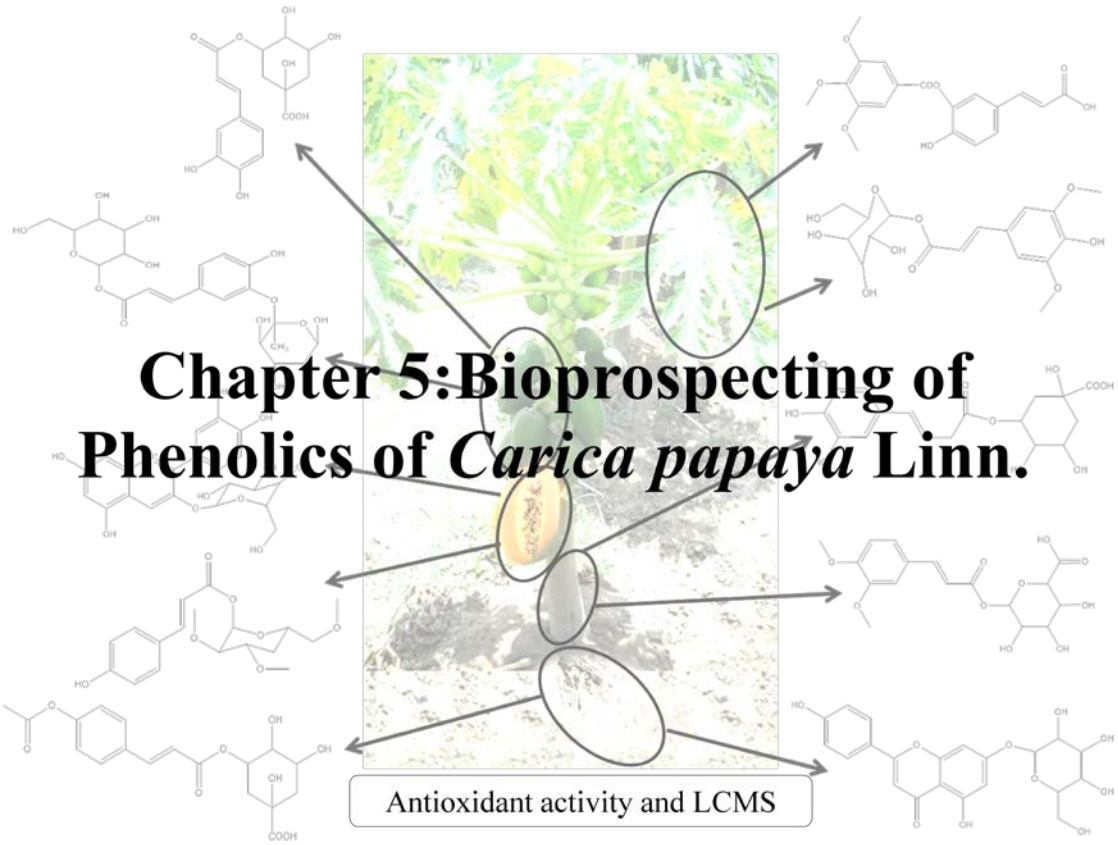



# Chapter 5:Bioprospecting of Phenolics of *Carica papaya* Linn.

# 5: Bioprospecting of Phenolics of *Carica papaya* Linn.

## 5.1 Introduction

Phenolics are class of secondary metabolites consisting of at least one common functional group – phenol. There are about 8000 phenolic compounds that are believed to be derived through shikimic path way. Phenolics are distributed throughout the plant kingdom however the type of phenolic compound present varies considerably as these are produced in response to the adaptation during evolution. The botanical survey reveals that a full range of phenolics are found in vascular plants and normally accumulated in vacuoles of guard cells, epidermal and sub-epidermal cells of leaves and shoots, plant cell wall and on the external surfaces of plant organs. Phenolic compounds being widely distributed in many organs of the plants, they are integral part of human diet.

The five major groups, namely, phenolic acids, flavanoids, tannins, stilbenes and lignans constitute the plant phenolics as shown in Scheme 5.1.

The Phenolic acids can be further classified as: derivatives of benzoic acid such as gallic acid, and derivatives of cinnamic acid such as coumaric, caffeic and ferulic acid.

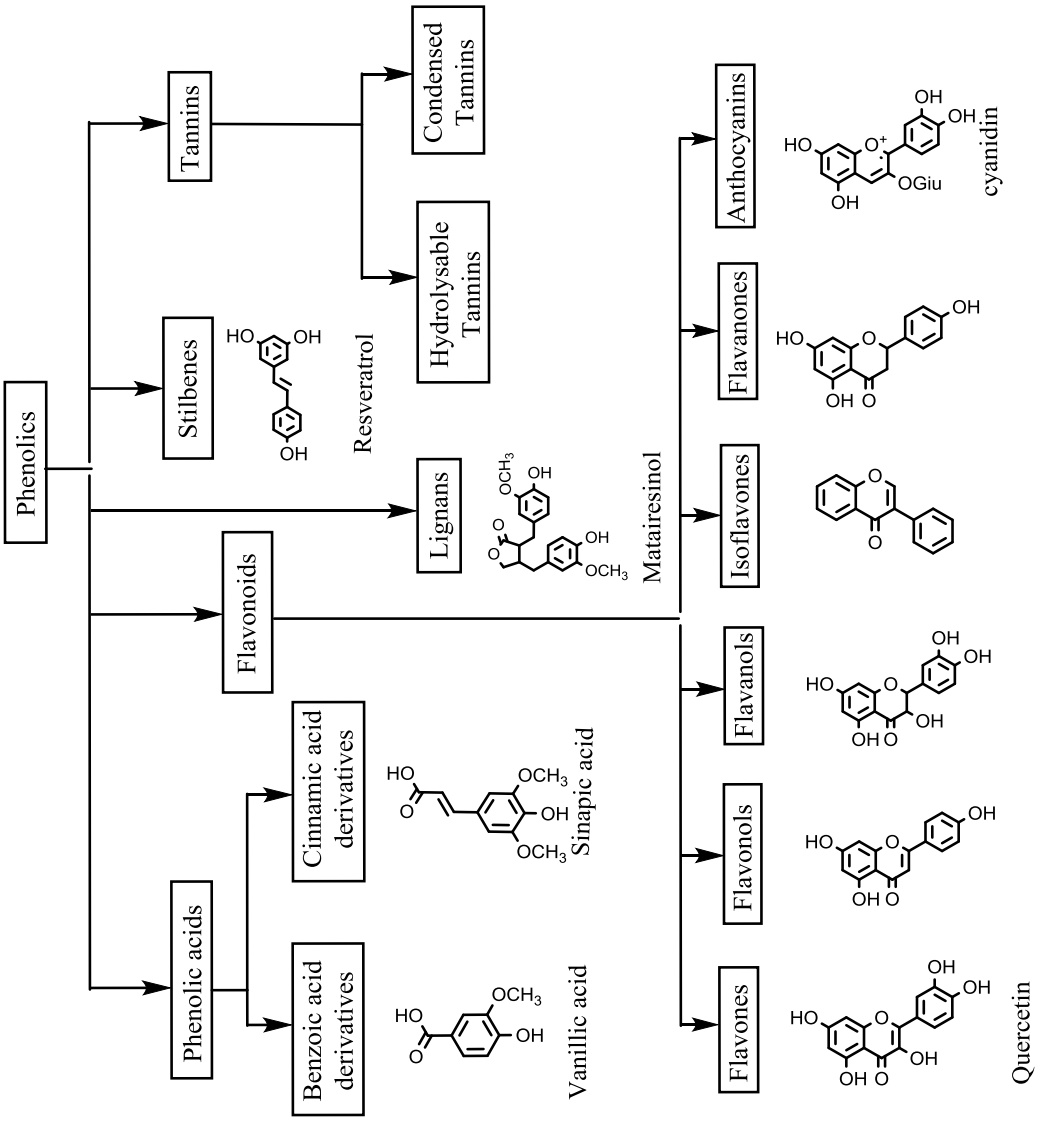
The basic structure of flavonoids is derived from the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6-C3-C6), based on the degree of oxidation of their central pyran ring flavonoid are divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins. The degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation of the three rings give rise to a structural variation in each subgroup. The study of plant flavanoids can be used to establish the phylogenetic relations between higher plants [1].

Stilbenes are phenolic compounds which contain C6-C2-C6 basic skeleton [2]. They are widely distributed in higher plants and their main physiological roles relate to their action as phytoalexins and growth regulators [3].

Apart from flavonoids, tannins form another major group of polyphenols in our diets and which are hydrolysable tannins or condensed tannins.

Hydrolysable tannins are compounds containing a central core of glucose or another polyol esterified with gallic acid, also called gallotannins, or with hexa-hydroxydiphenic acid, also called ellagitannins. The great variation in the structure of these compounds is due to many possibilities in forming oxidative linkage [4].

Condensed tannins, also known as proanthocyanidins are oligomers or polymers of flavan-3-ol linked through an interflavan carbon bond yield anthocyanidins through acid-catalyzed oxidation reaction upon heating in acidic alcohol solutions.




**Scheme 5.1:** Classification of phenolic compounds

Lignans are phytoestrogens structurally characterized by the coupling of two phenylpropanoid units through a bond between the β-positions in the propane side chains [5] present in seeds, vegetable oils, cereals, legumes, fruits and vegetables as aglycones, glycosides, esterified glycosides or as bio-oligomers [6], [7]. Frequently occurring lignans are lariciresinol, pinoresinol, secoisolariciresinol, syringaresinol, matairesinol, 7-hydroxymatairesinol, sesamin, sesamolin and sesamol [6].

Plant phenolics have a diverse functionality in the plant growth, development, and defences [8], [9]. They protect plant leaves from excessive ultraviolet radiations. All the plant cell wall mainly consists of cellulose, hemicelluloses (mostly xylans) and lignin which provide structural and functional reinforce to plant as well as provide resistance towards climate change and pathogens [10]–[13]. The bitter and astringent properties of phenolics provide a defence asset to plants against microbes and other animals [14], [15], [16]. Various colours of flowers due to different phenolics attract many insects which become carriers of pollens from one flowers to another and became a parts of pollination [17].

The phenolics play a rather different role for mankind, with extensive applications in diverse fields of medicine, cosmetics, nutraceuticals, dyeing etc. Phenolic compounds being widely distributed in many organs of the plants, they are integral part of human diet as vegetables and fruits which are responsible for the organoleptic properties of food. These phenolic compounds affect the sensory characteristics of food with impacts on color, flavor and astringency.

The role of phenolics as nutraceuticals probably due to its antioxidant properties which act as reducing or metal chelating agents, hydrogen donors and singlet oxygen quenchers [18].

Phenolic compounds, due to presence of different functional groups, effectively absorb UV-B radiations (between 280 and 315 nm) from the sun and are used in cosmetics as sunscreens [19]–[21].

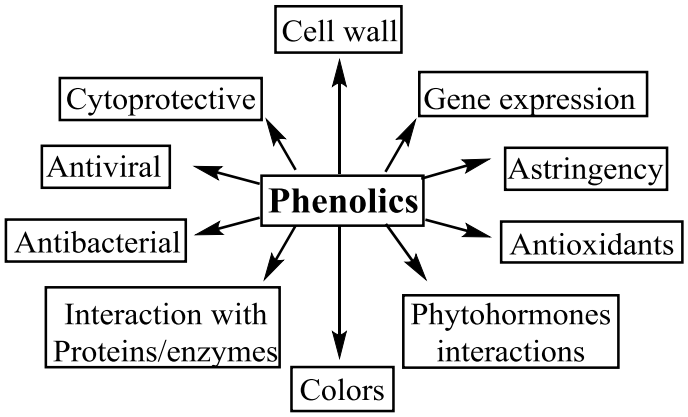



**Figure 5.1:** Significant applications of phenolics

Anthocyanins, with different oxidation state to flavilium salt, are responsible for the pH dependent orange, red, blue and purple pigments of many fruits and vegetables such as apples, berries, beets and onions. Phenolics impart different colors with metals which has been used traditionally for dyeing [4], [22]–[24].

The oldest medical application of phenolic compounds is the use of phenol as an antiseptic [9]. The pharmacological action of phenolic compounds like antiviral [25]–[27], anti-allergic [28], [29], anti-platelet [30]–[32], anti-estrogenic [33], [34], anti-cancerogenic [4], [35], [36], anti-inflammatory [37], [38], anti-angiogenic [39], [40], [41] and antioxidant properties[42]–[46] have been reported. These activities are through mechanisms, such as cellular signaling, gene expression, and modulation of enzymatic activity [47].

## 5.2 Detection techniques

Phenolics being water soluble the tests are performed in the water extract of the plant. Phenolic phytochemical groups contain phenyl ring which absorbs UV-visible radiation hence detection techniques based on UV/Visible absorption are known. Hydroxyl functional group makes the phenolic group actively participate in redox reactions, nucleophilic reactions and also forms colored chelate which is useful in detection of phenolics. Many chromatographic techniques (paper, thin layer 1-D, 2-D, GC, HPLC, LCMS) are developed for the quantitative and qualitative analysis of the phenolics [4], [48]–[59].

## 5.3 Phenolics present in *Carica papaya* Linn.

*Carica papaya* has abundant phenolics in different parts. The presence of ferulic acid, caffeic acid and rutin in the exocarp of 'Maradol' papaya fruit and lycopene, *β*-cryptoxanthin and *β*-carotene in mesocarp has been reported [60]. GC-MS profiling of *Carica papaya* leaves has been reported and reflect occurrence of quercetin, kaempferol, 5,7-Dimethoxycoumarin, protocatechuic acid, p-coumaric acid, caffeic acid in major amounts and chlorogenic acid in minor amounts [61]. There are few reports on the study of antioxidant properties of phenolics in different cultivars of *Carica papaya* [62]–[69].

However there are no reports on the study of phenolics in different parts of *Carica papaya.* ***S*o the aim of present work was to identify and study the antioxidant properties of various phenolics present in different cultivars of *Carica papaya*.**

The present work was carried out in two parts
Part 1 consists of isolation and identification of phenolic groups by 1D and 2D paper chromatography, HPLC and LCMS-MS method.
The part 2 is about quantitative analysis of phenolics and flavonoids and its antioxidant properties.

## 5.4 PART I

## 5.4.1 Materials and methods

### *5.4.1.1 Plant material*

The leaves, stems, seeds, fruits, and roots of *Carica papaya* L. collected in February 2011 from Vadodara, Gujarat, India. The whole plant was identified and authenticated by Prof. Dr. M Daniel at Department of Botany, The M. S. University of Baroda, Gujarat, India. The voucher specimen of this plant (No. BARO/2010/51) was deposited at the Herbarium, BARO, Department of Botany, The M. S. University of Baroda. The plant material was washed, shade dried for a day and then dried completely in an oven at 38°C. The plant materials were coarsely powdered using a rotary grinder and stored in airtight plastic containers.

### *5.4.1.2 Chemicals*

HPLC grade methanol (99.9%) was purchased from Merck (India) trifluroacetic acid (99%) from Sigma–Aldrich (India) and acetic acid (99.8%) from (Spectrochem, India). ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid, 99%), caffeic acid (3,4-dihydroxycinnamic acid, 97% predominantly trans), syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid, 98%), gallic acid (3,4,5-trihydroxybenzoic acid, 97%), protocatechuic acid(3,4-dihydroxybenzoic acid, 98%), gentisic acid (2,5-dihydroxybenzoic acid, 98%), veratric acid (3,4-Dimethoxybenzoic acid, 99%), p-coumaric acid (trans-4-hydroxycinnamic acid, 98%), o-coumaric acid (trans-2-hydroxycinnamic acid, 99%), m-coumaric acid (trans-3-hydroxycinnamic acid, 98%), sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid, 99%), p-hydroxybenzoic acid (4-hydroxybenzoic acid, 98%), vanillic acid (4-hydroxy-3- methoxybenzoic acid, 97%), chlorogenic acid [1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate), 95%] were purchased from Sigma–Aldrich (India). HPLC water was obtained from Merck (India).

### *5.4.1.3 Sample Preparation*

The air-dried and finely powdered parts of *C. papaya* (280 g) were macerated overnight, in petroleum ether:cyclohexane (50%) for defatting at room temperature. The residue was extracted with methanol, distilled and the remaining residue was further hydrolyzed with 7% $CF_3COOH$. The hydrolyzed fraction was filtered and subjected to sequential extraction with ethyl acetate three times. Using 15% acetic acid as a mobile phase for paper chromatography which was used for elution of the concentrated ethyl acetate extracts and then phenolic bands were cut and eluted in HPLC grade methanol, filtered through 0.2 µ Millipore filter and used for LCMS-MS studies.

## 5.4.2 Different techniques used for qualitative estimation of phenolics

### *5.4.2.1 1-Dimensional (1D) paper chromatography*

Ethyl acetate extract was concentrated upto 1 mL and spiked over Whatman paper 1 (22.3×14.3 cm) and eluted in 30% acetic acid in a closed chromatographic chamber.

After complete elusion, one fourth of the paper was dried and sprayed with 1% sodium carbonate to confirm the presence of phenolics.

### *5.4.2.2 2-Dimensional (2D) paper chromatography*

Ethyl acetate extract was spotted at the corner (1inches from base and left side) of the square sheet of Whatman paper 1 (23.2×18.8 cm). The elution was done in 2 different planes of paper with different solvent system to enhance resolution of phenolic acids. First elution in one direction was done using toluene: acetic acid: water (6:7:3) solvent system and after the elution, paper was dried and turned to 90° for the second elution with sodium formate: formic acid: water (10:1:200). After complete elution, paper was dried and sprayed with two staining agents: *p*-nitro aniline and sulphanilic acid.

**Staining reagents:**

**Stock solution:** *p*-nitroaniline/ sulphanilic acid was prepared by dissolving 0.7g of *p*-nitroaniline/ sulphanilic acid in 9mL of conc. HCl and diluted to 100 mL.

Both the spraying reagent were prepared by dilution of 4mL of the stock solution with 5mL of 1% $NaNO_2$ to 100mL at 0 °C to 4 °C. After spraying this reagent the wetted papers were sprayed with sodium carbonate solution.

### *5.4.2.3 High Performance Liquid Chromatography (HPLC)*

HPLC–DAD analyses were carried out on a Shimadzu (LC-20AT), a Rheodyne injection valve with a 20 µL loop and a diode array detector (DAD) (SPD-M20A). The data acquisition and instrument control were performed by Class VP (versions Rev.A.10.0 [1757], Analyses were performed on a Hypersil column, 250×4.6 mm, 5 µm particle size (leknokroma, Barcelona, Spain). The mobile phase consisted on a mixture of Acetonitrile (solvent A) and 1% acetic acid aqueous solution (solvent B). The applied gradient was 0–10 min: 100–90% A, 10–20 min: 90–85% A and hold at 30% A for 5 min and the flow rate was 0.5 mL $min^{-1}$. (The analyses were performed at 25 °C and the injection volume was 10 µL with a draw speed of 1mL $min^{-1}$). The

detector was set at 280 nm and 320 nm. For identification purposes, the retention parameters of each extract were compared with the standard controls and the peak purity with the UV–visible spectral reference data.

#### *5.4.2.4 Ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS)*

Liquid chromatographic separation was performed on an EXSIGENT UPLC system (AB SCIEX Framingham, USA) with ANALYST Software. Hypersil C-18 column 250 × 4.6 mm, 5 μm particle size (Leknokroma, Barcelona, Spain) was used for separation of phenolics. Prior to mass spectrometric (MS) analysis, a binary mobile phase consisting of 0.1% acetic acid (A) and 100% methanol (B) was used under the gradient conditions: 0.01 min, 100% B; 10.0 min, 90% B; 20.0 min, 85% B; 30 min, 70% B; 50 min, 50% B; and at 55.5 min until the end of 60 min run, 100% B, for re-equilibration of the column before the next injection. The eluting stream from the UPLC was introduced into AB SCIEX 3200 Q TRAP atmospheric pressure ionization (API) mass spectrometer (ANALYST SOFTWARE, AB SCIEX Corp, Framingham, USA) equipped with electrospray ionization (ESI) multi-mode ionization probe (ESI APCI). All spectra were obtained in negative mode ESI and the scan was set at m/z100-1200 Da. The LC-ESI source operation parameters were as: ion spray voltage, -2700V. Nitrogen was used as curtain and desolvation gas at the respective pressure of CUR: 20, GS1: 50, GS2: 50 (arbitrary unit). Block source temperature was maintained at 700 °C, with the respective voltages: ISV: 4500 V, EP: -10 V, CXP: 35 V.

### 5.4.3 Result and Discussion

Highly polar nature of phenolic compounds are confirmed by 1-D paper chromatography which showed bluish-white fluorescence with Rf ~1 which was reported for phenolics in pure form [48]. These phenolic groups were directly used for further qualitative analysis of phenolics present.

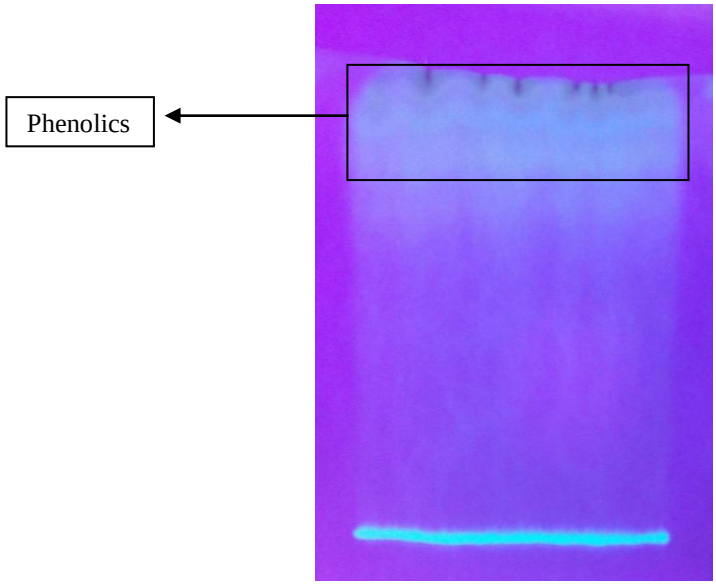



**Figure 5.2:** 1-D paper chromatography of phenolics of *C. papaya* leaves

2-D paper chromatography showed different phenolics (mainly protocatechuic acid) with different colour forming derivatives with p-nitro aniline and sulphanilic acid.

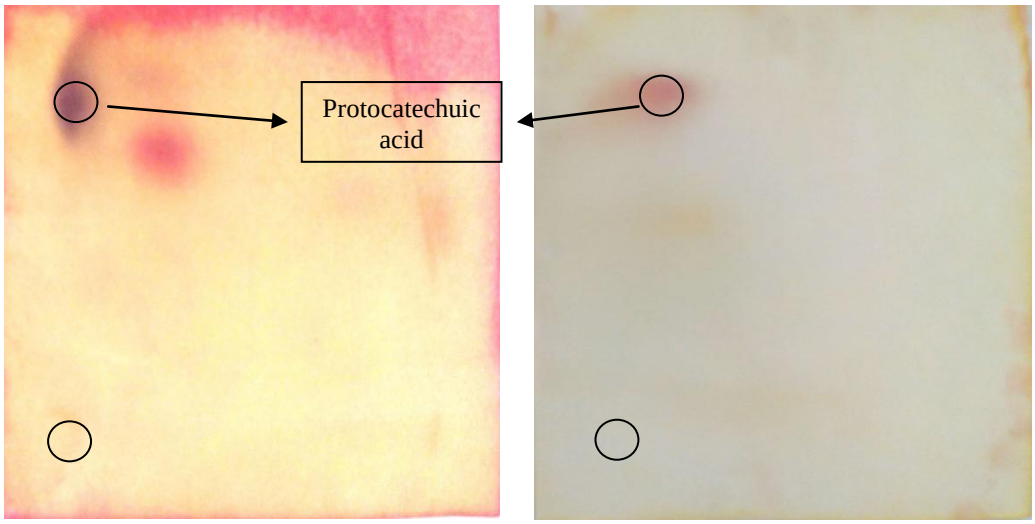



**Figure 5.3:** 2-D paper chromatography of phenolics of *C. papaya* leaves

**5.3 (a):** Derivatized by *p*-nitro aniline **5.3 (b)**: Derivatized by sulphanilic acid

From HPLC bioprospecting of phenolics between different parts as well as with other specie was performed. The results are tabulated below.

**Table 5.1:** Data of HPLC analysis

| Sl No. | Samples | Phenolic Acid Detected |
|---|---|---|
| 1 | Leaves | protocatechuic acid (12.37min), chlorogenic acid (19.21 min), caffeic acid (19.6 min), p-coumaric acid (25.53 min) |
| 2 | Fruits | Protocatechuic acid (12.38min), caffeic acid (19.6 min), syringic acid (21.12 min), p-coumaric acid (25.53 min) |
| 3 | Stem | Protocatechuic acid (12.38 min),caffeic acid (19.6 min), syringic acid (21.12 min) |
| 4 | Seeds | Protocatechuic acid (12.37 min), p- hydroxy benzoic acid (17.12 min),caffeic acid (19.6 min), syringic acid (21.12 min), p-coumaric acid (25.53 min) |
| 5 | Root | Protocatechuic acid (12.37 min), caffeic acid (19.6 min), syringic acid (21.12 min), p-coumaric acid (25.53min) |

The isolated phenolic fractions of aqueous extract were further analysed by LCMS-MS. Twenty compounds were characterized on the basis of mass fragmentation pattern. Most of them belonged to a typical hydroxy cinnamic acid and phenolic acid derivatives.

In the phenolic fraction of leaf extract the LCMS-MS analysis indicated presence of *p*-coumaryl trimethyl glycoside, 5-hydroxy caffeoyl-O-glycoside, kaempherol 3-O-pentoside, feruloyl quinic acid, syringic acid hexoside, chlorgenic acid, kaempherol-3-O-rhamnoside, syringic-caffeic acid ester and sinapic acid-O-hexoside.

The phenolic fraction of seeds contained syringic acid hexoside, 5-hydroxy caffeic quinic acid, 5-hydroxy feruloyl quinic acid, acetyl *p*-coumaryl quinic acid, feruloyl quinic acid, *p*-coumaryl trimethyl glycoside, kaempferol-3-O-rhamnoside, n-methyl feruloyl quinic acid, quercetin-3-O-glycoside, quercetin-3-O-rhamnoside, chlorgenic acid, cyanidin-3-O-glucose.

The phenolic fraction of stem was composed of kaempferol-3-O-pentoside, chlorgenic acid, syringic-caffeic acid ester, n-ethyl-*p*-coumaryl quinic acid, 5-hydroxy caffeoyl quinic acid, peonidin-3-O-glucoside, feruloyl-O-hexoside, syringic acid hexoside, cyanidin-3-O-glucose, methyl feruloyl glycoside, feruloyl quinic acid.

The phenolic fraction of roots contained quercetin-3-O-rhamnoside, chlorgenic acid, kaempherol-3-O-rhamnoside, feruloyl quinic acid, n-acetyl *p*-coumaryl quinic acid.

The phenolics present in the fruit were chlorgenic acid, feruloyl quinic acid, 5-hydroxy caffeoyl-O-glycoside, quercetin-3-O-glycoside, caffeoylhexose deoxyhexoside, *p*-coumaroyl quinic acid derivative, feruloyl-O-hexoside. Only feruloyl quinic acid and chlorgenic acid are found to be present in all parts of *C. Papaya*.

The retention time (R.T.), m/z of the molecular ions $[M-H]^-$, and m/z of the major diagnostic fragments of the main peaks are tabulated below.

**Table 5.2:** Retention times (min) and observed mass of phenolics in all parts of *C. Papaya* plant

| | **Compounds** | **R.T. (min)** | **MS $[M-1]^{-1}$ (m/z)** | **$MS^2$ (m/z)** | **Root** | **Leaf** | **Stem** | **Fruit** | **seed** |
|---|---|---|---|---|---|---|---|---|---|
| 1 | kaempferol-3-O-pentoside | 18.13 | 417 | 373, 238 | -- | √ | √ | -- | -- |
| 2 | syringic-caffeic acid ester | 32.51 | 373.1 | 358, 345.1 | -- | √ | √ | -- | -- |
| 3 | 5-hydroxy feruloyl quinic acid | 37.09 | 384.2 | 370, 355.7, 340 | -- | -- | -- | -- | √ |
| 4 | acetyl *p*-coumaryl quinic acid | 37.13 | 379.3 | 337 | √ | -- | -- | -- | √ |
| 5 | chlorgenic acid | 38.03 | 353 | 309.5 | √ | √ | √ | √ | √ |
| 6 | *p*-coumaryl trimethyl glycoside | 38.21 | 351.1 | 325.1 | -- | √ | -- | -- | √ |
| 7 | 5-hydroxy caffeoyl-O-glycoside | 38.23 | 356.9 | 330, 315.1 | -- | √ | -- | √ | -- |
| 8 | quercetin-3-O-rhamnoside | 38.46 | 446.9 | 223.1 | √ | -- | -- | -- | √ |
| 9 | quercetin 3-O-glycoside | 40.45 | 463.1 | 445.3, 405, 362.3 | -- | -- | -- | √ | √ |
| 10 | syringic acid hexoside | 40.81 | 359.5 | 315.1 | -- | √ | √ | -- | √ |
| 11 | 5-hydroxy caffeic quinic acid | 41.80 | 371 | 353.3, 343 | -- | -- | √ | -- | √ |
| 12 | kaempherol-3-O-rhamnoside | 42.52 | 431 | 416.2, 235.1 | √ | √ | -- | -- | √ |
| 13 | peonidin 3-O-glucoside | 44.59 | 462.8 | 420 | -- | -- | √ | -- | -- |
| 14 | feruloyl-O- hexoside | 45.02 | 355.3 | 337.8, 311.2 | -- | -- | √ | √ | -- |
| 15 | caffeoyl-hexose deoxyhexoside | 45.53 | 486.7 | 443.2 | -- | -- | -- | √ | -- |
| 16 | sinapic acid-O-hexoside | 46.42 | 385.3 | 370.3, 357.2 | -- | √ | -- | -- | -- |
| 17 | methyl feruloyl glycoside | 46.87 | 383 | 368.3, 355 | -- | -- | √ | -- | -- |
| 18 | *p*-coumaroyl quinic acid derivative | 48.82 | 351 | 336, 323 | -- | -- | -- | √ | -- |
| 19 | feruloyl quinic acid | 48.84 | 367.2 | 323, 199.1 | √ | √ | √ | √ | √ |
| 20 | cyanidin 3-O- glucose | 52.30 | 449 | 388.9 | -- | -- | √ | -- | √ |

# 5.5 Part II

## 5.5.1 Phenolics and its Antioxidant property

Phenolics are having high chemical diversity and the complexity in terms of composition in plant samples. Phenolics are recognized as sources of natural antioxidants and show a vital role in the chemoprevention of diseases. Isolation methods of each phenolic are very expensive as well as tedious [4] therefore, a variety of antioxidant assays were established to study the antioxidant property of phenolics present in different plant parts. Antioxidant assays such as Folin-Ciocalteu antioxidant capacity, DPPH scavenging activity, ferric ion reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), total radical-trapping antioxidant parameter (TRAP) and cupric ion reducing antioxidant capacity (CUPRAC) have been widely used for quantification of antioxidant capacity of phenolic samples from different plant parts. Most of the antioxidant assay are based on either hydrogen transfer or electron transfer mechanisms [70]–[72].

In the present study four methods, namely Folin-Ciocalteu antioxidant capacity, DPPH scavenging activity, ferric ion reducing antioxidant power (FRAP) and aluminium chloride colorimetric methods were used for quantitative determination of total phenolics, flavonoids and their corresponding antioxidant properties. The selected methods were cheap, fast and include all aspects of the antioxidant assay.

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## 5.5.2 Material and Method

### *5.5.2.1 Plant material*

Collection of plant material was described in Chapter 2 section 2.2.1.1.

### *5.5.2.2 Chemicals*

All chemicals used were of analytical reagent (AR) grade. Folin–Ciocalteu reagent, gallic acid, quercetin, DPPH, trichloroacetic acid were purchased from the Sigma Aldrich (India), Merck (India), and Sisco Research Laboratory (SRL) Mumbai India.

HPLC water was used for all the analysis. HPLC grade methanol (99.9%) was purchased from Merck (India) and acetic acid (99.8%) from Spectrochem (India).

***5.5.2.3 Sample Preparation***

Aqueous extract was prepared by Soxhlet extraction *Carica papaya* (20 g) in a 500ml flask with 250 ml distilled water for 6h. The aqueous extract was evaporated to dryness under reduced pressure and controlled temperature in a rotary evaporator.

## 5.5.3 Antioxidant properties (*in vitro*)

### 5.5.3.1 Total phenolic contents (TPC)

The Folin–Ciocalteu spectrophotometric method was used to determine the content of the total phenolic compounds in each extract [73], [74]. Each plant extract (0.5 mL) was mixed with 5 mL of the Folin-Ciocalteu reagent (1:10 dilution with water) and 4 mL of $Na_2CO_3$ (75 gm/L) and the absorbance was measured with a double beam UV/Vis spectrophotometer (Perkin Erlmer, lamda 25) at 765 nm after 30 mins of incubation at room temperature. TPC were expressed as gallic acid equivalents.

### 5.5.3.2 Total flavonoids determination (TFC)

Aluminium chloride colorimetric method was used for flavonoid determination [75], [76], [77]. Each plant extract (0.5 mL of 5 mg/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The solutions were incubated at room temperature for 30 mins. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Vis spectrophotometer (Perkin Elmer, Lamda 25). The calibration curve was prepared by employing quercetin solutions of concentrations 12.5 to 100 g/mL in methanol.

### 5.5.3.3 DPPH radical scavenging activity

The DPPH radical scavenging activity of *C. papaya* parts extract was estimated according to the method of Brand-williams (1995) [78]–[81]. After 0.5 mL of *C. papaya* parts extract had been mixed with 3.5 mL of 90 µM DPPH in methanol for 10

min, the absorbance of the sample was measured at 513.6 nm. The quenching of DPPH radical were monitored by a decrease in absorbance at 513.6 nm, which was recorded after 0, 1, 2, 3, 4 and 5 mins and subsequently every 5 mins up to 30 min, during which time the radical was stable. The percentage of remaining DPPH radical scavenging activity was expressed as percent inhibition IC 50 by plotting the DPPH% remaining in the steady state against various concentrations of each extract (5, 2.5, 1.25, 0.625, 0.312 mg/mL) and was calculated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = (1 - \text{sample OD/control OD}) \times 100$$

### 5.5.3.4 Ferric-reducing antioxidant power (FRAP)

This method determines the potential of antioxidants in different parts of *C. papaya* extract to reduce the ferricyanide complex to the ferrous form [82], [83]. One millilitre of extracts in different dilutions was added to 2.5 mL phosphate buffer (1 mL, 0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1% w/v). The mixture was then incubated in a water bath at 50 °C for 20 mins followed by 2.5 mL trichloroacetic acid (10% w/v) solution. The contents of the tubes were mixed well and 2.5 mL of solution was removed from each tube, to which 2.5 mL water and 0.5 mL ferric chloride solution (0.1% w/v) were added. The mixtures were allowed to stand for 30 min before absorbance measurements were taken at 700 nm. Triplicate solutions were prepared for each extract. The FRAP values, expressed in mg GAE/g, were derived from a standard curve.

**Table 5.3:** DPPH scavenging activity, FRAP activity, total flavonoid content (TFC) and total phenol content (TPC) of different parts of *C. papaya* plant

| Samples | DPPH $IC_{50}$(mg/mL) | FRAP $IC_{50}$(mg/mL) | TPC ± SE (µg GAE/mg) | TFC ± SE (µg Qtn/mg) |
|---|---|---|---|---|
| **Seed** | 3.11 ± 0.11 | 4.85 ± 0.27 | 865.73 ± 0.35 | 31.41 ± 0.01 |
| **Fruit** | 3.36 ± 0.13 | 12.43 ± 0.34 | 981.53 ± 0.53 | 22.99 ± 0.02 |
| **Leaf** | 13.31 ± 0.48 | 13.54 ± 0.71 | 815.29 ± 0.48 | 9.09 ± 0.01 |
| **Root** | 13.87 ± 0.08 | 48.15 ± 2.59 | 315.59 ± 0.19 | 3.48 ± 0.01 |
| **Stem** | 39.48 ± 0.4 | 94.24 ± 9.48 | 298.34 ± 0.11 | 5.82 ± 0.01 |

### 5.5.3.5 Statistical analysis

Results were expressed as the mean ± the standard deviation of triplicate analysis. Statistical comparisons were performed using the Student's t-test. Differences were considered significant at $p < 0.05$. The correlation coefficient ($r^2$) between the parameters tested was established by regression analysis.

## 5.5.4 Result and Discussion

The results for DPPH scavenging activity, FRAP activity, total phenolic content and flavonoid content in the aqueous extract of stem, seed, root, fruit and leaf of *C. papaya* plant are presented in Table 5.4 and figure 5.4, 5.5, 5.6 & 5.7.

The antioxidant properties was determined on the basis of the scavenging capacity of the free DPPH radical indicated the highest radical scavenging capacity of the seed (3.11 ± 0.11 mg/mL) and fruit (3.36 ± 0.13 mg/mL) which is somewhat lower than that of gallic acid (1.53 ± 0.01 mg/mL).

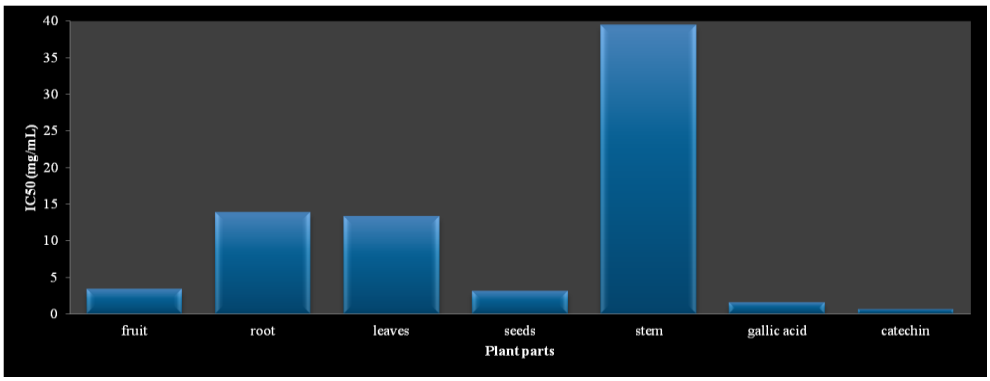


**Figure 5.4**: DPPH scavenging activity of different parts of *Carica papaya*

Reducing capacity of a compound may serve as a significant indicator of its potential antioxidant properties and prevents ROS formation by reacting with certain precursors. As shown in Table 1, the reducing power determined by FRAP was highest for the seeds (4.85 ± 0.27 mg/mL), and lowest for the stem (94.24 ± 9.48 mg/mL).

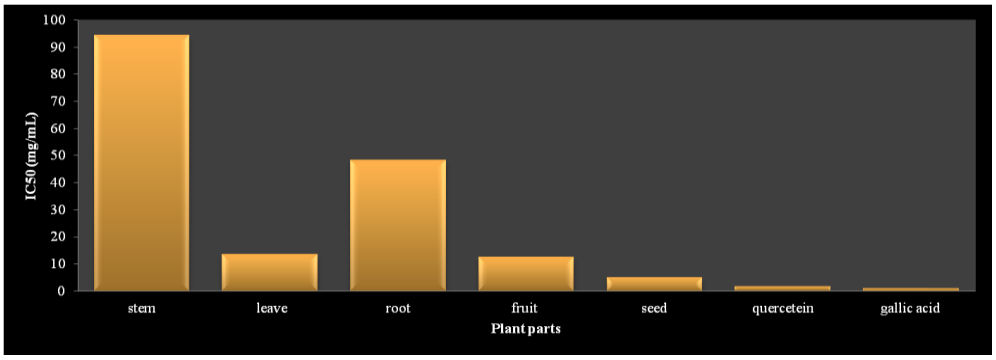


**Figure 5.5**: Ferric reducing property of different parts of *Carica papaya*

The total phenolic content based on gallic acid equivalent (GAE) determined by Folin-Ciocalteau method was found to be highest in the fruit (981.53 ± 0.53 µg GAE/mg) and lowest in the stem (298.34 ± 0.11 µg GAE/mg).

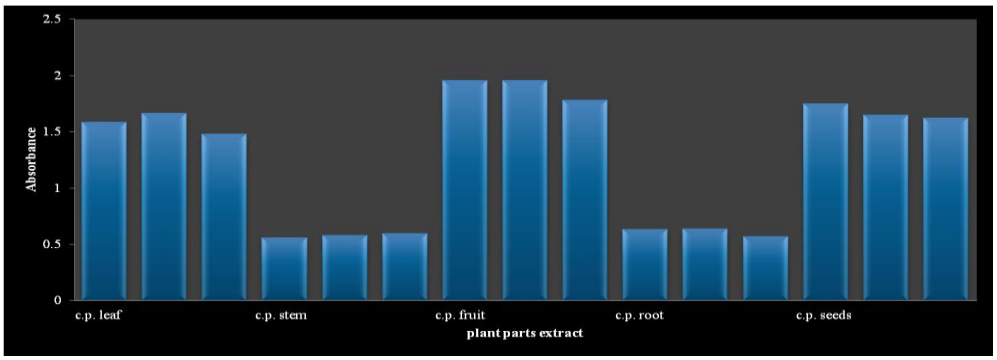


**Figure 5.6**: Total phenolic content present in different parts of *Carica papaya*

The flavonoid content based on quercetin equivalent, found to be maximum in the seed extract (31.410 ± 0.0073 µg Qtn/mg) and minimum in the root (3.483 ± 0.0056 µg Qtn/mg).

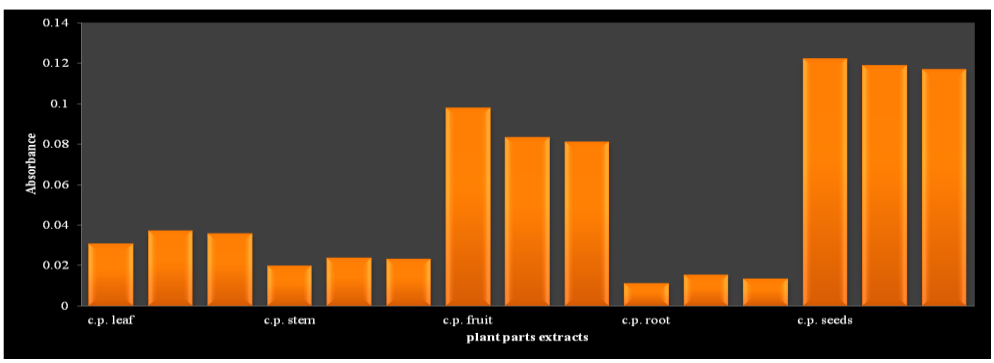


**Figure 5.7**: Total flavonoid content present in different parts of *Carica papaya*

A direct relationship is observed between the phenolic and flavonoids contents and the antioxidant properties.

## 5.6 Conclusion

For the isolation, purification and identification of phenolics different chromatographic techniques were used. Almost 20 new flavonoids and phenolic acids have been identified in different parts of *Carica papaya*.

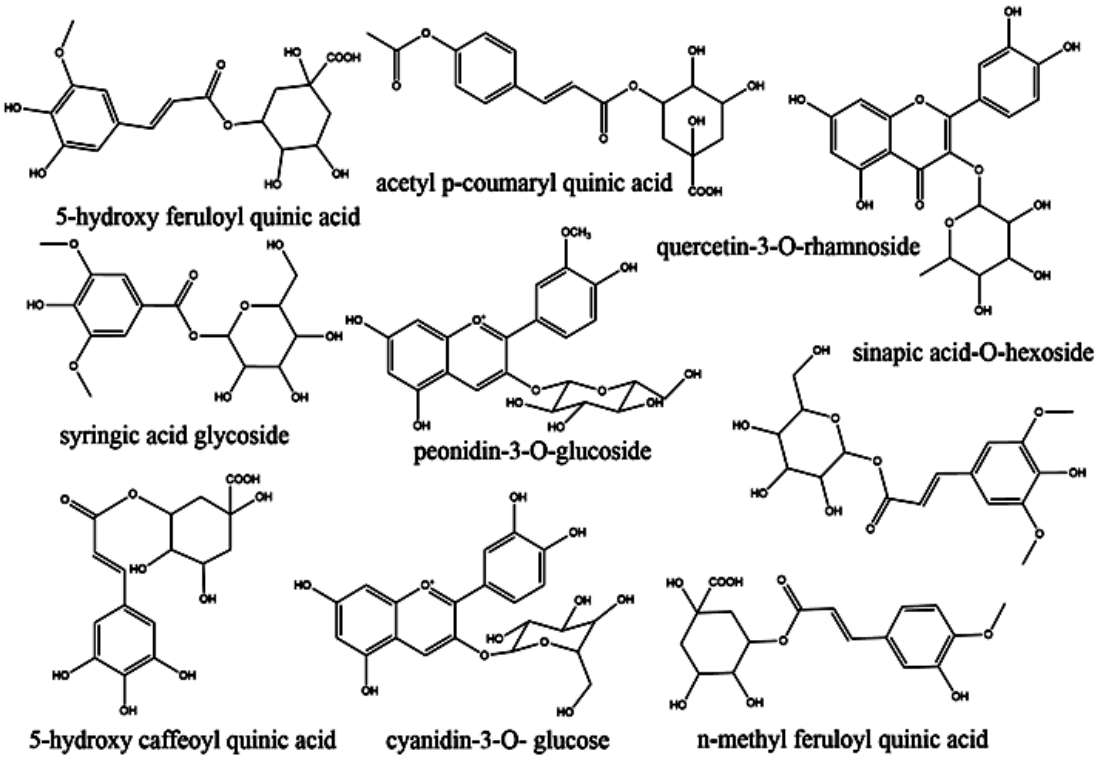


**Figure 5.8:** New Phenolics identified from different parts of papaya plant

It is the first comprehensive study of antioxidant potential of various parts of *Carica papaya*. This study reveals a number of phenolic components that have established antioxidant properties. The seeds of *Carica papaya* normally discarded, but they seem to have even greater antioxidant potential than the fruit itself. Thus, this work is a valuable addition to the knowledge of chemical spectrum of this medicinally valued and commercially useful plant.

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