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COMPARATIVE LC-MS ANALYSIS OF PHYTOCONSTITUENTS IN RIPE AND UNRIPE PAPAYA FERMENTA

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Research Paper

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ABSTRACT

Around the world, papayas (*Carica papaya Linn*.) are well-known for their culinary and nutritional benefits. In conventional medicine, papaya fruit and other plant parts are very well known for their therapeutic qualities. Papaya is grown commercially because every component of the tree has a marketable value. The biological activity and therapeutic uses of papaya have advanced significantly over the past few decades, and it is today regarded as a valuable fruit plant with nutraceutical properties. In addition to being tasty and nutritious, the ripe fruit and unripe fruit pulp have been shown to offer therapeutic benefits. The study reveals that ripe and unripe papaya fermenta have various phytoconstituents including: spirohexane-5-one (linolenic acid),1(+)-lactic acid, tert-butyldimethylsilylester (Propanoic acid), Caffeic acid (CA), myristic acid, myristin and linolelaidic acid. These compounds have various biological activities and are responsible for papaya's numerous health advantages.

No. of Pages: 6 **References: 28**

Keywords: *Carica papaya*, medicinal plant, phytoconstituents, LCMS, Biological activity.

INTRODUCTION

Carica papaya is herbaceous plant belongs to Caricaceae family. It is found in tropical region of the world. The papaya tree is a tiny tree with few branches that typically has a single stem that grows between 6- 10 meters (16 and 33 feet) tall and has spirally arranged leaves. According to Yogiraj *et al*., (2014) large, long-stemmed, weak, and usually unbranched terminal cluster of leaves produces an abundance of white latex, growing up to 10 meters tall. It is an herbaceous perennial with copious amounts of milky latex (Fayziyeva *et al*., 2021; Carvalho *et al*., 2012). The enormous, strongly palmately lobed leaves have seven lobes. Latex is present in all plant parts with articulated laticifers (Ogunlakin *et al*., 2023). Papayas reproduce sexually. The male flowers have their stamens fused to their petals, whereas the female blooms have five parts and are very dimorphic. The female flowers feature five twisted petals that are loosely attached at the base, along with a superior ovary (Ashish *et al*., 2021).

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The goal of phytochemical analysis is to define phytochemical standards for medicinal plant material for quality control purposes as well as to screen, identify, extract, and isolate phytoconstituents in order to assess the therapeutic potential of the plant. Nutraceuticals, food supplements, traditional medicine, pharmaceutical intermediates, bioactive products, and traditional medical systems all contain new pharmaceuticals derived from medicinal plants.

It also offers the plant extract's chemical fingerprint. This fingerprinting is carried out through qualitative examination, and quantitative assay can also be used to confirm it. Biological samples containing pharmaceuticals and plant metabolites have also been analyzed and quantified using LCMS.

MATERIALS AND METHODS

Plant materials

The fresh ripe and unripe papaya fruits were purchased from market of Gwalior, India. The authentication of fruit is done by Jiwaji University Institute of Ethnobiology.

Preparation of ripe and unripe papaya fermenta

After purchasing ripe and unripe papaya from the market, the skin (peel) and seeds were carefully removed and the pulp was salvaged. After being mechanically crushed, the pulp was diluted 1:1 (w/v) with purified water for both ripe and unripe papaya. The Panda et al., (2014) modified method is used for fermentation. After fermentation the product was filtered and kept in cold store for further analysis.

LC-MS (Liquid chromatography- mass spectrometry)

The analytical chemistry technique known as liquid chromatography–mass spectrometry (LC-MS) blends the mass analysis powers of mass spectrometry (MS) with the physical separation capabilities of liquid chromatography (or HPLC). Coupled chromatography - mass spectrometry systems are widely used in chemical analysis due to their ability to increase the specific capabilities of each technique while working in concert. Mass spectrometry offers the structural identification of the individual components with high molecular specificity and detection sensitivity, whereas liquid chromatography separates mixtures with many components. Organic, inorganic, and biochemical substances that are frequently present in complex samples with biological and environmental origins can all be analyzed using this tandem approach. Thus, LC-MS can be used in many different fields, such as biotechnology, food processing, environmental monitoring, pharmaceutical, agrochemical, and cosmetic industries (Jaspreet *et al*., 2022).

An LC-MS system includes instruments for mass spectrometry and liquid chromatography in addition to an interface that effectively moves the separated components from the LC column into the MS ion source (Mukherjee, 2002; Kamboj, 2000). The LC and MS devices are essentially incompatible, necessitating the interface. Although the mobile phase in an LC system is a pressurized liquid, high vacuum (about 10−6 torr / 10−7 "Hg) is typically used by MS analyzers. Consequently, the eluate from the LC column cannot be pumped straight into the MS source. In general, the interface is a mechanically straightforward component of the LC-MS system that transfers the most analyte possible, eliminates a sizable percentage of the LC mobile phase, and maintains the chemical identity of the chromatography products (which are chemically inert). The interface must not impede the MS system's ionizing efficiency or vacuum conditions (Dr. Mukherjee, 2002). These days, atmospheric pressure ionization (API) techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo-ionization (APPI) represent the foundation of the majority of widely used LC-MS interfaces. Following a two-decade research and development process, these interfaces were made available in the 1990s (Verma & Singh, 2006).

Analysis of fermented papaya products by LCMS

For LC-MS analysis, Liquid Chromatography-Mass Spectroscopy Shimadzu model no. 8030 was used. LC-MS analysis of fermented Papaya products was carried out using a mobile phases A (water/acetonitrile 90:10 (v/v) 0.1% HCOOH) and B (acetonitrile 0.1 % HCOOH) at a flow rate of 0.6ml/min. The following gradient was applied linear increases from solution 30% B to 100% B in 17 min, hold at 100% solution B for 20 min. The various details of LC-MS were that the m/z scanning range was 50-500 [Q3 scan (+) (-)] with a run time of 15 min. along with the event speed of 0.005sec. The scan speed is 1857 with nebulizing crus flow of 2L/min. The heat block temperature of the instrument is 400˚C with drying crus flow of 15 l/min. The total flow rate is 0.9ml/min and the pump concentration of B: A is 65:35%. The maximum and the minimum pressure limit of both the pumps A & B re 300 kg f/cm^3 & 0 kg f/ cm^3 resp. The detector used in UV detector along with the wavelength of 254nm with auxillary range 1.04 Au/m and recercler range of 1.0000. The intensity unit is calculated in Volt & the temp. of the column oven is 40˚C. The mobile phases of pump A are 0.1 formic acid and the mobile phase of pump B is 100% methanol. Analytical HPLC analysis (Shimadzu LC20 AD) was carried out on a C reversed phase analytical column (150mm_4.6mm, particle size 5mm) at 37˚C using mobile phase A (water/acetonitrile, 90:10 (v/v) 0.1 TFA and B (acetonitrile, 0.1TFA) at a flow rate of 1.5ml/min. The following gradient was applied linear increase from solution 30% B to 100% B in 10 min. Their condition of columns as well as the flow rates etc. where same as LCMS.

RESULT AND DISCUSSION

The LC-MS investigation of fermented Papaya products reveals the presence of three compounds in ripe papaya fermenta and ten compounds in unripe papaya fermenta. Detailed information about the identified components including molecular weight, molecular formula are shown in fig 1,2 and table 1,2.

In the ripe papaya fermenta, the LC-MS analysis indicated the presence of 2-butanone, 4-hydroxy-3-methyl, myristic acidand spirohexane-5-one(linolenic acid).

The unripe papaya fermenta contained (+)-3,4 dihydroproline amide; N, N-Dimethyl-o-(1-methylbutyl)-hydroxylamine; 5-fluoro-6-methyl-5-heptene-2-one; pyrimidine-2,4 (1H, 3H)-dione, 5 amino, 6 nitroso; DL-glyceraldehyde dimer; 2-butanone, 4-hydroxy-3-methyl and 1(+)-lactic acid, tert-butyldimethylsilylester.

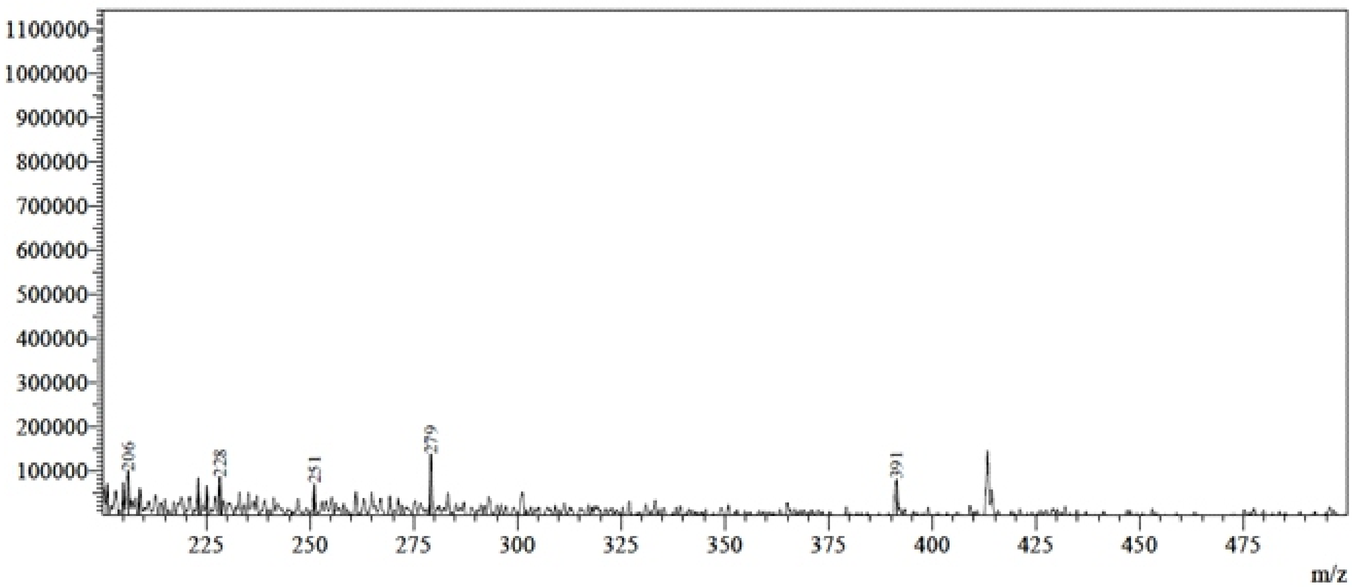

Fig. 1: LC-MS chromatogram of Ripe Papaya Fermenta.

Table 1: Phytochemical compounds identified in Ripe Papaya Fermenta by LC-MS.

S. NO.	Molecular weight	Molecular formula	Bioactive component
1.	206	$C_{13}H_{16}O_{2}$	2-butanone, 4-hydroxy-3-methyl
2.	228	$C_{14}H_{28}O_{2}$	Myristic acid
3.	279	$C_{18}H_{30}O_{2}$	spirohexane-5-one(linolenic acid)

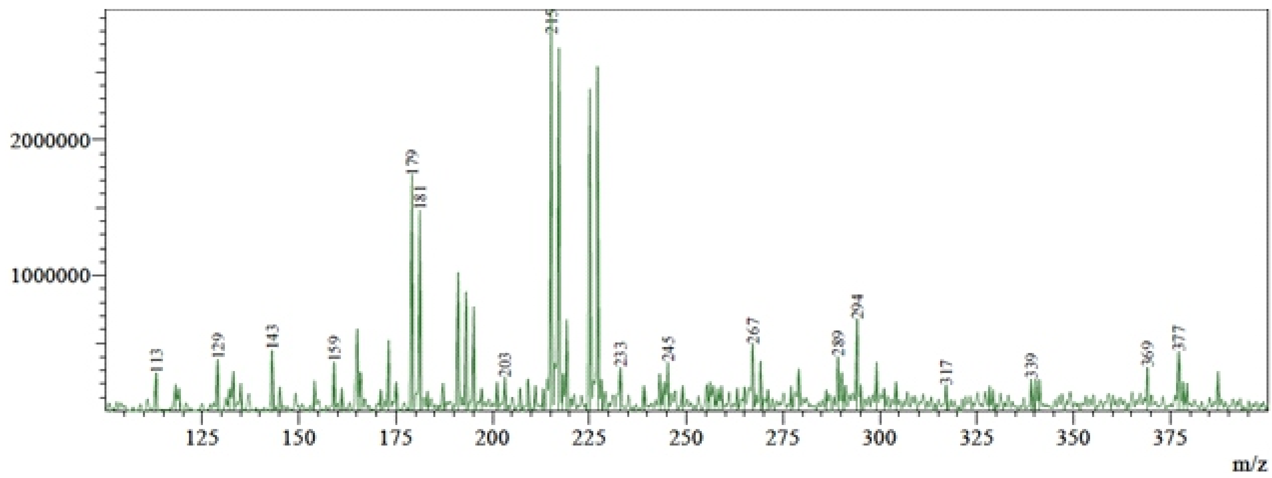

Fig.2: LC-MS chromatogram of Unripe Papaya Fermenta.

Table 2: Phytochemical compounds identified in Unripe Papaya Fermenta by LC-MS.

S. NO.	Molecular weight	Molecular formula	Bioactive component
1	113	$C_{5}H_{8}N_{2}O$	(+)-3,4 dihydroproline amide
2	129	$C_{7}H_{17}NO$	N,N-Dimethyl-o-(1-methylbutyl)-hydroxylamine
3	143	$C_{8}H_{13}FO$	5-fluoro-6-methyl-5-heptene-2-one
4	159	$C_{4}H_{4}N_{4}O_{3}$	pyrimidine-2,4 (1H, 3H)-dione, 5 amino, 6 nitroso
5	179	$C_{9}H_{8}O_{4}$	Caffeic acid
6	181	$C_{10}H_{15}NO_{2}$	[Bis-(2-hydroxyethyl)amino]benzene
7	204	$C_{13}H_{16}O_{2}$	2-butanone, 4-hydroxy-3-methyl
8	294	$C_{19}H_{3}4O_{2}$	Linolelaidic acid, methyl ester
9	317	$C_{15}H_{10}O_{8}$	Myricetin
10	318	$C_{15}H_{34}O_{3}Si_{2}$	1(+)-lactic acid, tert-butyldimethylsilylester (Propanoic acid)

Some of these compounds possess several biological activities. Spirohexane-5-one(linolenic acid) showed Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Antihistaminic, Antiarthritic, Anti-coronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic (Akhilesh & Anusha, 2013), Anti-inflammatory (Zhao *et al*., 2007), lowers high blood pressure and also used to prevent heart attack (Stanley *et al*., 2007) and cancer; 1(+)-lactic acid, tert-butyldimethylsilylester (Propanoic acid) have Anti-microbial activity (Shahraz *et al*., 2011), Anti-inflammatory activity (Nakajima *et al*., 2018), exhibiting analgesic and antipyretic properties (Loaiza-Ambuludi *et al*., 2013; Turan-Zitouni *et al*., 2015), herbicides, controlling both monocotyledonous and dicotyledonous plants

(Degenhardt *et al.*, 2011; Eş I *et al.*, 2017) as a preservatives in bakery and cheese products (Sabra *et al.*, 2013; Del *et al.*, 2013), also used as artificial flavours and fragrances (Liu *et al.*, 2012), pharmaceuticals (Shams *et al.*, 2019). Anti-obesity, Antidiabetic, Lower fatty acid content in liver and plasma reduces food intake, exerts immunosuppressive action & probably improves tissue insulin sensitivity (Heimann *et al.*, 2022). It also plays an important role in inflammation, oxidative stress, lipid metabolism, and mitochondrial function (Macfabe, 2012; Nankova *et al.*, 2014; MacFabe, 2015). Caffeic acid (CA) is a phenolic compound synthesized by plant species and is present in foods such as wine, coffee, tea. It is a phenolic acid and its derivatives have antioxidant, anti-inflammatory and anticarcinogenic activity (Kaio *et al.*, 2019).

CONCLUSION

Papaya is a potential source of phytoconstituents with several biological activities. From the study, it has been discovered that the fermented papaya productscontain alkaloids, flavonoids, phenols, terpenoids and tannins etc. A few compounds with different biological activities have been identified from fermented papaya products including spirohexane-5-one, 1(+)-lactic acid, tert-butyldimethylsilylester and Caffeic acid. Fermented papaya products are a potential therapeutic functional food which help to reduce degenerative diseases such as diabetes, cancer, obesity. It also has anti-inflammatory, anti-oxidants, anti-microbial, Hypocholesterolemic and hepatoprotective potential. The investigation of these products major bioactive components laid the foundation for further investigation into the possible health advantages of these plants and the necessity of more pharmacological studies.

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