Isolation of carpaine from *Carica Papaya* leaves by using LCMS

Aishwarya Avinash Shinde and Dr. DP Hase

Abstract

Papaya (*Carica papaya* Linn.) is commonly known for its food and nutritional values throughout the world. The medicinal properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine. Since, each part of papaya tree possesses economic value, it is grown on commercial scale. During the last few decades considerable progress has been achieved regarding the biological activity and medicinal application of papaya and now it is considered as valuable nutraceutical fruit plant. *Carica papaya*, is an lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties.

Keywords: Carica Papaya, LCMS

Introduction

Herbal medicine (Herbalism) is the study of botany and the use of medicinal plants. Plants have been the basis of medical treatments through much of human history, and such traditional medicine is still widely practiced today. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based drugs. Although herbalism may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exits. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts.

Herbal Medicine is also called Phytomedicine or Phototherapy. Paraherbalism describes alternative and pseudoscientific practices of using unrefined plant or animal extract as unproven medicines or health-promoting agents. Para herbalism differs from plant-derived medicines is standard pharmacology because it does not isolate or standardize biologically active compounds, but rather relies on the belief that preserving various substances from a given source with less processing is safer or more effective for which there is no evidence. Herbal dietary supplements most often fall under the Phytotherapy category.

Aim and Objectives

Phytochemical analysis is intended to screen, identify, extract and isolate the phytoconstituents to evaluate the therapeutic potential of plant and to develop phytochemical standards for medicinal plant material for the quality control purpose. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive products. It also provides with chemical fingerprint of said herbal extract. This fingerprinting is done by qualitative analysis and further can be validated by quantitative assay too.

LCMS has been also used to analyses and quantify drugs and plant metabolites contained in biological samples.

To explain the basic principle of LCMS/MS to describe the general characteristics of analytical LCMS/MS applications and to comprehensively discuss the application of technology in the field.
**Carica Papaya**

The papaya is a small, sparsely branches tree, usually with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves. Confined to the top of the trunk. The lower trunk conspicuously scarred where leaves and fruit were borne. The leaves are large, 50-70 cm (20-28 in) in diameter, deeply palmately lobed, with seven lobes. All part of the plants contain latex in articulated laticifers. Papayas are dioecious. The flowers are 5-parted and highly dimorphic, the male flowers with the stamens fused to the petals. The female flowers have a superior ovary and five contorted petals loosely connected at the base.

![Carica papaya tree, flower, and ripe fruit.](image)

**Chemical Constituents of Carica papaya**

Carica papaya Linn is one of the valuable plant used for various purposes in medicinal field. Leaves, fruit and seeds of the Carica papaya are used as ethno medicine this work describes biochemical constituents of leaves of Carica papaya. Chemical composition of various part of Carica papaya plant are describe.

**Fig 1: Carica papaya tree, flower, and ripe fruit.**

*Table 2: Chemical composition of various parts of Carica papaya Plant.*

<table>
<thead>
<tr>
<th>Part</th>
<th>Constituents</th>
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<tbody>
<tr>
<td>1. Fruit</td>
<td>protein, fat, fibre, carbohydrates, minerals, calcium, phosphorus, iron, vitamin C, thiamine, riboflavin, niacin, and caroxene, amino acid, citric acids and molic acid (green fruits), volatile compounds: linalol, benzylisothiocynate, cis and trans 2, 6-dimethyl-3,6 epoxo-7 octen-2-ol, Alkaloid, α-carpine, benzyl-b-glucoside, 2-phenylethyl-β-D-glucoside, 4-hydroxyl -phenyl-2-6 glucoside, carin and an enzyme myrosin</td>
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<tr>
<td>2. Juice</td>
<td>N-butyric, n-hexanoic and n-octanoic acids, lipids: myristic, palmitic, stearic, linoleic, linolenic acids-vaccenic acid and oleic acids</td>
</tr>
<tr>
<td>3. Seed</td>
<td>Fatty acids, crude proteins, crude fibre, papaya oil, carpine, benzylisothiocynate, benzyglucosinolate, glucotropacolin, benzylihourea, hentriacontane, β-sitosterol, carin and an enzyme myrosin</td>
</tr>
<tr>
<td>4. Root</td>
<td>Arposide and an enzyme myrosin</td>
</tr>
<tr>
<td>5. Leaves</td>
<td>Alkaloids carpine, pseudocarpain and dehydrocarpaine I and II, choline, carposide, vitamin C and E</td>
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<tr>
<td>6. Bark</td>
<td>β-sitosterol, glucose, fructose, sucrose, galactose and xyitol</td>
</tr>
<tr>
<td>7. Latex</td>
<td>proteolytic enzymes, papain and chemopapain, glutamine cyclotransferase, chymopapain A, B and C, peptidase A and B and lysozymes</td>
</tr>
</tbody>
</table>

**Plant material and authentication**

*Carica Papaya,* Family Caricaceae matured healthy leaves of male and female of from Sangamner, Maharashtra, India in September 2019. Mature leaves of male and female plant of Carica Papayawere thoroughly washed with distilled water to remove dust. Petiole and veins were removed. Remaining portion was shade dried for a day followed by complete drying in an oven at 38 C, grounded using a mortar and pestle for 10-15 min and the powder was preserved.

**Solvents and reagents**

Analytical grade Methanol (Sigma Aldrich), Acetonitrile (Thomas Baker) were used for LC-MS analysis, Anisaldehyde (Sigma Aldrich) were used for derivatising reagent, formic acid & water etc.

**Extraction procedure**

**Preparation of leaf extract**

Healthy, fresh young leaves of Carica papaya were collected from Sangamner, Maharashtra, India in September 2019. The leaf extract was prepared with slight modification. Leaves were rinsed under tap water and then by double distilled water then dried at room temperature for 15 days. 20g dried leaves were grounded to powder using a mixer grinder. 20g dried leaves powder was soaked in chloroform for extraction in a rotary shaker for 3–5 days. Dried leaves of C. papaya L. were ground in a cross beater mill equipped with a 1 mm sieve. The C. papaya leaves were extracted and extract partitioned into fractions using slightly modified methods as follows: 500 g of dark brown, smooth textured powdered sample was extracted with 1.5 l of petroleum ether using a Soxhlet extractor for six hours. The petroleum ether extract obtained was discarded. The marc was further extracted with 1.5 l of aqueous methanol (1:3 v/v) using the soxhlet extractor for another six hours. The obtained methanolic extract was then evaporated to dryness using vacuum rotary evaporator. The methanolic extract was then dissolved in 10% sulfuric acid solution and partitioned with hexane, chloroform, ethyl acetate and n-butanol successively to give hexane fraction - 23.9 g, chloroform fraction- 5.23 g, ethyl acetate fraction - 2.68 g, n-BuOH fraction 5.50 g and remaining aqueous fractions - 34.2 g. All extracts and fractions were encoded as: CE – crude methanol extract; HF- hexane fraction; CF- chloroform fraction; EF-ethyl acetate fraction; BF-nbutanol fraction; AF- aqueous fraction remaining after fractionation. All extract fractions were freeze dried and stored at 4°C.

**LC-MS (Liquid chromatography- mass spectrometry)**

Liquid chromatography–mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds.
commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical, and cosmetic industries. In addition to the liquid chromatography and mass spectrometry devices, an LC-MS system contains an interface that efficiently transfers the separated components from the LC column into the MS ion source \[2, 3\]. The interface is necessary because the LC and MS devices are fundamentally incompatible. While the mobile phase in a LC system is a pressurized liquid, the MS analyzers commonly operate under high vacuum (around 10⁻⁶ torr / 10⁻⁷ "Hg). Thus, it is not possible to directly pump the eluate from the LC column into the MS source. Overall, the interface is a mechanically simple part of the LC-MS system that transfers the maximum amount of analyte, removes a significant portion of the mobile phase used in LC and preserves the chemical identity of the chromatography products (chemically inert). As a requirement, the interface should not interfere with the ionizing efficiency and vacuum conditions of the MS system \[2\]. Nowadays, most extensively applied LC-MS interfaces are based on atmospheric pressure pressure ionization (API) strategies like electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo-ionization (APPI). These interfaces became available in the 1990s after a two decade long research and development process \[4\].

**Table 3.**

<table>
<thead>
<tr>
<th>Acronym</th>
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<tr>
<td>Classification</td>
<td>Chromatography Mass spectrometry</td>
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<td>Thermo Fisher Scientific</td>
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**Result and Conclusion**

Study of Morphological Characters of Plant *Carica papaya* leaves.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Morphological Character</th>
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<tbody>
<tr>
<td>1</td>
<td>Color</td>
<td>Green</td>
</tr>
<tr>
<td>2</td>
<td>Shape</td>
<td>Star shaped</td>
</tr>
<tr>
<td>3</td>
<td>Height</td>
<td>50-70 cm diameter</td>
</tr>
<tr>
<td>4</td>
<td>Odour</td>
<td>Aromatic</td>
</tr>
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</table>

**Powder characteristics of *Carica papaya* (Lin)**

The powder of *Piper nigrum* fruit is Blackish grey, with aromatic odour and pungent in taste. The microscopic examination of the powder shows epicarp, mesocarp, sclereid, yellow colour tests, beaker shape stone cells, starch and isolated oil cells.

**Fig 2:** Liquid chromatography - mass spectrometry

**Carpaine analysis with LC-MS**

Carpaine compounds were tested using LC-MS/MS equipment [UHPLC (Thermo Scientific ACCELLA type 1250)]. The column used was Hypersil Gold (50mm x 2.1mm x 1.9 im). The mobile phase A comprised 0.1% sulfuric acid diluted in distilled water, phase B consisted of 0.1% formic acid diluted in acetonitrile A linear gradient with a speed of 300 ᵇL/min with motion phase settings was used as follows: 0-0.6 minutes 10% B, 2.5-4.0 minutes 100% B and 4 minutes 10% B. The LC injection volume was 2 ᵇL. The columns were set to 30° C and the autosampler compartment was set to 16° C. The operation of MS/MS Triple Q (quadrupole) TSQ QUANTUM ACCESS MAX mass spectrometer from Thermo Finnigan with an ionization ion ESI (Electrospray Ionization) was controlled by TSQ Tune software positive mode.

**Fig 3:**

Papaya (*Carica papaya* L.) contains alkaloid compounds, saponins and flavonoids in its leaves, root, and bark. The phytochemical screening of simplicial powder and plants samples in wet form can identify the presence of alkaloid compounds, flavonoids, steroids, tannins, and saponins \[1, 2\]. *Carica papaya* L. is one of the plants that has the anticancer ability by increasing apoptosis and inhibiting proliferation of cells. Papaya leaves methanol extract has inhibitory activity against DNA enzyme Topoisomerase II. This is an enzyme that plays an important role in the process of replication, transcription, DNA recombination and proliferation of cancer cells \[1, 3\]. This study was aimed to observe papaya leaves extract as anticancer through inhibition of cell proliferation and apoptosis induction. It was exploratory research to observe the presence of chemical compounds contained in papaya leaves from LIPI (Indonesian Research Center) Conservation Hall Plant Garden Purwodadi Pasuruan, East Java. This research has ethical innovation established by Research Ethics Commission of Faculty of Veterinary Medicine of Airlangga University with Number: 408-KE. The papaya leaves which were slightly darker green coloured were taken during sunny weather conditions \[2\]. Papaya leaves were extracted using methanol solvent and adjusted to 9 pH using 5% NH4OH. The chloroform fraction obtained was then evaporated with rotavapor to obtain a chloroform fraction \[2, 4\]. The carpaine test was performed using 0.1 g in 10 mL methanol. The sonication was done for 10 minutes at 4500 rpm. The supernatant was filtered with PTFE filter 0.2 microns. The filtrate was inserted in vial bottle and 2 ᵇL injection sample volume was analyzed by LC-MS / MS.

~ 3 ~
The quantity determination by the SRM (Selected Reaction Monitoring) method was set to 479 m/z as the precursor ion and at 240 m/z as the product ions. The ionization of ESI were following these conditions: spray voltage 3 kV; evaporation temperature 250 °C; capillary temperature, 300 °C; nitrogen as a 40 psi sheath gas pressure and Aux gas pressure 10 psi with argon gas. All operating conditions were set using the x-calibur.

From the analysis of papaya leaves extract, there are carpaine compounds based on LC-MS/MS identification (Fig. 1). There are two chromatograms shown for carpaine identification. There are two peaks on the upper chromatogram as TIC (Total Ion Chromatogram), indicating that there are two compounds that have molecular ions of 479 m/z. In bottom chromatograms as XIC (Extracted Ion Chromatogram), the identification of the carpaine compound shows that there is one peak where the precursor ion is at 479 m/z and the product ion at 240. There is a peak in RT 3.83 minutes suspected as carpaine. The results of this study indicated the identification of alkaloid compound detected in papaya leaves extract. Knowing the potential of alkaloid compounds contained in papaya leaves, they can be used as further study material for the utilization of chemical compounds as drugs. Carpine contained in papaya leaves has antitumors, anticancer and antimicrobes properties. Duke reported that the secondary metabolite content of the papaya leaves is, among others, carpaine alkaloids and pseudocarpaineam which are piperidine group of alkaloids. The piperidine-type alkaloid compounds that have anticancer activity and have anticancer mechanism by inducing apoptosis are f lavopiridol compounds which are the resultant compounds of piperidine alkaloids with flavonoid compounds.

References


