among the great diversity of plants, leafy vegetables and fruits consumed by the Mayan people of the Yucatan peninsula, there are a number of native species including “chaya” [*Cnidioscolus aconitifolius* (Mill.) I.M. Johnst. (Euphorbiaceae)], “cocoyol” [*Acrocomia aculeata* (Jacq) Lodd. Ex Mart (Arecaceae)], “grosella” [*Phyllanthus acidus* (L) Skeels (Phyllanthaceae)], “nance amarillo” [Sweet craboo; *Byrsonima crassifolia* (L.) Kunth (Malpighiaceae)], “ciricote” [*Cordia dodecandra* A. DC. (Boraginaceae)], “zapote negro” [Black sapote; *Diospyros digyna* Jacq. (Ebenaceae)], “zapote” [Sapodilla; *Manilkara zapota* (L.) P. Royen (Sapotaceae)], “Kanisté” [Egg-fruit-tree; *Pouteria campechiana* (Kunth) Baehni (Sapotaceae)], “nance agrio” [*Byrsonima bucidifolia* Standl (Malpighiaceae)], “ciruela” [Green yellow mombin; *Spondias purpurea* L. (Anacardiaceae)], and “huaya india” [*Melicoccus oliviformis* (Kunth) Raldk (Sapindaceae)]; other fruits from species that became part of the local biodiversity after their introduction include “saramuyo” [Sugar apple; *Annona squamosa* L. (Annonaceae)], “caimito” [Purple star apple; *Chrysophyllum cainito* L. (Sapotaceae)], “mamoncillo” [Mamoncillo; *Melicoccus bijugatus* Jacq (Sapindaceae)], “mamey” [Mamey apple; *Pouteria sapota* (Jacq.) H.E. Moore & Stearn, (Sapotaceae)] and “calabaza yucateca” [*Cucurbita moschata* Duchesne ex Lam, (Cucurbitaceae)].

In spite of their consumption as part of the daily diet, “chaya” and many of the fruit species previously described are not widely available in Yucatecan or national supermarkets, and can only be found in local markets or with street vendors. Additionally,

most of the fruits have not been studied in terms of their biological activity, their bioactive constituents, or the potential health benefits associated with the prevention and/or treatment of chronic noncommunicable diseases (NCDs) such as metabolic syndrome, cancer, diabetes, and cardiovascular diseases [5,6]. Since the investigation of the health-promoting properties (e.g., antioxidant, antimicrobial, and antidiabetic activity) of these native and exotic tropical fruits could promote their general consumption and ensure their preservation by recognizing their economic potential as functional foods [7,8], the main objective of this study was to evaluate the biological activity and the bioactive components in the leaves or fruits of sixteen selected plants traditionally consumed by the people of the Yucatan peninsula.

Materials and Methods

Chemical reagents

All reagents and enzymes [ascorbic acid, gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, *b*-carotene, 2,6-Di-*tert*-butyl-4-methylphenol (BHT), *p*-nitrophenyl- α -D-glucopyranoside (PNPG), α -glucosidase from *Saccharomyces cerevisiae*, blocked *p*-nitrophenyl- α -D-maltoheptoside, α -amylase from porcine pancreas, acarbose, Folin-Ciocalteu reagent, 2,4,6-tris (2-pyridyl)-*s*-tri-azine (TPTZ), ferric chloride] were purchased from Sigma-Aldrich (Ottawa, ON, Canada).

Sample collection and handling

Some of the leaves and fruits of the different species (*C. aconitifolius*, *B. crassifolia*, *C. cainito*, *M. zapota*, *P. sapota*, *P. acidus*) were purchased at the local market in Merida, Yucatan, Mexico; others (*C. dodecandra*, *D. digyna*, *P. campechiana*) were collected from trees growing in the Botanical Garden of “Centro de Investigación Científica de Yucatán” or in a private backyard garden (*M. oliviformis*, *S. purpurea*, *A. aculeata*, *B. bucidifolia*) in Felipe Carrillo Puerto, Quintana Roo, Mexico. The collection took place between March and August of 2017; all fruits were purchased/collected when ready for consumption. The leaves or edible parts (pulp with or without skin) of the fruits were frozen at -80 °C and lyophilized (72 h). An average yield of approximately 40% of lyophilized material was obtained for all species.

Preparation of crude extracts

The lyophilized and powdered material was extracted following the methodology reported by Paz et al. [9], with some modifications. In short, 2.5 g of material was added to 100 mL of ethanol:water (1:1) and allowed to stir (100 rpm) for 24 h. The suspension was filtered and the filtrate subjected to centrifugation (4000 rpm for 35 min); the supernatant was gravity-filtered (Whatman No. 1 filter paper) and concentrated in vacuo at 35 °C. The aqueous residue was frozen, lyophilized (72 h) and stored at 4

°C until used. All extractions were carried out in triplicate.

Phytochemical analysis

Determination of total phenolic content (TPC)

The TPC of the different extracts was determined following the Folin-Ciocalteu method as described by Huber & Rupasinghe [10]; different concentrations (10, 20, 40, 80, 100, 150 and 250 mM) of gallic acid in methanol were used to prepare the standard curve. The test solutions were prepared fresh under reduced light conditions and the reaction was carried out under dark conditions. Twenty microliters of the diluted extract, or gallic acid standard, were combined with 100 mL of 0.2 N Folin-Ciocalteu phenol reagent in a 96-well clear polystyrene microplates. After 5 min, 80 mL of 7.5% (w/v) sodium carbonate solution was added to each well and mixed. The mixture was incubated for 2 h at room temperature before measuring the absorption at 760 nm using the plate reader (Tecan Infinite[®] M200 PRO microplate reader; Morrisville, NC, USA). Results are expressed as mg of gallic acid equivalents (GAE) per gram dry weight (mg GAE/g DW).

Determination of total carotenoid content (TCC)

The TCC was measured using the method reported by Parmar & Rupasinghe [11]. A solution of 20 mg of extract in 15 mL of a 6:4 mixture of methanol and ethylacetate was kept for 20 minutes at 60 °C under shake conditions (100 rpm). The samples were cooled in ice until they reached room temperature and the liquid phase was decanted. A portion (10 mL) of the liquid phase was combined with 10 mL of a 9:1 mixture of hexane and diethylether and then washed with 20 mL of a saturated NaCl solution. The organic phase was separated and evaporated under a nitrogen flow. The dried extract was dissolved in 5 mL of methanol and a 200 mL aliquot was taken and transferred to a 96-well plate, where the absorbance was measured in a plate reader (Tecan Infinite[®] M200 PRO microplate reader; Morrisville, NC, USA) at 470 nm. The TCC was calculated using the formula shown below:

$$TCC = \frac{(A * 10^6 * V)}{(A^{(1\%)} * 100 * G)}$$

Where A is the absorbance at 470 nm, V is the total volume of the extract, A^(1%) is the extinction coefficient for a mixture of solvents arbitrarily set a 2500, and G is the weight of the sample [13].

Total antioxidant capacity

DPPH-radical scavenging assay

The total antioxidant capacity assay measuring the DPPH radical reduction capacity of all extracts was carried out following the methodology reported by Castillo-Avila et al. [14]. A 25 μM ethanolic solution of DPPH was prepared minutes before being

used to prevent decomposition. The experiment was carried out in 96-well plates and a combination of 180 μL of DPPH solution (25 mM) and 20 μL of the test sample (1 C 10⁻¹ - 1 C 10⁻⁴) was added to each well. The mixture was allowed to stand for 30 minutes in the dark, then the absorbance was read at a wavelength of 517 nm; the absorbance values were extrapolated to a calibration curve prepared with ascorbic acid at different concentrations (1 C 10⁻¹ to 1 C 10⁻⁴), to determine the antioxidant activity of the samples tested.

Ferric reduction antioxidant power (FRAP) assay

The total antioxidant capacity determined by the FRAP method was carried out according to Huber & Rupasinghe [10]. The reaction reagent (FRAP solution) was prepared just before the assay by mixing 300 mM of acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride solution in a ratio of 10:1:1. The TPTZ solution was prepared the same day of the analysis. Standard solutions of Trolox were prepared by diluting a 1 mM Trolox stock solution in methanol to obtain concentrations of 5, 10, 25, 75, 150 and 300 μM of Trolox. The FRAP analysis was performed by combining 20 μL of blank, standard (5, 10, 15, 20, 25 mg/mL) or sample (10 mg/mL) with 180 μL of FRAP solution in 96-well plates. The reading was taken at 595 nm. Both the FRAP solution and the samples in the microplate were heated to 37 °C before the test. The FRAP values were expressed as milligram equivalents of Trolox (TE) per gram dry weight of the sample (mg TE/g DW).

In vitro antidiabetic capacity

α-amylase inhibitory assay

The pancreatic porcine α-amylase assay was adapted from Parmar & Rupasinghe [11], with modifications. The enzyme (10 mg) was dissolved in 3 mL of PBS 0.01 M (PBS: sodium phosphate buffer at pH 6.9). Solutions of the extracts at different concentrations (1-500 mg/mL) were prepared by dissolving in PBS. The tests were carried out in a 96-well plate, where 20 mL of pancreatic porcine α-amylase and 20 mL of test extracts at different concentrations were added to each well. The mixtures were incubated at 37°C for 10 min and after this time, 20 mL of the substrate (Blocked p-nitrophenyl-alpha-D-maltoheptaoside) were added and the mixture was incubated for 20 min at 37 °C. After incubation, 240 mL of stop solution (trisodium phosphate at pH 11) were added. The plate was read at 405 nm using a plate reader (Tecan Infinite[®] M200 PRO microplate reader; Morrisville, NC, USA). The α-amylase (20 mL enzyme and 20 mL PBS) control without any inhibitor represented 100% enzyme activity. Appropriate test extract controls containing the reaction mixture with and without the enzyme were used to correct for color interference. Acarbose was used as a positive control. The percentage inhibition of α-amylase by the test sample was calculated using the formula:

$$\text{Inhibition (\%)} = 100 \times (\text{AC} - \text{AS}) / (\text{AC})$$

Where AS and AC are the absorbance values of the sample and the control, respectively. The results are expressed in terms of IC_{50} , representing the concentration of test extract required to cause 50% inhibition of the enzyme. Calculations were carried out using the GraphPad Prism 5 software. All determinations were made in triplicate.

***α*-glucosidase inhibitory assay**

The *α*-glucosidase inhibitory assay was adapted from Parmar & Rupasinghe [11] with modifications. Solutions of extracts were prepared at different concentrations (1, 10, 25, 50, 100, 125, 250, 500, 1,000 mg/mL) in 0.01 M PBS. A reaction mixture containing 20 mL of extract at different concentrations, 20 mL of *α*-glucosidase (0.5 U/mL), and 60 mL of PBS 0.01 M was added to each well of a 96-well clear plate; the plate was incubated at 37 °C for 15 min, prior to adding 20 mL of 5 mM 4-nitrophenyl-*α*-D-glucopyranoside (PNPG) as substrate. The mixture was then incubated at 37 °C for the reaction to take place (15 min). The reaction was terminated by adding 80 mL of 200 mM sodium carbonate and the absorbance at 405 nm was recorded using a plate reader (Tecan Infinite[®] M200 PRO microplate reader; Morrisville, NC, USA). The *α*-glucosidase control (20 mL enzyme and 120 mL PBS) without any inhibitor represented 100% enzyme activity. Appropriate test extract controls containing the reaction mixture with and without the enzyme were used to correct for color interference. The *α*-glucosidase inhibition (%) of the samples was calculated in the same way as described previously for the *α*-amylase assay. Acarbose, a prescribed drug for a *α*-glucosidase inhibition, was also used as a positive control. The results are expressed as the concentration of extract inhibiting the enzyme activity by 50% (IC_{50}), as calculated by the GraphPad Prism 5 software. All determinations were made in triplicate.

Anticancer activity

Cell cultures

Cell lines of cervix adenocarcinoma (HeLa, ATCC-CCL-2), cervix squamous carcinoma (SiHa, ATCC-HTB-35), breast adenocarcinoma (MCF-7, ATCC-HTB-22; MDA-MB-231, ATCC-HTB-26), and green monkey kidney cells (Vero, ATCC-CCL-81), from the American Type Culture Collection (ATCC), were kindly provided by Veronica Vallejo-Ruiz from the East Biomedical Research Center (IMSS, Mexico). The cells were cultured in sterile Costar T25 flasks containing D-MEM medium (Gibco), supplemented with fetal bovine serum (FBS) (10%, v/v), 100 U/mL penicillin G, and 100 μg/mL streptomycin at 37 °C under 5% CO₂ atmosphere (95% humidity).

Bioassay of cytotoxic activity

The growth inhibition of the cell lines was evaluated using the sulforhodamine B method [15]. At 70-80% confluence, cells

were detached from the culture flask by treatment with 0.05% trypsin-EDTA (Gibco) and a suspension of 1.5×10^4 cell/mL of viable cells were seeded in a 96-well microtiter plate (Costar) and incubated for 24 h. When the cells reached >80% confluence, the medium was replaced and cells were incubated with stock solutions of extracts serially diluted to reach concentrations of 50.0, 25.0, 12.5, and 6.25 μg/mL. After 48 h of incubation, the medium was discarded and 100 μL of ice-cold 40% trichloroacetic acid (TCA, Aldrich) were added to fix the cells, incubating for 1 h at 4°C. The cells were washed five times with water, left to dry, and then 50 μL of SRB stain (10 mg, 1% acetic acid, Sigma) were added to each well and left to stand for 30 min. Finally, the cells were washed with 50 μL 1% acetic acid and solubilized with tris(hydroxymethyl)aminomethane (10 nM). The optical density of the solution was measured at 560 nm using a spectrophotometer bioassay reader (BioRad, USA). Results are expressed as the concentration of agent that reduces cell growth by 50% (CC_{50}), calculated by GraphPad Prism 5 software. Docetaxel was used as a positive control. All determinations were performed in triplicate. In addition, the level of harmfulness on normal cells was evaluated by determining the selectivity index (SI) [16].

Bioassay of antiproliferative activity

The sulforhodamine B (SRB) assay was carried out according to the method reported by Skehan et al. [15], using D-MEM medium with 10% FBS to induce cell proliferation. After 48 h of incubation, the medium was discarded and 100 μL of ice-cold 40% trichloroacetic acid (TCA, Aldrich) were added to fix the cells, incubating for 1 h at 4 °C. The cells were washed five times with water, left to dry, and then 50 μL of SRB stain (10 mg, 1% acetic acid, Sigma) were added to each well and left to stand for 30 min. Finally, the cells were washed with 50 μL of 1% acetic acid and solubilized with tris(hydroxymethyl)aminomethane (10 nM). The optical density of the solution was measured at 560 nm using a spectrophotometer bioassay reader (BioRad, USA). Results are expressed as the concentration of agent that reduces cell growth by 50% (IC_{50}), calculated by GraphPad Prism 5 software. Docetaxel was used as a positive control. All determinations were performed in triplicate. In addition, the level of harmfulness on normal cells was evaluated by determining the selectivity index (SI) [16].

Identification of polyphenolic metabolites using Ultra-performance liquid chromatography and mass spectrometry (UPLC/MS)

Analyses of major individual phenolic compounds present in freeze-dried fruits and plant extracts were performed according to a previously reported method [17]. All analyses were conducted using an ultra-pressure liquid chromatograph (Model Waters Acquity CHA, Waters Corp, Milford, MA, USA) coupled with Micromass Quattro Micro API tandem mass spectrometry (UPLC-

MS/MS) system and controlled with Mass Lynx V4.2 data analysis system (Micromass, Cary, NC, USA). The column used was an Aquity BEH C₁₈ (100 × 2.1 mm, 1.7µm) (Waters, Milford, MA, USA), and the mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). A linear gradient profile was used with the following proportions of Solvent A applied at different times (min, A%): (0, 94%), (2, 83.5%), (2.61, 83%), (2.17, 82.5%), (3.63, 82.5%), (4.08, 81.5%), (4.76, 80%), (6.75, 20%), (8.75, 94%), and (12, 94%). Electrospray ionization in negative ion mode (ESI-) was used for the analysis of the flavonols, phenolic acids, and catechins, under the following conditions: capillary voltage 3,000 V, nebulizer gas (N₂) temperature 375° C at a flow rate of 0.35 mL/min. The settings for positive ion experiments were as follows: capillary voltage (25–50V), optimized for each component, and single ion monitoring (SIM) mode using specific precursor ion for quantification by comparing with standards [*m/z* 301 for quercetin (Q), *m/z* 608.5 for Q-3-*O*-rutinoside, *m/z* 463 for Q-3-*O*-glucoside and *m/z* 446.7 for Q-3-*O*-rhamnoside, *m/z* 594.7 for Q-3-*O*-arabinoglucoside (peltatoside), *m/z* 433.3 for Q-3-*O*-arabinoside, *m/z* 595 for phloritin, *m/z* 435 for phloridzin, *m/z* 352.7 for chlorogenic acid, *m/z* 179 for caffeic acid, *m/z* 288.7 for catechin and epicatechin, and *m/z* 305 for epigallocatechin].

Statistical analysis

The experiments were performed in a completely randomized design in triplicates, and data are expressed as mean ± standard error (± SE). The significant difference between, TPC, TCC,

FRAP, DPPH, α-amylase, and α-glucosidase inhibition values were tested using one-way analysis of variance (ANOVA) and the multiple mean comparison using LSD test (IBM SPSS Software[®]). Significant levels were defined as probabilities of 0.05 or less.

Results

A group of fifteen fruits and one plant, belonging to eleven different families, was identified as commonly consumed by the Yucatecan population either fresh or as part of Yucatecan traditional dishes (Table 1). The edible part(s) were frozen and lyophilized before extraction with an ethanol-water mixture.

Phytochemical analysis

Initial phytochemical analyses were carried out to determine the total phenolic (TPC) and carotenoid (TCC) content of the different extracts (Table 2). The highest TPC value (mg GAE/g DW) was found in the extract of the fruits of *B. bucidifolia* (93.76); a second group, showing moderate TPC values, included the extracts from the leaves of *C. aconitifolius* (22.84), and the fruits of *M. bijugatus* (25.14), *P. acidus* (22.99), *A. aculeata* (21.28), *D. digyna* (19.76) and *S. purpurea* (19.17). The rest of the fruit extracts showed values well below 15.76 mg GAE/g DW (Table 2). Finally, evaluation of the TCC in all extracts showed very low values for most of the extracts, with only the leaf extracts of *C. aconitifolius* (174.00 mg/g DW) and the fruit extracts of *C. moschata* (152.63 mg/g DW) showing a moderate TCC content (Table 2).

Table 1: Plants and fruits traditionally consumed in Yucatan investigated in this study.

Botanical family	Scientific name*	Common name	Edible part
Sapotaceae	<i>Manilkara zapota</i> (L.) P. Royen	Zapote (sapodilla ^b)	Pulp
Sapotaceae	<i>Pouteria sapota</i> (Jacq.) H.E. Moore & Stearn	Mamey (mamey apple ^a)	Pulp
Sapotaceae	<i>Chrysophyllum cainito</i> L.	Caimito (purple star apple ^a)	Pulp
Cucurbitaceae	<i>Cucurbita moschata</i> (Duchesne ex Lam.)	Calabaza yucateca	Pulp + peel
Phyllanthaceae	<i>Phyllanthus acidus</i> (L) Skeels.	Grosella	Pulp + peel
Anacardiaceae	<i>Spondias purpurea</i> L.	Ciruela (green yellow mombin ^a)	Pulp + peel
Sapindaceae	<i>Melicoccus oliviformis</i> Kunth	Huaya india	Pulp
Sapotaceae	<i>Pouteria campechiana</i> (Kunth) Baehni	Kanisté (egg-fruit-tree ^a)	Pulp
Ebenaceae	<i>Diospyros digyna</i> Jacq	Zapote negro (black sapote ^a)	Pulp
Sapindaceae	<i>Melicoccus bijugatus</i> Jacq	Mamoncillo	Pulp
Annonaceae	<i>Annona squamosa</i> L.	Saramuyo (sugar apple ^a)	Pulp
Malpighiaceae	<i>Byrsonima crassifolia</i> (L.) Kunth	Nance amarillo (sweet craboo ^a)	Pulp + peel

Malpighiaceae	<i>Byrsonima bucidifolia</i> Standl.	Nance agrio	Pulp + peel
Areaceae	<i>Acrocomia aculeata</i> (Jacq) Lodd. Ex Mart.	Cocoyol	Pulp
Boraginaceae	<i>Cordia dodecandra</i> A. DC.	Ciricote	Pulp + peel
Euphorbiaceae	<i>Cnidoscolus aconitifolius</i> (Mill.) I.M. Johnst.)	Chaya	Leaves

*Scientific and common names taken from ^aThe Plant List and ^bMoo-Huchin et al. (2014)

Antioxidant capacity

The antioxidant capacity of the different extracts was evaluated using two different methods: FRAP and DPPH-reduction. In the FRAP method, where the antioxidant activity is expressed as mg TE/g DW, four fruit extracts showed the highest values: *M. bijugatus* (0.272), *P. acidus* (0.250), *S. purpurea* (0.225) and *D. digyna* (0.193), while a second group, which included *A. aculeata* (0.162), *B. crassifolia* (0.160), and *B. bucidifolia* (0.137), had a moderate activity. The rest of the species showed values below 0.112 mg TE/g DW (Table 2). Finally, evaluation of the antioxidant activity of all extracts using the DPPH reduction assay showed the highest activity, with an IC₅₀ of 0.08 mg/ml, in the fruit extract of *B. bucidifolia*, followed by those from *M. bijugatus* and *P. acidus* showing a moderate activity (IC₅₀ 0.46 mg/ml and 0.50 mg/ml, respectively) (Table 2)

Table 2: Phytochemical analyses, antioxidant capacity and enzymatic inhibition of plants and fruits traditionally consumed in Yucatan.

Samples	Total phenols (mg GAE/g DW)	Total carotenoids content (mg/g DW)	FRAP (mg TE/g DW)	DPPH IC ₅₀ (mg/mL)	α -amylase IC ₅₀ (□g/mL)	α -glucosidase IC ₅₀ (□g/mL)
<i>Manilkara zapota</i>	2.38 ± 0.78 ⁱ	121.37 ± 0.28 ^d	0.026 ± 0.01 ^g	> 0.60	≥ 1000	184.2 ± 16.95 ^{abcd}
<i>Pouteria sapota</i>	3.88 ± 0.78 ⁱ	122.60 ± 0.43 ^{cd}	0.026 ± 0.01 ^g	0.57 ± 0.27 ^b	≥ 1000	158.73 ± 14.53 ^{abc}
<i>Chrysophyllum cainito</i>	6.22 ± 0.96 ^{hi}	122.23 ± 0.86 ^d	0.069 ± 0.01 ^{efg}	> 0.60	680.3 ± 71.31 ^a	155.76 ± 7.38 ^{abc}
<i>Cucurbita moschata</i>	5.89 ± 1.19 ⁱ	152.63 ± 1.04 ^b	0.031 ± 0.01 ^g	> 0.60	≥ 1000	114.43 ± 12.80 ^a
<i>Phyllanthus acidus</i>	22.99 ± 1.91 ^{bc}	124.63 ± 0.31 ^{cd}	0.249 ± 0.02 ^{ab}	0.50 ± 0.12 ^{ab}	≥ 1000	140.30 ± 18.60 ^{abc}
<i>Spondias purpurea</i>	19.17 ± 1.48 ^{cde}	124.70 ± 0.76 ^{cd}	0.225 ± 0.02 ^{ab}	0.56 ± 0.13 ^b	≥ 1000	≥ 200
<i>Melicoccus oliviformis</i>	10.48 ± 1.12 ^{gh}	124.67 ± 1.03 ^{cd}	0.067 ± 0.01 ^{fg}	> 0.60	≥ 1000	≥ 200
<i>Pouteria campechiana</i>	4.32 ± 1.03 ⁱ	123.77 ± 0.50 ^{cd}	0.024 ± 0.01 ^g	> 0.60	≥ 1000	≥ 200
<i>Diospyros digyna</i>	19.76 ± 1.20 ^{cd}	127.97 ± 0.82 ^c	0.193 ± 0.01 ^{bc}	> 0.60	≥ 1000	≥ 200
<i>Melicoccus bijugatus</i>	25.14 ± 1.5 ^b	121.13 ± 0.31 ^d	0.272 ± 0.02 ^a	0.46 ± 0.22 ^{ab}	≥ 1000	≥ 200
<i>Annona squamosa</i>	15.76 ± 0.66 ^{def}	121.63 ± 0.64 ^d	0.096 ± 0.01 ^{ef}	> 0.60	≥ 1000	183.63 ± 33.69 ^{abcd}

<i>Byrsonima crassifolia</i>	13.32 ± 0.97 ^{fg}	126.17 ± 2.22 ^{cd}	0.159 ± 0.01 ^{cd}	> 0.60	≥ 1000	≥ 200
<i>Byrsonima bucidifolia</i>	93.76 ± 1.42 ^a	125.57 ± 0.86 ^{cd}	0.137 ± 0.03 ^{cd}	0.08 ± 0.01 ^a	≥ 1000	180.63 ± 33.29 ^{abcd}
<i>Acrocomia aculeata</i>	21.28 ± 0.78 ^{bc}	125.13 ± 1.53 ^{cd}	0.162 ± 0.02 ^{cd}	> 0.60	≥ 1000	168.27 ± 40.46 ^{abcd}
<i>Cordia dodecandra</i>	15.06 ± 0.99 ^{ef}	121.27 ± 0.03 ^d	0.111 ± 0.02 ^{def}	> 0.60	≥ 1000	123.55 ± 22.40 ^{ab}
<i>Cnidioscolus aconitifolius</i>	22.84 ± 1.24 ^{bc}	174.00 ± 0.20 ^a	0.068 ± 0.01 ^{efg}	> 0.60	≥ 1000	140.63 ± 34.86 ^{abc}
EGCG					99.67 ± 13.42	35.54 ± 1.46
Acarbose					12.12 ± 1.14	26.23 ± 4.92
Ascorbic acid				0.041 ± 0.004		

The results are expressed as the average ± SE, for three independent experiments. Different letters within rows denote significant differences between values as determined by one-way ANOVA analysis

***α*-glucosidase and *α*-amylase activity**

The potential antidiabetic activity of the different extracts was evaluated measuring their capacity to inhibit the activity of the carbohydrate-hydrolyzing enzymes *α*-glucosidase and *α*-amylase. While all the extracts showed inhibition of *α*-glucosidase, with IC₅₀ values ranging from 114.43 to 287.06 μg/mL (Table 2), the highest inhibitory activity was observed for the fruit extracts of *C. moschata* (IC₅₀ 114.43 μg/ml) and *C. dodecandra* (IC₅₀ 123.55 μg/ml). Alternatively, when tested for their inhibition of the *α*-amylase, only six fruit extracts showed inhibitory activity, with the extract of *C. cainito* exhibiting the strongest *α*-amylase inhibitory activity (IC₅₀ 680.3 μg/mL), and none of the remaining extracts showing significant inhibition of the enzyme (Table 2).

Cytotoxic and antiproliferative activity

The cytotoxic and antiproliferative activity of the different extracts was evaluated against five tumor cell lines (Vero, SiHa, HeLa, MDA, MCF-7). The results showed that none of the extracts had antiproliferative activity and that only the fruit extract of *A. squamosa* showed cytotoxic activity, with a CC₅₀ of 51.92 ± 1.1 μg/mL and a selectivity index of 1.9.

Identification and quantification of major phenolics

The chromatographic profiles of all extracts were determined by UPLC. A number of common phenolics were detected and identified by comparing their retention times and *m/z* of deprotonated parent ions with those of commercial flavonols (quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, quercetin, quercetin-3-*O*-rutinoside, quercetin-3-arabino-furanoside), chalcones (phloridzin and phloretin), phenolic acids (cafeic acid and chlorogenic acid), and flavan-3-ols (catechin, epicatechin, epigallocatechin, epicatechin-3-gallate and epigallocatechin-3-gallate) (Fig. 1). The concentration of the identified phenolics was estimated using the external standard curves of each compound and the results are expressed as mg/g DW of extract. Of the three groups of the phenolic quantified, the flavonols (quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside), were found in greater amounts in *P. acidus* (quercetin-3-*O*-arabinoside, 0.941), *S. purpurea* (quercetin-3-*O*-arabinoside, 0.772), *C. aconitifolius* (quercetin-3-*O*-rhamnoside, 0.608) and *C. dodecandra* (quercetin-3-*O*-rutinoside, 0.571) (Table 3). Alternatively, the phenolic acids were particularly abundant in *P. acidus* (chlorogenic acid, 0.299) and *C. dodecandra* (cafeic acid, 0.286), while only one catechin (epicatechin, 0.114) was found in *A. aculeata* (Table 3).

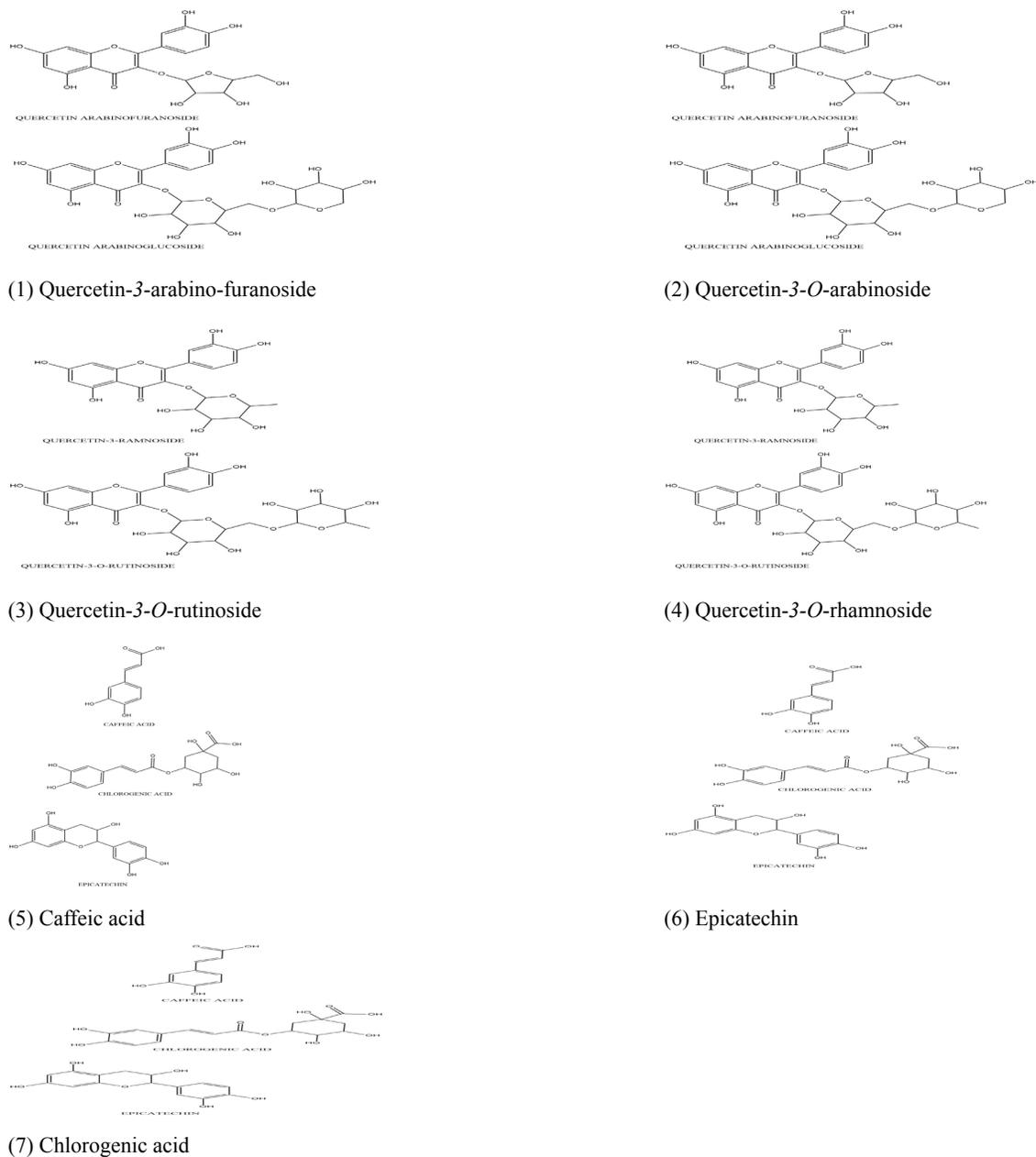


Figure 1. Phenolic metabolites quantified in extracts from Yucatecan plants and fruits.

Table 3: Quantification of phenolic metabolites present in crude extracts from plants and fruits traditionally consumed in Yucatan

Sample	Flavonols (mg /g extract)				Phenolic acids (mg /g extract)		Catechins (mg /g extract)
	Quercetin ArabinoGlu	Quercetin Rhamnoside	Quercetin Rutinoside	Q3Arabinofuro	Cafeic acid	Chlorogenic acid	Epicatechin
<i>Cucurbita moschata</i>	< 0.1	< 0.1	0.145	< 0.1	< 0.1	< 0.1	< 0.1
<i>Phyllanthus acidus</i>	0.941	0.409	0.137	< 0.1	< 0.1	0.299	< 0.1
<i>Spondias purpurea</i>	0.772	0.255	0.1	< 0.1	< 0.1	0.238	< 0.1
<i>Byrsonima bucidifolia</i>	< 0.1	< 0.1	< 0.1	0.159	< 0.1	< 0.1	< 0.1
<i>Acrocomia aculeata</i>	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.114
<i>Cordia dodecandra</i>	< 0.1	< 0.1	0.571	< 0.1	0.286	< 0.1	< 0.1
<i>Cnidioscolus aconitifolius</i>	0.213	0.608	0.452	<0.1	< 0.1	<0.1	< 0.1
Results are expressed as the average for three independent determinations.							

Discussion

To the best of our knowledge, the results of the present study provide, for the first time, information about the biological activities, together with the qualitative and quantitative content of the main biologically active secondary metabolites, of sixteen plants and fruits traditionally consumed in Yucatan. The fruits of *B. bucidifolia* showed the highest TPC, followed by the leaves of *C. aconitifolius* and the fruits of *M. bijugatus*, *P. acidus*, *A. aculeata*, *D. digyna* and *S. purpurea*; the TPC content of this group of species is comparable with that previously reported for similar or related species [18,19]. However, the TCC contents of different extracts were significantly lower than those previously reported for some of the species [18]; these differences might be due to the differences in the ripeness of the fruits at the time of collection [20].

The presence of phenolic metabolites in foods, particularly in fruits, is of particular interest mainly because of their antioxidant properties [21,22]. To date, there are several methods used to measure the total antioxidant capacity of extracts obtained from plant tissues [23,24], and it is often recommended that the antioxidant activity of a given sample is evaluated using at least two different methods since each method follows a different

reaction mechanism [25]. In this investigation, the antioxidant activity of the sixteen extracts was evaluated using the FRAP and DPPH assays, two methods that share the same mechanism (i.e. Single Electron Transfer), but differ in the way they measure the antioxidant activity; while DPPH reports the activity as the amount of the sample needed to reduce the biological effect by 50% [half maximal inhibitory concentration (IC_{50})], FRAP reports the activity as the equivalent to a reference antioxidant agent [25]. As expected, the extract of the fruits of *B. bucidifolia* showed the highest antioxidant activity in the DPPH radical reduction assay, confirming the direct correlation between antioxidant activity and the TPC. While there are no reports on the phytochemistry of the fruits of *B. bucidifolia*, a study carried out on the antioxidant activity of the leaf extract of the plant resulted in the isolation of two bioactive metabolites identified as artifacts originating from gallotanin-type precursors [14]. Alternatively, testing of the antioxidant activity of the different extracts using the FRAP assay showed the highest activity in the extracts from the fruits of *M. bijugatus*, *P. acidus*, and *S. purpurea*. These results are similar to those reported in the literature [18], where it is also mentioned that the drying process can influence the antioxidant activity of the extracts [26].

One of the chronic health conditions that affect a large percentage of the population in Mexico is type 2 diabetes mellitus, characterized by chronic hyperglycemia [27]. A key approach for controlling this complication is to decrease the postprandial increase in blood glucose levels through the consumption of fruits and vegetables, which could inhibit the carbohydrate-hydrolyzing enzymes (*α*-glucosidase and *α*-amylase). Currently, drugs such as acarbose, miglitol, and voglibose are recognized as potent inhibitors of these two enzymes; however, all of these pharmaceuticals have undesirable side effects that limit their use [28]. Due to this, the search for natural inhibitors present in common foods, such as fruits and vegetables, represents a potential alternative for the preventive treatment of hyperglycemia, with minimal side effects [29]. Testing of all the extracts for their inhibition of *α*-glucosidase and *α*-amylase inhibition showed that while none of the extracts had a significant inhibitory activity on *α*-amylase, the extracts of the fruits of *C. moschata* and *C. dodecandra* exhibited the highest inhibitory activity against *α*-glucosidase. It is interesting to point out that, even though it has been reported that the activity against *α*-glucosidase is highly favored by the content of polyphenols (Wang et al., 2013), neither the extract from the fruits of *C. moschata*, nor that from the fruits of *D. dodecandra*, showed a significant TPC, suggesting that the activity in the extracts of these two fruits might be due to non-phenolic secondary metabolites. These findings are in agreement with several reports in the literature describing the hypoglycemic potential of different fruit species from the Cucurbitaceae family [30] and the identification of a series of cucurbitane-type triterpene glucosides with *α*-glucosidase inhibitory activity from the extract of *Momordica charantia* [31]. It is interesting to mention, however, that a number of fruits from other plants belonging to the Boraginaceae family are reported to have antioxidant activity and high TPC values [32]. To date, the phytochemical and biological potential of *C. dodecandra* remains largely unknown, even though its fruits and wood represent an important source of income for the local population.

Testing of all the extracts for their cytotoxic and antiproliferative activity showed that none of the extracts had antiproliferative activity and that only the extract of *A. squamosa* had moderate cytotoxic activity; the cytotoxic activity of *A. squamosa* can be explained by the fact that plants of the Annonaceae family are reported to contain acetogenins, recognized as antitumor agents [33,34].

Although many studies on functional foods are particularly directed towards evaluating the antimicrobial activity of fruits and their extracts, since it has been argued that natural products with antioxidant activity generally show good antimicrobial activity and vice versa [9], none of the extracts tested in this investigation showed antimicrobial activity against a wide variety of human and plant pathogens (data not shown). Taking into consideration the

fact that some of the fruits evaluated showed a high TPC, it can be said that the antimicrobial activity cannot be attributed exclusively to the presence of antioxidant metabolites.

Similarly, none of the extracts showed significant inhibition of the formation of advanced glycation end products (AGEs; data not shown); this could be due to the absence of anthocyanins in the different extracts, since previous studies have suggested that a high content of anthocyanins is favorably correlated with anti-AGEs activity [35,36].

Qualitative and quantitative UPLC-MS/MS analyses of the extracts showed that the main polyphenolic metabolites in *P. acidus* and *C. dodecandra* were flavonols such as quercetin-3-*O*-arabinoglucoside, quercetin-3-*O*-ramnoside, quercetin-3-*O*-arabinofuranoside and phenolic acids such as caffeic acid and chlorogenic acid, while those in *S. purpurea* and *C. aconitifolius* were the flavonols 3-*O*-arabinoglucoside, quercetin-3-*O*-ramnoside, quercetin-3-*O*-arabinofuranoside and epicatechin in *A. aculeata*. Flavonols such as the identified quercetin-3-*O*-arabinoglu, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-rutinoside and quercetin-3-*O*-arabinofuranoside have been reported to be effective in reducing lipid peroxidation *in vitro* [37,38] and a diet rich in this type of flavanols is also associated with a lower incidence of cardiovascular diseases and some types of cancer [39].

Conclusions

This study represents the initial step in the investigation of the functional properties of one plant and a number of fruits consumed traditionally in Yucatan, Mexico. Given the limited or non-existent knowledge of the phytochemical content and biological activity of these selected species, the results of this study contribute to advancing the knowledge of their benefits and their potential use as functional foods in the future. Being that functional foods demonstrate beneficial biological functions beyond basic nutrients, the results of this investigation suggest that the fruits of *B. bucidifolia*, *M. bijugatus*, *P. acidus*, *S. purpurea*, *C. moschata*, *C. dodecandra* and the leaves of *C. aconitifolius*, could be further investigated as potential functional foods because of their comparatively greater antioxidant capacity and anti-diabetic properties.

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