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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 **Review Article**

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 collecte d 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 6000ml 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 bef ore 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 Ecli pse Plus C18 1.8mm, 3.0100 mm column. The autosampler injected 4mL of sample per run, with automatedneedle 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-dicaffeoyl shikimic acid, sucrose stearic acid) and n- hexadenoic 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).

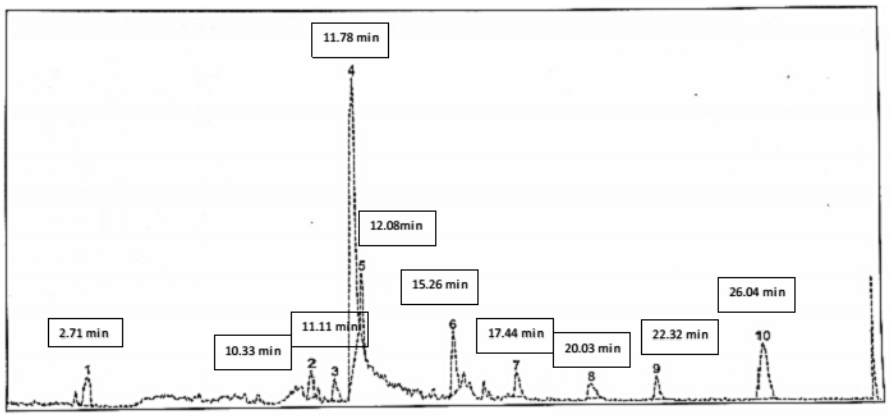

Fig-1: LC- TOF- MS of total compounds in CPL sample extracted with water. The chromatograph shows ten peaks at different retention times, each one of which represents the molecule with a different number of charge

Table-1: Compounds Detected in CPL from aqueous Extract Using LC–TOF-MS

Peak area	RT/min	Possible compound	Formula	[M-H] (m/z)	Intensity	Type
1	1, 2.71	9-12-octadecenoic acid a-octadecenoic acid chrysoeriol corchori fatty acid D o-feruloylquinic acid	C18H32O2 C18H34O2 C16H11O6 C18H25O4 C17H17O9	280 282 300 305 365	2983 2039 3326 2307 7545	doubly unsaturated Fatty acid fatty acid flavonoid fatty acid phenolic acid
2	2, 10.31	n-hexadenoic acid Naringenin methyl ether	C16H32O2 C16H12O5	256 284	2007 1353	Fatty acid flavanoid
3	3, 11.11	catechin	C15H11O6	287	803	Phenol and antioxidant
4	4, 11.78	Isorhamntin-30-glucoside Sphingolipid conjugate Dicaffeoylquinnic acid	C22H23O12 C23H45 NO7P C25H24O12	479 480 515	19538 7009 4229	flavanoid Antioxidant activity
5	5, 12.08	o-dicaffeoyl shikimic acid	C22H25O13	497	33545	Phenolic acid
6	6, 15.26	4-o-caffeoylquinic acid o-dicaffeoyl shikimic acid	C16H17O9 C22H25O13	353 497	654 665	Antioxidant activity Phenolic acid
7	7, 17.44	3-(4-hydroxyeinnamoyl) quinic acid Acyl sucrose Caffeoyl feruoyl tartaric acid	C16H17O8 C17H29O12 C30H15O7	337 425 485	7375 756 666	 sucrose Phenolic acid
8	8, 20.03	Luteolin hexoside	C21H21O12	449	593	flavonoid
9	9, 22.23	sucrose	C12H21O11	341	2259	sucrose
10	10, 26.04	Stearic acid	C18H33O2	281	5437	Fatty acid

Compounds 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- dicaffeoyl 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 isoquercetrin 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.

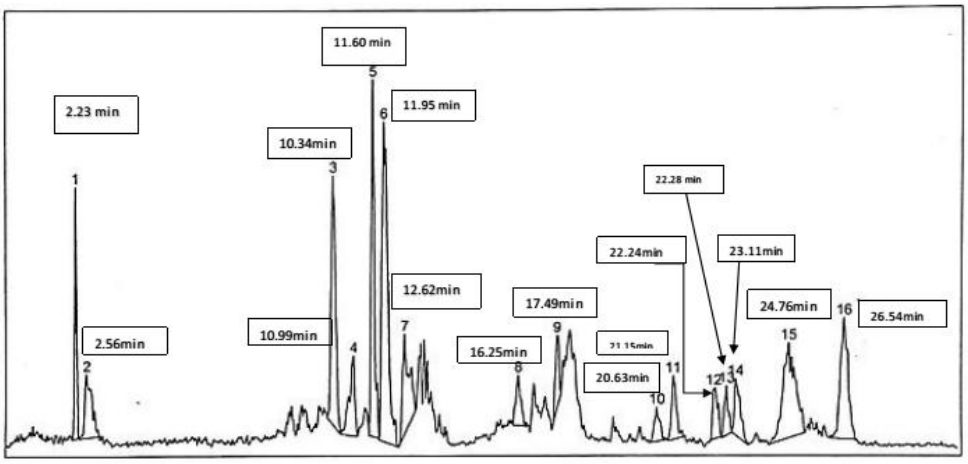

Fig-2: LC- TOF- MS of total compounds in CPL sample extracted with methanol. The chromatograph shows sixteen peaks at different retention times, each one of which represents the molecule with a different number of charges

Table-2: Compounds Detected in CPL from Methanol Extract Using LC–TOF-MS

Peak area	RT/min	Possible compound	Formula	[M-H] (m/z)	Intensity	Type
1	1, 2.23	a-hydroxy-10,12-octadecadienoic	C18H30O3	292	6731	Fatty acid
3	3, 10.34	Dihydroxyoctadecadienoic acid	C18H31O4	311	1880	Fatty acid
4	4, 10.99	Luteolin Pinene-ol-o-gulcoside	C15H9O6 C18H30O15	285 326	2137 2671	Flavonoid flavanoid
5	5, 11.60	Megastigman hexoside	C19H29O8	385	2035	phenolic
6	6, 11.95	o-dicaffeoyl shikimic acid	C22H25O13	443	3031	Phenolic acid
7	7, 12.62	Palmitic acid Apigenin p-coumaroyl malate sucrose sinapic acid hexoside	C16H31O2 C15H9O5 C13H11O7 C12H21O11 C17H21O10	255 269 297 341 385	7993 3335 2352 2191 5199	Fatty acid Flavonoid Phenolic acid Sucrose phenolic
8	8, 16.25	Linolenic acid o-caffeoylshikimic acid	C18H29O2 C16H15O8	277 335	1144 1843	fatty acid phenolic acid
9	9, 17.49	Linolenic acid Dihydroxyoctadecadienoic acid	C18H29O2 C18H31O4	277 311	1217 114	fatty acid
10	10, 20.63	Corchorifatty acid D Pinene-ol-o-gulcoside	C18H25O4 C18H31O5	305 327	3893 1941	Fatty acid flavanoid
11	11, 21.15	Linoleic acid	C18H31O2	279	1317	fatty acid
13	13, 22.82	sucrose	C12H21O11	341	2344	Sucrose
14	14, 23.11	n-hexadecanoic acid p-coumaroyl malate o-caffeoyshikimic acid	C16H32O2 C13H11O7 C16H15O8	265 279 335	2238 822 869	Fatty acid phenolic phenolic
16	16, 26.54	Chrysoeriol Isoquercetrin acetate	C16H11O6 C23H20O13	299 504	10534 1773	Flavonoid Flavonoid

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 to 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 triterepenoids, 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.

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