Journal of Medicinally Active Plants

Volume 12
Issue 1 *Vol 12 Issue 1*

3-30-2023

# Antimicrobial and Antioxidant Properties of Theobroma cacao, Bourreria huanita, Eriobotrya japonica, and Elettaria cardamomum - Traditional Plants Used in Central America

# Antimicrobial and Antioxidant Properties of *Theobroma cacao*, *Bourreria huanita*, *Eriobotrya japonica*, and *Elettaria cardamomum* - Traditional Plants Used in Central America

Nadjet Cornejal[1,2Ψ], Evian Pollack[1Ψ], Rajvinder Kaur[1], Ashanna Persaud[1], Marlena Plagianos[3], H. Rodolfo Juliani[4], James E. Simon[4], Martin Zorde[4], Christine Priano[1], Adolfina Koroch[1], and José A. Fernández Romero[1,3*]

[1]Borough of Manhattan Community College, City University of New York, New York, NY 10007
[2] Brooklyn College, City University of New York, New York, NY, 11210
[3]Center for Biomedical Research, Population Council, New York, NY, 10065
[4]New Use Agriculture and Natural Plant Products, Department of Plant Biology, Rutgers University, NJ, 08901
[Ψ]Equal first author

***Corresponding author:** jfernandezromero@bmcc.cuny.edu

**Manuscript received:** January 14, 2023

**Keywords:** polyphenols, medicinal properties, antiviral, antibacterial, SARS-CoV-2 delta pseudoviral model

## ABSTRACT

**The search for alternative naturally occurring antimicrobial agents will always continue, especially when emerging diseases like COVID-19 provide an urgency to identify and develop safe and effective ways to prevent or treat these infections. The purpose of this study was to evaluate the potential antimicrobial activity as well as antioxidant properties of commercial samples from four traditional medicinal plants used in Central America: *Theobroma cacao*, *Bourreria huanita*, *Eriobotrya japonica*, and *Elettaria cardamomum*. Ethanolic extracts were prepared from commercial products derived from the seeds or flowers of these plants. Total phenolics and antioxidant activity were assessed using commercial kits. The cytotoxicity and antiviral activity against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) were evaluated using the XTT colorimetric assay and a SARS-CoV-2 delta pseudoviral model. The half-maximal cytotoxic concentration ($CC_{50}$) and half-maximal effective concentration ($EC_{50}$) were used to calculate the therapeutic index (TI). Additionally, the antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis* was tested using a spectrophotometric method. The extracts showed total phenolics in the range of 0.06 to 1.85 nM/µL catechin equivalents, with *T. cacao* bean extract showing the highest content. The antioxidant activity showed values between 0.02 and 0.44 mM Trolox equivalents. *T. cacao* bean extract showed the highest antioxidant activity. Most plant extracts showed zero to moderate selective antiviral activity; however, one *T. cacao* beans sample showed excellent antiviral activity against SARS-CoV-2 with a TI**

**value of 30.3, and one sample of *E. japonica* showed selective antiviral activity with a TI value of 18.7. Significant inhibition of *E. coli* and *S. epidermidis* by an *E. japonica* ethanolic extract ($p<0.001$) was observed using a spectrophotometric method that monitors bacterial growth over time. Additionally, ethanolic extracts of *E. cardamomum* showed significant inhibition of *S. epidermidis* growth ($p<0.001$). The results warrant further investigation of the antimicrobial and antioxidant properties of these plant extracts.**

## INTRODUCTION

Central America contains a rich history of medicinal plants as essential sources of medical treatments. Most of the traditional knowledge comes from Mayan medicine, and the Mayan rainforest provides many plants used for healing purposes (Cáceres, 1996). The Mayan civilization flourished from A.D. 250 to A.D. 900 and had about 200,000 square miles of land, including areas in southern Mexico, Guatemala, western Honduras, El Salvador, and northern Belize (Fusi, 2015). In the Q'eqchi, a Mayan community of the eastern lowlands, the native flora was used to treat different illnesses as part of their traditional medical experience and spiritual beliefs (Michel et al., 2007).

The health benefits of these plants have been associated with the accumulation of secondary metabolites such as phenolics. Secondary metabolites are often synthesized in the chloroplast or cytoplasm via a phenylpropanoid pathway (Biala and Jasiński, 2018; Santiago et al., 2000). The synthesis of these metabolites can be affected by pathogens, drought, and other external factors. Phenolic compounds are widely distributed in plants (Bravo, 1998) and provide ecological advantages to those plants, for example as protection against ultraviolet radiation, pathogens, and parasites (Pandley and Rizvi, 2009; Dai and Mumper, 2010). Phenolic compounds are involved in plant defense mechanisms to protect against herbivores, and in different plant growth processes. The concentration of phenolics can vary due to seasonal changes, growth, and developmental processes (Bhattacharya et al., 2010).

Free radicals, or reactive oxygen species (ROS), are naturally produced during normal physiological reactions that are part of cell metabolism. Because of the unpaired electrons, they are very unstable and react to either accept or donate electrons to achieve a more stable state. The ROS can attack cellular components and lead to deficient antioxidant defense and oxidative stress (Betteridge, 2000). Free radicals are associated with many chronic degenerative diseases, aging, and pathologies including cancer, central nervous system disorders, diabetes, atherosclerosis, rheumatoid arthritis, and liver damage (Aruoma, 1998; Finkel and Holbrook, 2000; Halliwell, 2012). Plants have many antioxidant molecules that might play a central role in preventing oxidative stress (Miller et al., 1997; Rice-Evans et al., 2000; Proteggente et al., 2002; Huang et al., 2005).

Phenols contain hydroxyl groups on their aromatic ring, making them suitable electron donors and contributing to their antioxidant activity. Many studies have shown that phenols inhibit free radicals, prevent oxidative cellular damage, and participate in peroxide decomposition, metal inactivation, and oxygen scavenging in biological systems (Rice-Evans et al., 1996, 1997; Zhang et al., 2018; Aryal et al., 2019). In addition, they have also been shown to have antimicrobial, anti-inflammatory, and antiallergic properties (Lanfer-Marquez et al., 2005; Stalikas et al., 2007; Santos-Buelga et al., 2012; Saowapa et al., 2015).

This study focuses on commercial samples from four traditional plants used in Central America: *Theobroma cacao, Eriobotrya japonica, Bourreria huanita,* and *Elettaria cardamomum*. While *T. cacao* is the only native to Central America, the rest of the plants have been introduced and naturalized into this region and are now widely used in traditional medicine or extensively cultivated in the area (Alam et al., 2021; Cruz-Castillo et al. 2005; Torres, 2021).

*Theobroma cacao*

*Theobroma cacao* (Fam. Malvaceae) has a rich and expansive history. *Theobroma's* Greek translation means "food of the gods" (Baharum et al., 2016). In Mayan mythology, cacao was given to

humans by the hero Hunahpú (Fusi, 2015). As Elis Fusi stated in the book The Hidden Maya Forest, the Aztecs, like the Mayans, believed that the cacao bean had divine properties (Fusi, 2015). Early reports of cacao consumption date as early as 460 A.D. (Katz et al., 2011). The earliest mention of cacao being pronounced as "kakawa" was from the ancient Olmecs (Powis et al., 2011), the earliest civilization formed in Mesoamerica. However, recent genomic research indicates that cacao was initially used and domesticated in the upper Amazon tributary regions (Zarrillo et al., 2018). The study identified that cacao domestication in South America happened about 1,500 years before these tribes migrated to Central America and Mesoamerica. At one point in history, cacao beans (Figure 1) were also used for currency (Nelson, 2017), for trade, for ritualistic practices, and for large feasts among Mayans (Zarrillo et al., 2018) and among Aztec elites (Steinberg, 2002).

Much of what is known about the beneficial properties of cacao has been passed down from generation to generation through oral history that can be found imprinted in jade, obsidian, stonework, and pottery (Fusi, 2015). In Guatemala, the seed shells (Figure 1) are used in tea and help to decrease blood pressure. Furthermore, it has been proposed that cacao beans can protect nerves from injury and inflammation, protect the skin from oxidative damage caused by the sun's UV rays, improve cognitive function and mood, improve platelet function, and reduce blood clots (Rojo-Poveda et al., 2020).

The cacao shell is also rich in protein, dietary fiber, ash, and other valuable bioactive compounds such as methylxanthines and phenolics (Katz et al., 2011). Cacao's phenolic compounds have antioxidant properties that stop the further oxidation of low-density lipoprotein (LDL) cholesterol (Andújar et al., 2012). The oxidation of LDL has been noted to cause an inflammatory response that can have harmful effects in the body (Tekin et al., 2013).

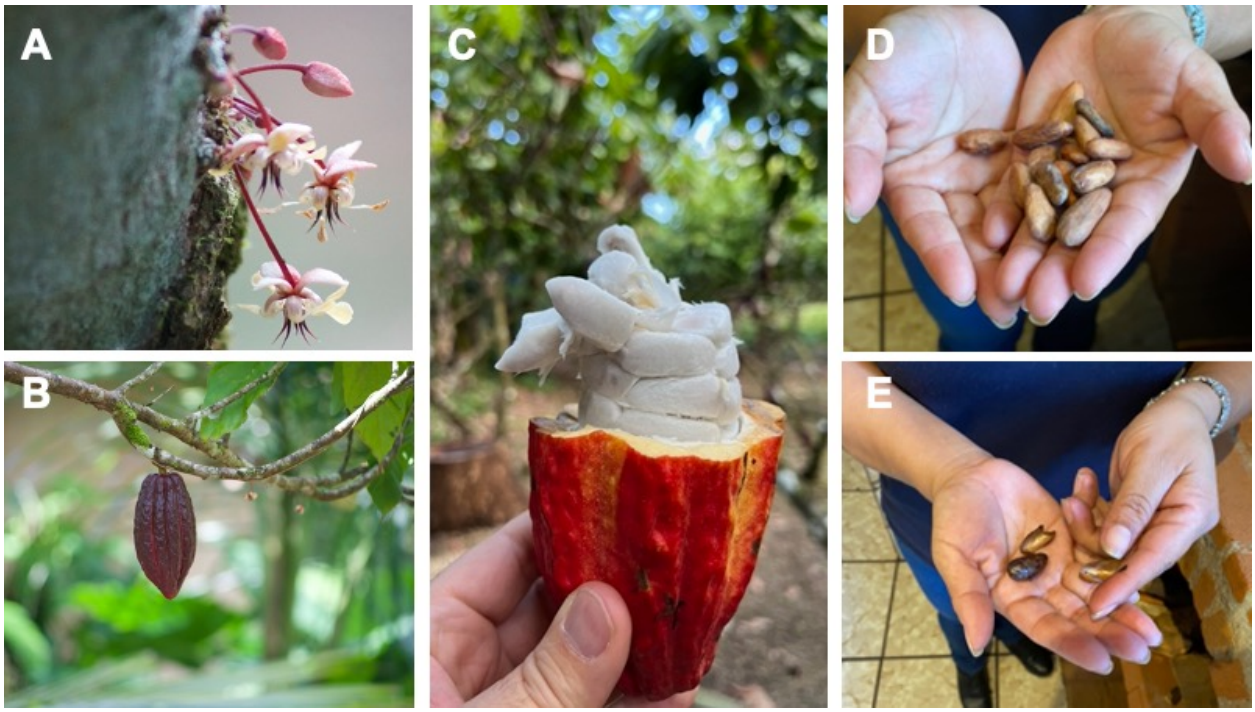


**Figure 1. From a flower to the valuable seed of *Theobroma cacao*.** The figure shows the different stages to obtain, in the end, the cacao seeds and tea. A) Cacao flower, B) Cacao fruit, C) Opened cacao fruit, D) Roasted cacao seeds, E) Cacao seeds shell. Photos taken by José Fernández Romero.

*Eriobotrya japonica*

*Eriobotrya japonica* (Fam. Rosaceae) is known as the loquat tea (Figure 2). The fruits, leaves, and flowers are widely used in Guatemala and many other countries. The earliest domestication of loquat was during the Han Dynasty in China 2000 years ago (Wang, 2021) and it was later cultivated in Japan (Lin et al., 1999). It has also been used to treat coughs, chronic bronchitis, inflammation, diabetes, and cancer (Jian et al., 2020). The seeds are used to prepare alcoholic beverages, and in Japanese tradition it is believed that this type of drink is good for the health. (Koba et al., 2007). Treatments with seed extracts have decreased the production of immunoglobulin E in rats exposed to dinitrofluorobenzene, thus indicating anti-inflammatory properties (Sun et al., 2010).

Many beneficial properties of loquat come from the high concentrations of phenolics, triterpenes, carotenoids, flavonoids, and vitamins found in different parts of the plant (Wang, 2003; Zhou et al., 2011; Pareek et al.,2014; Liu et al., 2016). Ethanolic extracts of loquat peel and seeds were shown to decrease low-density lipoprotein (LDL) oxidation *in vitro* (Koba et al., 2007), and have significant antimicrobial and antioxidant activities (Rashed and Butnariu, 2014).

**Figure 2. The fruit of *Eriobotrya japonica* is used in traditional medicine.** Photo taken by José Fernández Romero.

*Bourreria huanita*

*Bourreria huanita* (Fam. Boraginaceae) is also known as "Flor del árbol de Esquisuchil" and *"*The tree of Hermano Pedro" (Hellmuth, 2013) (Figure 3). It was first introduced in La Antigua, Guatemala in 1657 by Saint Hermano Pedro in the garden Los Laureles, from El Calvario, Colombia (Torres, 2021). Natives believed that the dried flowers have miraculous properties. Locals used the dried flowers in tea, and it is believed that the Aztecs used the flowers for flavoring, and that the Mayans used them as a spice (Mayan-ethnobotany, 2022). Traditionally *B. huanita* has been used to treat respiratory ailments, gastrointestinal infections, burns, and heart diseases (Cruz et al., 2008). In Guatemala, it is also used as a sedative or relaxant (Beteta and Haydee, 2006). Ethanolic extracts of *B. huanita* have been reported to exhibit antimicrobial activity and to inhibit growth of *Escherichia coli* (Cruz et al., 2008). The extracts contain alkaloids, flavonoids, tannins, and volatile oils (Beteta and Haydee, 2006).

**Figure 3. The *Bourreria huanita* tree with its flowers.** The dried flowers are collected to prepare an herbal tea. Photo taken by José Fernández Romero.

*Elettaria cardamomum*

*Elettaria cardamomum* (Fam. Zingiberaceae), also known as the true cardamon, has been cultivated and grown for a long time in Sri Lanka, India, Nepal, Indonesia, and Tanzania (Abu-Taweel, 2018), and was brought to Guatemala in 1914. It is a small spindle-like light green seed pod and is commonly known as the queen of spices. The seeds are used for flavoring coffee, pastries, and curry dishes, and to freshen breath (Abu-Taweel, 2018). They are also used in culinary preparations and in traditional medicine to control asthma, to treat tooth and gum infections, and to treat digestive and kidney disorders (Agnihotri and Wakode, 2010; Ashokkumar, et al., 2020).

Cardamon is rich in volatile oils such as alpha-pinene, beta-pinene, sabinene, myrcene, alpha phellandrene, limonene, cineole, terpinene, cymene, terpinolene, and linalool (Ashokkumar et al., 2020). These compounds have been associated with antioxidant, anticancer, anti-inflammatory, anti-fungal, anti-bacterial, anti-diabetic, and antiviral activities (Das et al., 2012). They have been used to control cardiovascular, pulmonary, kidney, and lung-associated disorders (Vaidya et al., 2014).

The objective of this research was to assess the antimicrobial activity, antioxidant activity, and phenolic levels of extracts from: *Theobroma cacao* beans and shells; *Eriobotrya japonica* leaves, stems, and flowers; *Bourreria huanita* flowers; and *Elettaria cardamomum* seeds.

## MATERIALS AND METHODS

**Extracts preparation.** Commercial plant samples purchased in local marketplaces (Table 1) were ground for 30 seconds using a coffee grinder (Mr. Coffee, Cleveland, Ohio). While all the finished botanical product samples used in our experiments came from the Central American region, additional commercial samples from different regions including India and Peru were also included for comparative purposes. For each sample, 10 mL of 95% ethanol (Sigma Aldrich, St Louis, MO) were added to 1 g of powdered grains and samples were mixed using a vortex mixer. Each sample was then sonicated three times at 10-second intervals and 30% intensity using a Bransonic® Ultrasonic bath. Samples were centrifuged at 300 x g for 5 min, and the supernatant

was collected and filtered through a 0.45 µm syringe filter (ThermoFisher Scientific, Waltham, MA). These ethanolic extracts were used as prepared to test total phenolic and antioxidant activities. For antiviral assays, the ethanol was evaporated, and the dried samples resuspended in 20% dimethyl sulfoxide (DMSO, Sigma Aldrich, St. Louis MO) in ultrapure water (ThermoFisher Scientific, Waltham, MA). The ethanolic and DMSO extracts were aliquoted and stored at -20°C.

**Total phenolic.** The Phenolic Compounds Assay Kit (Sigma Aldrich, MAK365) was used to quantify total phenolics in the ethanolic extracts. Each test was performed in duplicate in a 96-well flat bottom clear microplate (Fisher Scientific, Waltham, MA). For each extract, 20 µL of sample were mixed with 30 µL of ultrapure water (ThermoFisher Scientific), 10 µL of PC probe and 40 µL of PC assay buffer. For each sample, a parallel sample well was prepared to serve as a background control containing 20 µL of sample, 30 µL of ultrapure water, and 50 µL of PC assay buffer. Additionally, catechin standards with concentrations between 0 and 10 nM were included. The plate was incubated at room temperature for 10 min with gentle shaking and absorbance was measured at 480 nm using the Spectramax iD3 microplate reader (Molecular Devices, San Jose, CA). A total of four independent experiments were performed. Results were expressed as total nM/µL catechin equivalents.

**Antioxidant capacity.** The Antioxidant Activity Assay Kit (Sigma Aldrich, CS0790) was used to estimate the antioxidant capacity in ethanolic extracts. Each test was performed in duplicate in a 96-well flat bottom clear microplate (Fisher Scientific). For each extract, 5 µL of sample or Trolox standard were mixed with 10 µL of myoglobin working solution (3.5 mg/mL) and 75 µL of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) substrate working solution. For each sample, a parallel sample well was prepared to serve as a background control containing 5 µL of sample, 10 µL of ultrapure water, and 75 µL of ABTS substrate working solution. Trolox standards were prepared at concentrations between 0 and 0.42 mM. The plate was incubated at room temperature for 5 min with gentle shaking and the reactions were stopped by adding 50 µL of the stop solution provided by the manufacturer. The absorbance was measured at 405 nm using the Spectramax iD3 microplate reader (Molecular Devices, San Jose, CA). A total of two independent experiments were performed. Results were expressed as total mM Trolox.

**Antiviral Activity.** Antiviral activity was determined by testing the cytotoxicity of each extract using the XTT colorimetric assay and the PsV entry inhibition assay (Melo et al., 2021). The therapeutic index was used to determine potential selective antiviral activity.

***Cytotoxicity assay.*** The cytotoxicity was determined using the XTT colorimetric assay (Fernandez-Romero et al., 2012). Different concentrations of each extract were added in triplicate to human angiotensin-converting enzyme 2 (hACE-2)-expressing HeLa cells (HeLa ACE-2, provided by Dennis Burton, The Scripps Research Institute, La Jolla, CA) seeded in clear bottom 96-well microplates and then incubated at 37ºC, 5% $CO_2$, and 98% humidity for 72 hours. Tween 20 (Sigma Aldrich) was used as a positive control for cytotoxicity. XTT (ThermoFisher Scientific) was added to all wells after 72-hour incubation, and the absorbance was measured at 450 nm using a the Spectramax iD3 microplate reader (Molecular Devices). A total of two independent experiments were performed.

***Production of SARS-CoV-2 pseudoviral (PsV) particles***. SARS-CoV-2 delta PsV particles were produced following the procedure described by Schmidt et al., 2020 with some modifications. 293T cells (ATCC, Manassas, VA) were seeded in 6-well plates (Fisher Scientific) at a concentration of 5 x $10^5$ cells/mL, 2 mL per well. The plates were incubated overnight at 37ºC, 5% $CO_2$, and 98% humidity. Plasmids containing the SARS-CoV-2 spike gene (pSARS-CoV-2-Strunc (Delta variant), pCRV1NHG GagPol, and pNanoLuc2AEGFP) were used to produce the PsVs (Schmidt et al., 2020). The plasmids were mixed gently with Lipofectamine 2000 (ThermoFisher Scientific, Waltham, MA) using the amounts indicated by the manufacturer.

The mixture was incubated for 20 min at room temperature. The supernatant of 293T cells, seeded the previous day, was aspirated and washed twice with 2 mL of OptiMEM medium (ThermoFisher Scientific). The DNA/lipofectamine 2000 mixture was added to 293T cell monolayers and incubated for 6 hours at 37°C, 5% $CO_2$, and 98% humidity. After this brief incubation, the cell monolayers were washed twice with D-PBS (ThermoFisher Scientific) and DMEM (ThermoFisher Scientific) with 10% FBS (ThermoFisher Scientific), and 100 I.U./mL penicillin and 100 µg/mL streptomycin (ThermoFisher Scientific) were added to each well. The plates were incubated for 48 hours at 37°C, 5% $CO_2$, 98% humidity, and the cell supernatants were collected, filtered using a 0.22 µm pore size PVDF filter, aliquoted, and stored at -80°C. The PsV titer was determined using a cell-based pseudoviral entry assay (Schmidt et al., 2020) and the TurboLuc™ Luciferase One-Step Glow Assay Kit (ThermoFisher Scientific).

***Pseudoviral entry inhibition assay.*** The antiviral activity for each of the extracts tested in the XTT assay was evaluated using the cell-based pseudoviral entry assay SARS-CoV-2 delta PsV (Melo et al, 2021). The same concentrations and number of replicates for each extract were used to test the antiviral activity against SARS-CoV-2 delta PsV. The different concentrations of plant extracts and the PsV were mixed and incubated in 96-well U bottom microplates for 30 min at 37ºC, 5% $CO_2$, and 98% humidity. After this brief incubation, the mixture was transferred to HeLa ACE-2 cell monolayers seeded in white opaque 96-well flat bottom microplates. The plates were incubated at 37ºC, 5% $CO_2$, and 98% humidity for 72 hours. After the 72-hour incubation, 50 µL of the medium were removed from each well and replaced with 50 µL of luciferase buffer and substrate mixture. The plates were shaken for 10 min, and luminescence was read using the Spectramax iD3 microplate reader (Molecular Devices). A total of two independent experiments were performed.

**Antibacterial activity.** The methods previously described by Melo et al., 2021 were used to determine antibacterial activity. Fresh overnight LB broth cultures of *Escherichia coli* ATCC™ 8739™ (Fisher Scientific, Hampton, NH) and *Staphylococcus epidermidis* NCIMB 8853 (Fisher Scientific) were prepared from each bacterial stock. The antibacterial activity was tested in 96-well flat bottom microplates (Fisher Scientific). Each plant extract and solvent control (20% DMSO in ultrapure $H_2O$) was tested by mixing 20 µL of each sample with 70 µL of Tryptic Soy broth and 10 µL of bacteria (~$10^5$ cells/mL). For each extract, background controls were prepared that included the extract and broth, but no bacteria. Blank wells were also included that contained 100 µL of Tryptic Soy broth. Plates were incubated at 37°C, and optical density (OD) was measured at 600 nm at time zero and at every hour for 6 hours using the Spectramax iD3 microplate reader (Molecular Devices). Five replicates of each sample were tested in each of two independent experiments.

**HPLC Analysis.** Analysis was conducted by Agilent 1290 Infinity II ultra-high performance liquid chromatography (UHPLC) equipped with an UV diode array detector (DAD). The column used was Agilent Polaris Amide-C18 column, 250 x 4.6 mm, 3 µm. The same samples prepared for the biological activities were used to perform the analysis. The sample initial concentration (1 g/mL) was diluted ten times (100 mg/mL) and centrifuged at 12,000 RPM to remove debris. The following standards were used: caffeine, theobromine, catechin, epicatechin, and procyanidins B1 and B2. For determination of alkaloids and proanthocyanins, water with 0.1% formic acid was used as mobile phase A and acetonitrile with 0.1% formic acid was used as mobile phase B. The gradient was 2% B at 0 min and held for 3 min, raised to 15 % B at 15 min and held until 25 min, then raised to 35% B at 50 min, 60% B at 51 min, and held until 55 min. The flow rate was 0.8 mL/min. The column was equilibrated with 2% B for 3 min between injections. The column was set at 40°C. The autosampler was maintained at 4°C. The injection volume was 2.5 µL. The diode array detector (DAD) was set at 280 nm, with bandwidth 4 nm. The reference wavelength was 400 nm, with reference bandwidth at 10 nm.

**Data analysis.** The mean value ± SE of total

phenolic and antioxidant activities was graphed for each sample using GraphPad Prism v9.0.2 software (San Diego, CA). The percent cell viability and percent pseudoviral entry were calculated in Microsoft Excel and transferred to GraphPad Prism. Graphs of percent cell viability or percent PsV entry versus the logarithmic value of each extract concentration were created in GraphPad Prism. The half-maximal cytotoxic concentration ($CC_{50}$) and half-maximal effective concentration ($EC_{50}$) were determined for each extract, using a dose-response-inhibition analysis in GraphPad Prism. The therapeutic index (TI= $CC_{50}/EC_{50}$) was calculated for each extract. The absorbance versus time was graphed for the antibacterial assay, and the area-under-the-curve (AUC), standard error, and 95% confidence intervals were calculated using GraphPad Prism. To compare these AUCs, the AUCs, standard errors, and the number of data points were copied and pasted into a new grouped table. The AUC of each sample was compared to the AUC of sample 14 (control) using an unpaired t-test in GraphPad Prism. Finally, the total phenolic content in the samples was compared using a paired t-test in GraphPad Prism.

**Table 1.** Commercial samples of *Theobroma cacao*, *Eriobotrya japonica, Bourreria huanita*, and *Elettaria cardamomum* used in this study.

| Sample # | Plant specie | Description | Commercial Source (Origin) |
|---|---|---|---|
| **1** | *T. cacao* | Organic raw whole cacao beans | Kevala International LLC, Dallas, Texas (India) |
| **2** | *T. cacao* | Organic raw whole cacao beans | Tootsi Impex USA Inc., Montclair, NJ (Peru) |
| **3** | *T. cacao* | Organic raw whole cacao beans | Chocolate Antigua, San Juan del Obispo, Guatemala (Guatemala) |
| **4** | *T. cacao* | Cacao tea | |
| **5** | *E. japonica* | Loquat natural dried leaves and stems | EidolonGreen* |
| **6** | *E. japonica* | Loquat leaf and flower tea | San Juan del Obispo, La Antigua, Guatemala (Guatemala) |
| **7** | *B. huanita* | Esquisuchil dried flower | |
| **8** | *E. cardamomum* | Organic Cardamom seeds | Pinch, Louisville, KY (Guatemala) |
| **9** | *E. cardamomum* | Organic Cardamom seeds | San Juan del Obispo, La Antigua, Guatemala (Guatemala) |
| **10** | *E. cardamomum* | Organic Cardamom seeds | Frontier CO-OP, Norway, IA* |
| **11** | *E. cardamomum* | Organic Cardamom seeds | Whole Foods, Austin, Texas* |
| **12** | *E. cardamomum* | Organic Cardamom whole | Spice and Tease, Grand Central Station, New York * |
| **13** | *E. cardamomum* | Organic Cardamom whole | |

*Country of origin not specified

## RESULTS AND DISCUSSION

The Phenolic Compounds Assay Kit used in the experiments provided a quick, sensitive, and selective method for measuring the total amount of phenolic compounds in the ethanolic extracts. In this assay, phenolic compounds react with diazonium salts under alkaline conditions to form a stable diazo chromophore that can be detected by absorbance at 480 nm (Lanfer-Marquez et al., 2005; Stalikas et al., 2007; Santos-Buelga et al., 2012; Saowapa et al., 2015). An important advantage of this method, unlike the Folin-Ciocalteu procedure, is that this assay kit cannot be affected by non-phenolic reducing substances found in plant extracts such as sulfites, reducing sugars, or ascorbic acid. In the 13 samples of fermented whole cacao beans, the amount of total phenolics ranged from 0.06 to 1.85 nM/μL catechin equivalents, with a sample from La Antigua, Guatemala (sample #3) showing the highest total phenolic content at 1.35 nM/μL catechin equivalents (Figure 5A; $p<0.0368$).

The antioxidant activity measured the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide that oxidizes the ABTS to produce ABTS•+, a soluble green color product that can be quantified spectrophotometrically at 405 nm (Miller et al., 1997; Rice-Evans et al., 2000; Proteggente et al., 2002; Huang et al., 2005). Consistent with the results obtained with the total phenolic assay, fermented organic raw whole cacao beans from La Antigua, Guatemala (Sample #3) showed the highest antioxidant activity at 0.398 mM Trolox equivalents (Figure 5B).

Antioxidants in plants are important as they neutralize free radicals or reactive oxygen species (ROS) that are naturally produced during normal physiological reactions that occur during cell metabolism. ROS react with cellular components and, if not neutralized, cause oxidative damage. Such oxidation can potentially initiate various pathologies including cancer, central nervous system disorders, diabetes, atherosclerosis, rheumatoid arthritis, and liver damage. Plants contain many antioxidants that might play a central role in preventing oxidative stress (Miller et al., 1997; Rice-Evans et al., 2000; Proteggente et al., 2002; Huang et al., 2005).

Cacao beans are rich in bioactive compounds, including proanthocyanidins, a group of phenolic compounds (Katz et al., 2011). These compounds in cacao include polyphenols of four different types of flavonoids including catechins, 92% of which constitute (-) epicatechin (Jalil and Ismail, 2008). Cacao is also rich in methylxanthines including caffeine, theobromine, and theophylline (Lee, 2000). The four cacao samples studied in these experiments included fermented organic cacao beans and cacao tea. Prior studies demonstrated that the country of origin and the preparation method can impact the content and concentration of the polyphenols in cacao beans (Miller et al., 2006; Jalil and Ismail, 2008; Ortega et al., 2008; Miller et al., 2009). Other studies have shown that phenolic content can vary depending on climate, soil characteristics, drying methods, storage, and transportation (Albertini et al., 2015). Another potential factor that might contribute to the different phenolic contents of *T. cacao* samples is their fermentation process. The bitter and acidic taste of cacao, due to phenolic content, can be reduced through fermentation (Jalil and Ismail, 2008). Polyphenols can diffuse from their storage cells and undergo oxidation during cacao seed fermentation leading to the formation of insoluble tannins (Hansen et al., 1998). High temperatures and prolonged processing times can further decrease the concentration of soluble polyphenols, which might explain the lower total phenolic compounds and antioxidant activities found in the cacao tea obtained from the shells of fully roasted cacao beans from Guatemala (sample #4).

Antimicrobial properties of these plant extracts were also explored. Antiviral activity against SARS-CoV-2 was evaluated using a pseudoviral model. Extracts prepared from whole cacao beans from Guatemala (sample # 3) resulted in the highest level of antiviral activity against SARS-CoV-2, having the lowest $EC_{50}$ value (14 μg/mL) and the highest therapeutic index (30.3) when comparted with the other samples from India and Peru (Table 2).

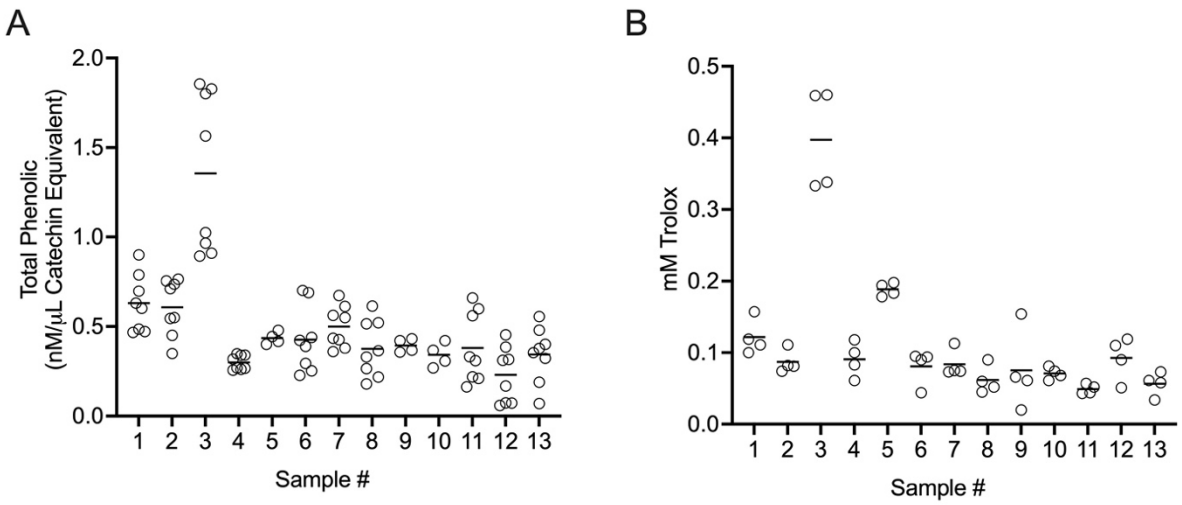


**Figure 5.** Phenolic content (A) and antioxidant activity (B) of extracts of *Theobroma cacao* (samples #1-4), *Eriobotrya japonica* (samples #5-6), *Bourreria huanita* (sample #7), and *Elettaria cardamomum* (samples #8-13). *Theobroma cacao,* fermented raw whole cacao beans from Guatemala (sample #3), shows the highest phenolic content and the highest antioxidant activity. Total phenolics are expressed as nM/µL of catechin equivalents and antioxidant activity is expressed as mM Trolox equivalents. The graphs show the results of two or four independent experiments.

**Table 2.** Antiviral activity of the extracts for each species of *Theobroma cacao* (samples #1-4), *Eriobotrya japonica* (samples #5 and 6), *Bourreria huanita* (sample #7), and *Elettaria cardamomum* (samples #8-13) against SARS-CoV-2 delta PsV.

| **Sample #** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$CC_{50}$ (µg/mL) [a]** | 496.5 | 452.5 | 425.1 | 288.2 | 352.6 | 173.3 | 324.9 | 53.3 | 139.7 | 81.8 | 98.8 | 127.3 | 128 |
| **95% CI [b]** | 233.7 to 1273 | 217.3 to 1105 | 271.6 to 701.0 | 144.2 to 586.4 | 141.7 to 1145 | 91.5 to 352.8 | 113.5 to 1026 | 24.9 to 116 | 55.8 to 368.6 | 32.7 to 190.6 | 44.6 to 235.5 | 47.5 to 346.7 | 59.3 to 299.2 |
| **$EC_{50}$ (µg/mL) [c]** | 139.7 | 90.22 | 14 | 289.4 | 18.8 | 29.7 | 228.7 | 62.8 | 91.5 | 54.9 | 54.5 | 24.4 | 23.1 |
| **95% CI** | 83.3 to 230.9 | 58 to 138.3 | 10.3 to 19.1 | 177.2 to 485.8 | 13.9 to 25.2 | 22.3 to 39.8 | 141.4 to 370.4 | 41.7 to 94.7 | 64.5 to 129.2 | 36.4 to 81.8 | 38.8 to 76.3 | 18.5 to 32.4 | 16.6 to 32 |
| **TI ($CC_{50}/EC_{50}$) [d]** | 3.6 | 5.0 | 30.3 | 1.0 | 18.7 | 5.8 | 1.4 | 0.8 | 1.5 | 1.5 | 1.8 | 5.2 | 5.5 |

[a]$CC_{50}$= half maximal cytotoxic concentration, [b] 95% CI= 95% Confidence Interval, [c]$EC_{50}$= half maximal effective concentration, and [d]TI= therapeutic index.

Several publications indicate potential antiviral activity of compounds isolated from *T. cacao*. Procyanidin C1-flavonoids isolated from *T. cacao* have been shown to reactivate latent HIV through the MAPK pathway (Musarra-Pizzo et. al., 2021). Kamei et al., 2015 investigated the effect of *T. cacao* against the influenza virus in a cell-based assay and proposed that aqueous extract of *T. cacao* exhibits

dose-dependent inhibition of influenza virus A (H1N1 and H3N2), human influenza virus B, and avian influenza viruses (H5N1 and, H5N9). Additionally, Kamei et al., 2015, performed animal experiments to show that the aqueous extract significantly improved survival in mice after intranasal challenge with influenza virus.

From the available literature, this project is the first to report of a *T. cacao* extract showing selective antiviral activity against SARS-CoV-2 by interfering with viral attachment and/or viral entry, and that phenolic compounds are likely involved in this activity. It has been previously reported that other naturally occurring compounds interfere with the SARS-CoV-2 spike and ACE-2 receptor interaction, including flavonoids, proanthocyanidins, secoridioids, and xanthanoes (Al-Shuhaib et al., 2022). Inhibiting the virus binding to the ACE-2 receptor is an area of interest because of its crucial role in the pathogenesis of the SARS CoV-2 virus. The virus receptor-binding domain (RBD) of the spike (S) protein binding with the ACE-2 receptor allows the virus to enter the host cell (Ni et al., 2020; Boopathi et al.,2021). Using computational models, Al-Shuhaib et al., 2022, found that the flavonoid epicatechin might inhibit SARS CoV-2 binding to the ACE-2 receptor. This compound is of particular interest because it is a flavonoid present in cacao, and about 92% of cacao catechin is composed of epicatechin. Similarly, another study found that catechin had a high affinity for the ACE-2 receptor (Jena et al., 2021). Furthermore, other polyphenols present in different plants have been shown to inhibit activity of SARS CoV-1 helicase, a viral enzyme with a crucial role in viral replication. Yu et al., (2012) identified about 64 naturally occurring compounds as potential inhibitors of SARS CoV-1 helicase. They further report that myricetin and scutellarin might inhibit SARS CoV-1 helicase by inhibiting ATPase activity, but not the helicase activity itself.

In addition to *T. cacao*, antioxidant and antiviral activity were explored for other plant extracts including *E. japonica* leaves and stems (sample #5 Eidolon Green) and leaf and flower tea (sample #6) (Table 1). Although phenolic content and antioxidant activity were not as high as that of *T. cacao, E. japonica* (leaves and stems) had an $EC_{50}$ value of 18.8 µg/mL and a TI value of 18.7 (Table 2). This TI value indicates selective antiviral activity. Similar to *T. cacao,* there could be numerous explanations for the low phenolic and antioxidant activity present in *E. japonica* samples, for example variations in manufacturing, temperature, storage, and growth conditions. Compounds responsible for the potential antiviral activity seen in *E. japonica* have not been identified and could be a future area of investigation.

As previously mentioned*, E. japonica* extracts are used in traditional medicine to treat chronic bronchitis and cough. *E. japonica* is rich in secondary metabolites such as phenolics and triterpenoids (Zhou et al., 2011). Research has found that terpenoids might inhibit SARS CoV-2 viral replication (Giofrè et al., 2021). A report on *E. japonica* showed that products from this plant have antiviral activity against rhinoviruses (De Tommasi et al., 1992). Some of the most important phytochemicals found in *E. japonica* are quercetin, ursolic acid, oleanolic acid, tannins, chlorogenic acid, and caffeoylquinic acid (Seong et al., 2019). Quercetin, a type of flavonoid, inhibits the SARS CoV-2 protease *in vitro* (Park et al., 2017).

The potential antibacterial activity of these plant extracts was tested by monitoring bacterial growth over time using spectrophotometry. *E. japonica* extracts showed a broad-spectrum antibacterial activity with significant inhibition of bacterial growth ($p<0.0001$; Figure 6 A and B). Similarly, extracts of *E. japonica* and *E. cardamomum* showed significant inhibition of *S. epidermidis* ($p<0.001$; Figure 6B).

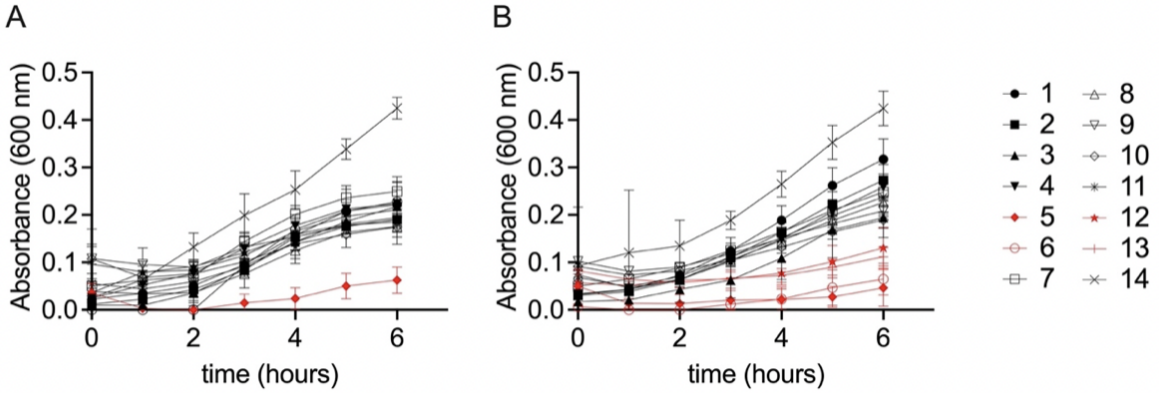


**Figure 6.** *E. coli* (A) and *S. epidermidis* (B) growth over time measured at 600 nm. *Theobroma cacao* (samples #1-4), *Eriobotrya japonica* (samples #5-6), *Bourreria huanita* (sample #7), and *Elettaria cardamomum* (samples #8-13). *E. japonica* (samples #5 and 6) and *E. cardamomum* (samples #12 and 13) showed significant inhibition ($p<0.0001$) of bacterial growth compared with the Tryptic Soy Broth control (sample #14). Each data point represents the mean value ± SE of 10 replicates from two independent experiments. Red curves indicate significant ($p<0.0001$) difference in the AUC compared to sample #14 (control).

Studies with *E. cardamomum* have been shown to have several pharmacological properties, including antibacterial and anti-inflammatory activities likely associated with volatile compounds (Souissi et al., 2020). An interesting observation is that although two extracts of *E. japonica* from different commercial sources were prepared, only one (leaves and stems) showed significant inhibition of *E. coli*. As mentioned before, samples from different countries of origin and different methods of preparation can impact the biological properties of plant products (Jalil and Ismail, 2008; Miller et al., 1997; Ortega et al., 2008; Miller et al., 2009).

Considering that whole cacao beans from Guatemala (sample #3) showed the highest phenolic content, the highest antioxidant activity, and the most selective anti-SARS-CoV-2 activity, we next examined the phytochemical profile of this particular sample. The HPLC analysis of whole beans of *T. cacao* showed the presence of catechin, epicatechin, caffeine, theobromine, and procyanidin B1. Epicatechin was the major polyphenol with 38.1 mg/g, followed by the alkaloids theobromine (2 mg/g), caffeine (0.58 mg/g), catechin (0.3 mg/g) and procyanidin B1 (0.17 mg/g) (Table 3). The composition of cacao beans is likely linked with high total phenolic content (Fig. 5A), high epicatechin content (Table 3), antioxidant activity (Fig. 5B), and antiviral activity (Table 2).

**Table 3.** Composition of alkaloids and polyphenols of whole cacao beans from Guatemala (*Theobroma cacao*)

| Retention Time | Component | mg/g dry cacao |
|---|---|---|
| 12.2 | Theobromine | 2 |
| 17.106 | Caffeine | 0.58 |
| 18.746 | Procyanidin B1 | 0.17 |
| 19.526 | Catechin | 0.3 |
| 24.693 | Epicatechin | 38.1 |

## CONCLUSIONS

In this study, four plants widely used in traditional medicine in Central America were screened for their antioxidant activity and potential antimicrobial activities. The total phenolics were determined for each. Antioxidant activities were detected in *T. cacao*, *E. japonica*, *B. huanita*, and *E. cardamomum* ethanolic extracts. Interestingly, whole *T. cacao* beans from La Antigua, Guatemala (sample #3) showed the highest total phenolic concentration, antioxidant activity, and selective antiviral activity. This study also showed that dry leaves and stems of *E. japonica* (sample #5) had selective antiviral activity with a TI value of 18.7. *E. japonica* and *E. cardamomum* showed significant inhibition of bacterial growth using spectrophotometry. The results suggest that phenolic

compounds are responsible for these activities; however, other secondary compounds might also contribute to the results observed. The selective antiviral activity with a TI value of 30.3 warrants further investigation of the fermented organic *T. cacao* beans. Extraction methods using alternative solvents should be further explored.

## ACKNOWLEDGMENTS

This project was funded by a National Institutes of Health Maximizing Access to Research Careers (MARC) Program (award #5T34GM008078-31), a National Science Foundation Research Experiences for Undergraduates (REU) grant (award #1756998), and the City University of New York (CUNY) Research Scholars Program (CRSP). Research reported in this publication was also supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T34GM137870. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Part of this research was an Honors by Contract project of Ashanna Persaud in Fundamentals of Microbiology (BIO 230) at Borough of Manhattan Community College (BMCC). We thank the Center for Biomedical Research, Population Council, the Science Department at BMCC, and Christopher Thompson at BMCC.

## REFERENCES

Abu-Taweel, G. M. 2018. Cardamom (*Elettaria cardamomum*) perinatal exposure effects on the development, behavior, and biochemical parameters in mice offspring. Saudi Journal of Biological Sciences *25*(1): 186–193.

Agnihotri, S., and Wakode, S. 2010. Antimicrobial activity of essential oil and various extracts of fruits of greater cardamom. Indian Journal of Pharmaceutical Sciences *72*(5): 657–659.

Al-Shuhaib, M., Hashim, H. O., and Al-Shuhaib, J. 2022. Epicatechin is a promising novel inhibitor of SARS-CoV-2 entry by disrupting interactions between angiotensin-converting enzyme type 2 and the viral receptor binding domain: A computational/simulation study. Computers in Biology and Medicine141: 105155.

Alam A., Rehman N.U., Ansari M.N. and Palla A.H. 2021. Effects of Essential Oils of *Elettaria cardamomum* Grown in India and Guatemala on Gram-Negative Bacteria and Gastrointestinal Disorders. Molecules 26(9): 2546.

Albertini, B., Schoubben, A., Guarnaccia, D., Pinelli, F., Della Vecchia, M., Ricci, M., Di Renzo, G. C., and Blasi, P. 2015. Effect of fermentation and drying on cocoa polyphenols. Journal of Agricultural and Food Chemistry 63(45): 9948–9953.

Andújar, I., Recio, M. C., Giner, R. M., and Ríos, J. L. 2012. Cocoa polyphenols and their potential benefits for human health. Oxidative Medicine and Cellular Longevity: 906252.

Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists Society 75 (2): 199-212.

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., and Koirala, N. 2019. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel, Switzerland*)*: *8*(4), 96.

Ashokkumar, K., Murugan, M., Dhany, M. K., and Warkentin, T. D. 2020. Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton] - A critical review. Journal of Ethnopharmacology 246: 112244.

Baharum, Z., Akim, A. M., Hin, T. Y., Hamid, R. A., and Kasran, R. 2016. *Theobroma cacao*: Review of the Extraction, Isolation, and Bioassay of Its Potential Anti-cancer Compounds. Tropical Life Sciences Research *27*(1): 21–42.

Beteta, O., and Haydee, G. 2006. Actividad antifúngica de los extractos etanolicos de la flor de *Bourreria huanita* y la hoja de *Lippia graveolens* y sus particiones hexanica, clorofórmica, acetato de etilo y acuosa contra los hongos *Sporothrix schenckii* y Fonsecaea pedrosoi. Universidad de San Carlos de

Guatemala. Retrieved May 2, 2022, from https://biblioteca-farmacia.usac.edu.gt/Tesis/QB854.pdf.

Betteridge, D. J. 2000. What is oxidative stress? Metabolism 49 (2 Suppl 1): 3-8.

Bhattacharya, A., Sood, P., and Citovsky, V. 2010. The roles of plant phenolics in defense and communication during Agrobacterium and Rhizobium infection. Molecular Plant Pathology 11(5): 705–719.

Biala, W. and Jasiński, M. 2018. The Phenylpropanoid Case - It Is Transport That Matters. Front Plant Sci. 9:1610. doi: 10.3389/fpls.2018.01610.

Boopathi, S., Poma, A. B., and Kolandaivel, P. 2021. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. Journal of Biomolecular Structure and Dynamics 39(9): 3409–3418.

Bravo, L. 1998. Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. Nutrition Reviews 56 (11): 317-333.

Cáceres, A. 1996. *Plantas de uso medicinal en Guatemala.* Guatemala City: Universidad de San Carlos. Universitaria, Guatemala.

Cruz-Castillo, J.G., Rodríguez-Bra Camontes, F., Vásquez-Santizo, J., Torres-Lima, P. 2005. Temperate fruit production in Guatemala. New Zealand Journal of Crop and Horticultural Science 34(4): 341-348.

Cruz, S.M., García, E.P., Letrán, H., Gaitán, I., Medinilla, B., Paredes, M.E., Orozco, R., Samayoa, M.C. and Cáceres, A. 2008. Caracterización química y evaluación de la actividad biológica de *Bourreria huanita* (Llave and Lex.) Hemsl. (Esquisuchil) y *Litsea guatemalensis* Mez. (Laurel). Informe Final del Proyecto, Dirección General de Investigación, Universidad de San Carlos, Guatemala. Retrieved May 2, 2022, from https://digi.usac.edu.gt/bvirtual/informes/puicb/INF-2008-087.pdf.

Dai, J., and Mumper, R. J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10): 7313–7352.

Das, I., Acharya, A., Berry, D. L., Se, S., Williams, E., Permaul, E., Sengupta, A., Bhattacharya, S., and Saha, T. 2012. Antioxidative effects of the spice cardamom against non-melanoma skin cancer by modulating nuclear factor erythroid-2-related factor 2 and NF-κB signalling pathways. The British Journal of Nutrition 108(6): 984–997.

De Tommasi, N., De Simone, F., Pizza, C., Mahmood, N., Moore, P. S., Conti, C., Orsi, N., and Stein, M. L. 1992. Constituents of *Eriobotrya japonica.* A study of their antiviral properties. Journal of Natural Products 55(8):1067–1073.

Fernandez-Romero, J. A., Abraham, C. J., Rodriguez, A., Kizima, L., Jean-Pierre, N., Menon, R., and Zydowsky, T. M. 2012. Zinc acetate/carrageenan gels exhibit potent activity in vivo against high-dose herpes simplex virus 2 vaginal and rectal challenge. Antimicrobial agents and chemotherapy 56 (1): 358-368.

Finkel, T., Holbrook, NJ. 2000. Oxidants, oxidative stress and the biology of aging. Nature 408 (6809): 239-247.

Fusi, E. 2015. *The Hidden Maya Forest: Sacred and Medicinal Plants and Rituals of Guatemala*. CreateSpace Independent Publishing Platform; Large Print Edition.

Giofrè SV, Napoli E, Iraci N, Speciale A, Cimino F, Muscarà C, Molonia MS, Ruberto G, Saija A. 2021. Interaction of selected terpenoids with two SARS-CoV-2 key therapeutic targets: An in-silico study through molecular docking and dynamics simulations. Comput Biol Med. 134:104538.

Halliwell, B. 2012. Free radicals and antioxidants: updating a personal view. Nutrition Reviews 70 (5): 257–265.

Hansen, C.E., del Olmo, M., and Burri, C. 1998. Enzyme activities in cocoa beans during fermentation. Journal of the Science of Food and Agriculture 77: 273–281.

Hellmuth, N. M. 2013. *Flowers from the Árbol del Hermano Pedro*. Retrieved May 2, 2022, from

https://www.revuemag.com/2013/07/flowers-from-the-arbol-del-hermano-pedro/.

Huang, D., Ou, B., and Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53(6):1841–1856.

Jalil, A. M., and Ismail, A. 2008. Polyphenols in cocoa and cocoa products: is there a link between antioxidant properties and health? Molecules (Basel, Switzerland) 13(9): 2190–2219.

Jena, A. B., Kanungo, N., Nayak, V., Chainy, G., and Dandapat, J. 2021. Catechin and curcumin interact with S protein of SARS-CoV2 and ACE2 of human cell membrane: insights from computational studies. Scientific Reports 11(1): 2043.

Jian, T., Chen, J., Ding, X., Lv, H., Li, J., Wu, Y., Ren, B., Tong, B., Zuo,, Y., Su K., and Li, W. 2020. Flavonoids isolated from loquat (*Eriobotrya japonica*) leaves inhibit oxidative stress and inflammation induced by cigarette smoke in COPD mice: the role of TRPV1 signaling pathways. Food and Function 11(4): 3516–3526.

Kamei, M., Nishimura, H., Takahashi, T., Takahashi, N., Inokuchi,, K., Mato T., and Takahashi, K. 2015. Anti-influenza virus effects of cocoa. Journal of the Science of Food and Agriculture 96(4): 1150–1158.

Katz, D. L., Doughty, K., and Ali, A. 2011. Cocoa and chocolate in human health and disease. Antioxidants and Redox Signaling 15(10): 2779–2811.

Koba, K., Matsuoka, A., Osada, K., and Huang, Y.S. 2007. Effect of loquat (*Eriobotrya japonica*) extracts on LDL oxidation. Food Chemistry 104: 308–316.

Lanfer-Marquez, U. M., Barros, R. M. C., and Sinnecker, P. 2005. Antioxidant activity of chlorophylls and their derivatives. Food Research International 38: 885–891.

Lee, C. 2000. Antioxidant ability of caffeine and its metabolites based on the study of oxygen radical absorbing capacity and inhibition of LDL peroxidation. Clinica Chimica Acta; International Journal of Clinical Chemistry 295(1-2): 141–154.

Lin, S., Sharpe, R. H., and Janick, J. 1999. Loquat: botany and horticulture. Horticultural Reviews 23: 233–276.

Liu, Y., Zhang, W., Xu, C., and Li, X. 2016. Biological Activities of Extracts from Loquat (Eriobotrya japonica Lindl.): A Review. International Journal of Molecular Sciences 17(12): 1983.

Mayan-ethnobotany. 2022. Reports by FLAAR Mesoamerica on Flora and Fauna of Parque Nacional Yaxha Nakum Naranjo Peten, Guatemala, Central America. Retrieved May 2, 2022, from https://www.maya-ethnobotany.org/.

Melo, C., Perdomo, R., Yerima, F, Mahoney, O., Cornejal, N., Alsaidi, S., Coron, S., Bonnaire, T., Zhang, S., Zydowsky, T.M., Priano, C., Fernández Romero, J.A., Juliani, H.R., and Koroch, A. 2021. Antioxidant, antibacterial, and anti-SARS-CoV activity of commercial products of xylopia (*Xylopia aethiopica*). Journal of Medicinally Active Plants 10: 11-23.

Michel, J., Duarte, R. E., Bolton, J. L., Huang, Y., Caceres, A., Veliz, M., Soejarto, D. D., and Mahady, G. B. 2007. Medical potential of plants used by the Q'eqchi Maya of Livingston, Guatemala for the treatment of women's health complaints. Journal of Ethnopharmacology 114(1): 92–101.

Miller, K. B., Hurst, W. J., Flannigan, N., Ou, B., Lee, C. Y., Smith, N., and Stuart, D. A. 2009. Survey of commercially available chocolate- and cocoa-containing products in the United States. 2. Comparison of flavan-3-ol content with nonfat cocoa solids, total polyphenols, and percent cacao. Journal of Agricultural and Food Chemistry 57(19): 9169–9180.

Miller, K. B., Stuart, D. A., Smith, N. L., Lee, C. Y., McHale, N. L., Flanagan, J. A., Ou, B., and Hurst, W. J. 2006. Antioxidant activity and polyphenol and procyanidin contents of selected commercially available cocoa-containing and chocolate products in the United States. Journal

of Agricultural and Food Chemistry 54(11): 4062–4068.

Miller, N. J., and Rice-Evans, C. A. 1997. Factors influencing the antioxidant activity determined by the ABTS.+ radical cation assay. Free Radical Research 26(3): 195–199.

Musarra-Pizzo, M., Pennisi, R., Ben-Amor, I., Mandalari, G., and Sciortino, M. T. 2021. Antiviral Activity Exerted by Natural Products against Human Viruses. Viruses 13(5): 828.

Nelson, S. 2017. *A brief history of Guatemalan chocolate*. Culture Trip. Retrieved May 2, 2022, from https://theculturetrip.com/central-america/guatemala/articles/a-brief-history-of-guatemalan-chocolate/.

Ni, W., Yang, X., Yang, D., Bao, J., Li, R., Xiao, Y., Hou, C., Wang, H., Liu, J., Yang, D., Xu, Y., Cao, Z., and Gao, Z. 2020. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Critical Care (London, England) 24(1): 422.

Ortega, N., Romero, M. P., Macià, A., Reguant, J., Anglès, N., Morelló, J. R., and Motilva, M. J. 2008. Obtention and characterization of phenolic extracts from different cocoa sources. Journal of Agricultural and Food Chemistry 56(20): 9621–9627.

Pandey, K.B., and Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2(5):270-8. doi: 10.4161/oxim.2.5.9498.

Pareek, S., Benkeblia, N., Janick, J., Cao, S., and Yahia, E. M. 2014. Postharvest physiology and technology of loquat (Eriobotrya japonica Lindl.) fruit. Journal of the Science of Food and Agriculture 94(8): 1495–1504.

Park, J. Y., Yuk, H. J., Ryu, H. W., Lim, S. H., Kim, K. S., Par, K. H., Ryu, Y. B., and Lee, W. S. 2017. Evaluation of polyphenols from *Broussonetia papyrifera* as coronavirus protease inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry 32(1): 504–515.

Powis, T. G., Cyphers, A., Gaikwad, N. W., Grivetti, L., and Cheong, K. 2011. Cacao uses and the San Lorenzo Olmec. Proceedings of the National Academy of Sciences of the United States of America 108(21): 8595–8600.

Proteggente, A. R., England, T. G., Rehman, A., Rice-Evans, C. A., and Halliwell, B. 2002. Gender differences in steady-state levels of oxidative damage to DNA in healthy individuals. Free Radical Research 36(2): 157–162.

Rashed, K. N., and Butnariu, M. 2014. Isolation and antimicrobial and antioxidant evaluation of bio-active compounds from *Eriobotrya japonica* stems. Advanced Pharmaceutical Bulletin 4(1): 75–81.

Rice-Evans, C. A. 2000. Measurement of total antioxidant activity as a marker of antioxidant status in vivo: procedures and limitations. Free Radical Research 33 Suppl: S59–S66.

Rice-Evans, C. A., Miller, N. J., and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20(7): 933–956.

Rice-Evans, C.A., Miller, N.J., Paganga, G. 1997 Antioxidant properties of phenolic compounds. Trends Plant Sci 2:152–159.

Rojo-Poveda, O., Barbosa-Pereira, L., Zeppa, G., and Stévigny, C. 2020. Cocoa bean shell-A by-product with nutritional properties and biofunctional potential. Nutrients 12(4): 1123.

Santiago, L.J.M., Louro, R.P., De Oliveira, D.E. 2000. Compartmentation of phenolic compounds and phenylalanine ammonia-lyase in leaves of *Phyllanthus tenellus* Roxb. and their induction by copper sulphate. Ann. Bot. 86:1023–1032.

Santos-Buelga, C., Gonzalez-Manzano, S., Dueñas, M., and Gonzalez-Paramas, A. M. 2012. Extraction and isolation of phenolic compounds. Methods in Molecular Biology 864: 427–464.

Saowapa, R., Benjakul, S. and Prodpran, T., 2015. Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornate* and *Sargassum polycystum*, grown in Thailand. Int. Aquat. Res., 7:1–16.

Schmidt, F., Weisblum, Y., Muecksch, F.,

Hoffmann, H. H., Michailidis, E., Lorenzi, J., Mendoza, P., Rutkowska, M., Bednarski, E., Gaebler, C., Agudelo, M., Cho, A., Wang, Z., Gazumyan, A., Cipolla, M., Caskey, M., Robbiani, D. F., Nussenzweig, M. C., Rice, C. M., Hatziioannou, T., and Bieniasz, P. D. 2020. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. The Journal of experimental medicine 217(11): e 20201181.

Seong, N. W., Oh, W. J., Kim, I. S., Kim, S. J., Seo, J. E., Park, C. E., Kim, D. Y., Ko, J. W., and Kim J. C. 2019. Efficacy and local irritation evaluation of *Eriobotrya japonica* leaf ethanol extract. Laboratory Animal Research 35: 4.

Souissi, M, Azelmat, J, Chaieb, K, and Grenier, D. 2020. Antibacterial and anti-inflammatory activities of cardamom (*Elettaria cardamomum*) extracts: Potential therapeutic benefits for periodontal infections. Anaerobe 61:102089

Stalikas, C. D. 2007. Extraction, separation, and detection methods for phenolic acids and flavonoids. Journal of Separation Science 30(18): 3268–3295.

Steinberg, M. K. 2002. The Globalization of a Ceremonial Tree: The case of cacao (*Theobroma cacao*) among the Mopan Maya. Economic Botany 56(1): 58–65.

Sun, G., Liu, Y., Zhu, J., Iguchi, M., Yoshioka, S., Miyamura, M., and Kyotani, S. 2010. Immunomodulatory effect of Eriobotrya japonica seed extract on allergic dermatitis rats. Journal of Nutritional Science and Vitaminology 56(2): 145–149.

Tekin, I. O., Orem, A., and Shiri-Sverdlov, R. 2013. Oxidized LDL in inflammation: from bench to bedside. Mediators of Inflammation 762759. doi.org/10.1155/2013/762759

Torres, M. F. 2021. *Las plantas que cultivaba el hermano Pedro en la Antigua Guatemala (1654-1658)*. Planeta Canario. Retrieved May 2, 2022, from https://planetacanario.com/plantas-cultivaba-el-hermano-en-la-antigua-guatemala-1654-1658/.

Vaidya, A. and Rathod, M. 2014. An in vitro study of the immunomodulatory effects of *Piper nigrum* (black pepper) and *Elettaria cardamomum* (cardamom) extracts using a murine macrophage cell line. American International Journal of Research in Formal, Applied and Natural Sciences 8: 18-27.

Wang, Y. 2021. A draft genome, resequencing, and metabolomes reveal the genetic background and molecular basis of the nutritional and medicinal properties of loquat (*Eriobotrya japonica* (Thunb.) Lindl). Hortic Res. 8: 231.

Wang, Z. M., Zhu, X. X., Cui, X. L., Liang, A. H., Du, G. H., Ruan, J. X., Sun, J. N., Li, P. T., and Wang, S. R. 2003. Screening of traditional Chinese remedies for SARS treatment. China Journal of Chinese Materia Medica 28(6): 484–487.

Yu, M. S., Lee, J., Lee, J. M., Kim, Y., Chin, Y. W., Jee, J. G., Keum, Y. S., and Jeong, Y. J. 2012. Identification of myricetin and scutellarein as novel chemical inhibitors of the SARS coronavirus helicase, nsP13. Bioorganic and Medicinal Chemistry Letters 22 (12): 4049–4054.

Zarrillo, S., Gaikwad, N., Lanaud, C., Powis, T., Viot, C., Lesur, I., Fouet,O., Argout,X., Guichoux, E., Salin,F., Solorzano,R.L., Bouchez, O., Vignes, H., Severts, P., Hurtado, J., Yepez, A., Grivetti, L., Blake, M., and Valdez F. 2018. The use and domestication of Theobroma cacao during the mid-Holocene in the upper Amazon. Nat Ecol Evol. 2: 1879–1888.

Zhang, T. J., Zheng, J., Yu, Z. C., Huang, X. D., Zhang, Q. L., Tian, X. S., and Peng, C. L. 2018. Functional characteristics of phenolic compounds accumulated in young leaves of two subtropical forest tree species of different successional stages. Tree Physiology 38(10): 1486–1501.

Zhou, C., Sun, C., Chen, K., and Li, X. 2011. Flavonoids, phenolics, and antioxidant capacity in the flower of *Eriobotrya japonica* Lindl. International Journal of Molecular Sciences 12(5): 2935–2945.