Phytochemical Studies of Aqueous and Methanol Extracts of Carica Papaya Leaves
Laila M Abdelrahim, Zetty NM Zain*, Siti NS Abdul Jalil, Zahidah Abu Seman, Fadlul AF M, Noradilah S Abdullah

Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Tingkat 13, Blok B, Persiaran MPAJ, Jalan Pandan Utama, Pandan Indah, 55100 Kuala Lumpur, Malaysia

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*Corresponding author: Dr. Zetty Nadia Mohd Zain

Abstract

The *Carica papaya* plant has been shown to be useful in a wide range of therapeutic use from anthelmintic effect of its latex to the aphrodisiac property of its root. *Carica papaya* leaves (CPL) is a popular remedy to treat dengue-induced thrombocytopaenia. This study was designed to explore the preliminary phytochemical analysis of CPL aqueous and methanol extracts. In this study flavonoids and phenolics were characterized by LC-TOF-MS, in which 19 compounds were detected in aqueous extract and 24 compounds were detected in methanol extract. This knowledge may help us devise better formulations to develop CPL into useful therapeutic agents for the management of thrombocytopaenia related disorders.

Keywords: CPL aqueous extract, CPL methanol extracts, LC-TOF-MS

INTRODUCTION

*Carica papaya* is a tropical plant popular for its delicious fruit. Other parts of the plant like root, bark, peel, seed and pulp are also known to be used in traditional folk therapies due to their nutraceutical property [1]. Biochemically, its leaves and fruits produce several proteins and alkaloids with important medical and industrial application.

Nowadays, modern sciences accepted the usage of herbs as a source of new bioactive compounds [2].

In this study we have validated the use of CPL for medicinal purpose. We used water and methanol as solvents

MATERIALS AND METHODS

Materials
CPL of Indian origin was obtained from NutriCargo, USA.

Methods
Plant Extract Preparation
Preparation of Aqueous Extract
The whole leaves of dried and powdered CPL were supplied from USA (the origin of CPL is from India). Two hundred grams of powdered leaves were dissolved in 2000 ml of distilled water and heated at 70°C for 1 hour. The extract was subsequently filtered through Whatman filter paper no.1 using a funnel. The filtrate was collected and further heated at 60–70 °C to reduce the volume to less than half (~600 ml). The concentrated extract was then dispensed into individual aliquots and dried in the oven at 40-50 °C for three days. The completely dried extract was then scraped and collected. A final derived extract (10g) was stored at room temperature for further use [3].

Preparation of Methanol Extract
Six hundred grams of powdered leaves were soaked in 6000 ml of methanol (24 hours). Later, the extract was filtered through the Whatman filter paper no.1. The filtrate was subsequently concentrated using a rotary evaporator maintained at 55°C. Finally, the concentrated extract was dispensed into individual aliquots and dried at 65°C for 10 days until completely dried. A final collected methanol extract (30g) was stored at 4°C for subsequent use. (This method was similar with that of the aqueous extract method).

Phytochemical Studies
The aqueous and methanol extracts of CPL were subjected to qualitative chemical analysis to detect the presence of various classes of phytoconstituents. The stock solution of plant
extracts for LC - TOF- MS analysis was prepared by re- dissolving the aqueous and methanol extract in HPLC grade aqueous and methanol to obtain a final extract concentration of 100 mg/ml concentration. Following this, the extracts were pretreated with ISOLUTE®C18 SPE Columns (Biotage, Sweden) and then the concentration was adjusted to about 20 ppm before injection of the samples into the LC- TOF- MS system at the Department of Chemistry, Faculty of Science, University of Kebangsaan, Malaysia. The mass spectra were acquired with a TOF mass spectrometer with a gas temperature of 250°C, a gas flow of 8 l/min and nebulizer on 35 psi. The mass spectrometer was operated in both negative and positive ion modes with a scanning range of 100 to 1000 m/z. Liquid chromatography separation was performed on a Hardware Kit ZORBAX Eclipse XDBC18 column (150 × 4.6 mm; particle size, 5μm; Agilent Technologies, USA).

Liquid Chromatography-Time of Flight-Mass Spectrometry (LC-TOF-MS) Analysis
The liquid chromatography analysis was performed according to the method described by Guale F et al. [4]. The liquid chromatograph was powered by an Agilent 1290 Infinity system consisting of binary pumps, degasser, column heater and autosampler. The internal control was provided by the manufacturer. Based on manufacturer recommendations, the pumps were programmed to deliver an increasing gradient of methanol against an aqueous mobile phase of 5 mM ammonium formate over the following time course: 5% methanol from 0 to 0.5 min, increasing to 30% methanol at 1.5 min, 60% methanol at 4.5 min, to 95% methanol at 6.5 min, and a reset to 5% methanol as acquisition stopped at 10 min with a 3 min post-run. Separations were performed using an Agilent Eclipt Plus C18 1.8mm, 3.0100 mm column. The autosampler injected 4mL of sample per run, with automated needle washes in between. The column flow rate was kept at 0.6 mL/min with the heater at a constant 50°C. The mass analyzer was an Agilent 6230 TOF- MS operated in positive ion scan mode with mass scanning from 100 to 1000m/z. The ion source was upgraded from the original Agilent Jet Stream (AJS) source to the dual - sprayer version for improved reference mass delivery. The instrument acquired data using the following parameters: drying gas temperature, 3508C; drying gas flow, 8.0 L/min; nebulizer, 35 psi; sheath gas temperature, 4008C; sheath gas flow, 11 L/min; VCap. 3.500 V; nozzle, 0 V; fragmentor, 125 V; skimmer, 65 V; and octopole RF peak, 750. A constant flow of Agilent TOF reference solution through the reference nebulizer allowed the system to continuously correct for any mass drift by using the reference mass ions purine at 121.05087 and HP-921 at 922.00979m/z.

Phytochemical analysis was carried out to detect the active compound of both extracts. The compounds detected in this work were tentatively characterised by mean of MSdata, together with the interpretation of the observed MS/MS spectra in comparison with those found in the literature. The following public databases were consulted: ChemSpider (http://www.chemspider.com), SciFinder Scholar (https://scifinder.cas.org), and Phenol Explorer (www.phenol - explorer.eu).

RESULTS
LC-TOF-MS Analysis
The compounds identification was performed by analysing the MS spectral pattern based on the LC-TOF- MS database library and comparison with literature data [5]. Most of the compounds identified had similarity index of more than 90 %. We observed different peaks at different retention times.

Compounds characterization in CPL Aqueous Extract
The chromatograph shows ten peaks at different retention times, each one of which represents the molecule with a different number of charges. The phytochemical screening of CPL aqueous extract revealed that the identified compound could be grouped into two broad classes, (a) phenolic acids and (b) flavonoids and flavonoid glycosides. As shown in Figure 1, compounds identified in the aqueous extract were (9- 12-octadecenoic acid, a-octadecenoic acid, corchori fatty acid D, o-feruloylquinic acid, and naringenin methyl ether, o-dicaffeyl shikimic acid, sucrose stearic acid) and n- hexadecenoic acid which is an ester of fatty acid with antioxidant activity. The phenolic acids identified are mainly o-feruloylquinic acid (peak 1) with retention time of 2.71, catechin (peak 3) with retention time of 11.11, o dicaffeoyl shikimic acid (peak 5) with retention time of 12.08 and (peak 6) with retention time of 15.26, caffeoyl feruoyl tartaric acid (peak 7) with retention time of 17.44. The flavonoid was represented by chrysoeriol (peak 1), naringenin methyl ether (peak 2), isorhamntin - 30-glucoside (peak 4), -o-caffeoylquinic acid (peak 6) and luteolin hexoside (peak 8).

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Fig-1: LC- TOF- MS of total compounds in CPL sample extracted with water. The chromatograph shows ten peaks at different retention times, each one of which represents the molecule with a different number of charge.

<table>
<thead>
<tr>
<th>Peak area</th>
<th>RT/min</th>
<th>Possible compound</th>
<th>Formula</th>
<th>[M-H] (m/z)</th>
<th>Intensity</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, 2.71</td>
<td>9-12-octadecenoic acid</td>
<td>C18H32O2</td>
<td>280</td>
<td>2983</td>
<td>doubly unsaturated fatty acid</td>
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<tr>
<td></td>
<td></td>
<td>a-octadecenoic acid</td>
<td>C18H34O2</td>
<td>282</td>
<td>2039</td>
<td>fatty acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chrysoeriol</td>
<td>C16H11O6</td>
<td>300</td>
<td>3326</td>
<td>flavonoid</td>
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<tr>
<td></td>
<td></td>
<td>corchori fatty acid D</td>
<td>C18H25O4</td>
<td>305</td>
<td>2307</td>
<td>fatty acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>o-feruloylquinic acid</td>
<td>C17H17O9</td>
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<td>7545</td>
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<tr>
<td>2</td>
<td>2, 10.31</td>
<td>n-hexadecenoic acid</td>
<td>C16H32O2</td>
<td>256</td>
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<td>Fatty acid</td>
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<tr>
<td></td>
<td></td>
<td>Naringenin methyl ether</td>
<td>C16H12O5</td>
<td>284</td>
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<td>3</td>
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<td>C15H11O6</td>
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<td>803</td>
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<td>Isorhammin-30-glucoside</td>
<td>C22H23O12</td>
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<tr>
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<td></td>
<td>Sphingolipid conjugate</td>
<td>C23H45 NO7P</td>
<td>480</td>
<td>7009</td>
<td>Antioxidant activity</td>
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<td></td>
<td>Dicaffeoylquinic acid</td>
<td>C25H24O12</td>
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<tr>
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<td>o-dicaffeoyl shikimic acid</td>
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<td>497</td>
<td>33545</td>
<td>Phenolic acid</td>
</tr>
<tr>
<td>6</td>
<td>6, 15.26</td>
<td>4-o-caffeoylquinic acid</td>
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<td>654</td>
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<td>665</td>
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<tr>
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<tr>
<td></td>
<td></td>
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<td>C30H15O7</td>
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<tr>
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<td>C21H21O12</td>
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<tr>
<td>9</td>
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<td>C12H21O11</td>
<td>341</td>
<td>2259</td>
<td>sucrose</td>
</tr>
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<td>10</td>
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<td>Stearic acid</td>
<td>C18H33O2</td>
<td>281</td>
<td>5437</td>
<td>Fatty acid</td>
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</tbody>
</table>

**Compound characterization in CPL Methanol Extract:**
The chromatograph shows sixteen peaks at different retention times, each one of which represents the molecule with a different number of charges. LC- TOF- MS analysis for methanol extract detected seven phenolic compounds comprising (a) phenolic acids (peaks 5, 6, 7, 8, 14) and (b) flavonoid and their derivatives (peaks 4, 7, 10, 16). The former group was represented by megastigman hexoside (peak 5), o- dicafeoyl shikimic acid (peak 6), p- coumaroyl malate and sinapic acid hexoside (peak 7), o- caffeoylshikimic acid (peak 8), and p- coumaroyl malate (peak 14).

The flavonoids and their derivatives include luteolin and pinene - ol - o- gulcoside (peak 4) with retention time of 11.78, apigenin (peak 7) with retention time of 17.44, pinene- ol - o- gulcoside (peak 10), isoquercitrin (peak 11), chrysoeriol and isouqueretin acetate (peak 16). The phytochemical screening of CPL aqueous extract revealed that the identified compound could be grouped into two broad classes, (a) phenolic acids and (b) flavonoids and flavonoid glycosides.
**DISCUSSION**

Phytochemical analysis is not only important for drug discovery, but also in discovering the actual value of traditional medicines. Since the plant material contains numerous chemicals, there is a need for more advanced standard methods or techniques that can at the same time perform both qualitative and quantitative analysis [6].

If the plant was selected on the basis of traditional uses [7], it is essential to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional ‘herbal’ drug. The selection of the solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound of natural products [8]. In this study we have validated the use of CPL for medicinal purpose. We used water and methanol as solvents. As water extraction is common in traditional medicine of natural products, we have included aqueous extraction in our research. The aqueous extract may either contain more nonphenolic compounds or possess phenolic compounds that contain a smaller number of active groups than the other solvents [9].

Methanol is also used for the extraction because it is commonly used for extraction of various polar
compounds. Polar solvents are frequently used for recovering polyphenols from plant matrices. Methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols [10]. Moreover, a certain group of non-polar compounds are fairly soluble in methanol and has a low boiling point of just 65˚C compared to other alcohols.

The challenge associated with the use of all medicinal plant extracts is that they may differ widely in their biochemical composition depending on the extract preparation.

As a result, they may possess different pharmacological properties, depending on the method of processing or preparation [11]. This accounts for the accompanying differences in their efficacy [12,13]. Therefore, we have performed LC-TOF-MS analysis for CPL extract. Phytochemical screening of plant extracts was done in the liquid form using LC-TOF-MS method. In general, the test for the presence or absence of phytochemical compounds using standard methods involves the addition of an appropriate chemical agent to all the extracts in a test tube and shaken. Nonetheless, different qualitative chemical tests are usually performed in establishing the profile and chemical composition of a given extract. Phytochemical screening of various fractions from aqueous and methanol extracts of CPL were carried out for metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols and triterpenoids, tannins, quinines, coumarins, resins, anthroquinones, phlobatannin, catechol, acidic compounds, reducing sugars, carbohydrates, proteins and amino acids, phenols and glycosides.

The efficiency of methanol in giving high extraction yield of phytochemicals has been reported in previous studies [14]. Methanol as an organic solvent will dissolve organic substances. In contrast, the organic solvent could not dissolve in water as water is inorganic. Water has only polar covalent bonds whereas alcohol has both polar and non-polar bonds [15]. Our results show that CPL extract contains high levels of methanol-soluble compounds. In this study, LC-TOF-MS analysis of extracts showed the presence of various types of compounds in CPL. Main compounds contained by CPL extracts include phenolic acids, alkaloids as well as chlorogenic acid, organic acid, flavonoids, steroids, quinones, alkaloids and sugar compounds.

Catechin is one of the phenols that have been detected in the extract which showed growth inhibitory and anti-inflammatory activity [16]. Dicaffeoylquinic Acid is an antioxidant agent that has been identified as a nontoxic cosmetic and pharmaceutical depigmenting [17]. Hexadecenoic acid has antimicrobial properties. Compounds 10-octadecenoic acid methyl ester, 9,12-octadecadienoic acid (Z, Z)-, 9,12-octadecadienoic acid methyl ester, and n-hexadecenoic acid have been reported to have antimicrobial activity [18]. Luteolin, one of the flavonoids, that has been detected in the aqueous extract shows anti-inflammatory, antioxidant, antimicrobial, cancer chemo-preventive activity, and chemotherapeutic activity. It also has cardioprotective and antidiabetic effects [19].

The presence of these compounds partially explained the pharmacological properties of this plant. The different bioactive phytochemicals found in Carica papaya possess a wide range of biological activities that can be of a valuable therapeutic index.

CONCLUSION

Based on our data, it could be concluded that CPL is a natural source of substances of high importance. It was shown that the highest concentrations of phenolic compounds in the extracts were obtained using solvents of high polarity. It is observed as well that the methanolic extract manifested greater power of extraction for the phenolic compounds from CPL.

REFERENCES


