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Spectrophotometric method of Ascorbic acid in *Carica papaya* L. extracts: An *in vitro* study

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Abstract
Carica papaya L. is one of the precious plants used for different use in medicinal herbalism for contagious diseases. *Carica papaya* leaves, fruit and seeds are used as complementary alternative ethano medicine. Fresh leaves samples of the plant were collected during the month of June 2019 from different areas of Perambalur district, Tamil Nadu, India. The purpose of the study was to evaluate the biochemical composition in leaves of *Carica papaya* growing in the Semi-arid region of Perambalur, Tamil Nadu and based on the result to validate its consequence in relation to chemoprevention of infectious diseases. The dried leaves were further analyzed for biochemical constituents like ascorbic acid and Cystatin using different solvent extraction. The result shows the leaf extract of *C. papaya* has high permeability of ascorbic acid and Plant Cystatin, phenols, flavanoids and saponins possess significance at α=0.05 in methanol, chloroform, ethyl acetate and water extraction. The high amount of total phenolics, saponins and total flavonoids in the ethyl acetate and methanol fractions show their permeability to their antioxidant activities. Papaya leaves contain vitamin C has a therapeutic effect in curing the skin rejuvenation which also supports the immune system and digestive ailment.

Keywords: *Carica papaya* leaves, ascorbic acid, Cystatin, infectious diseases

Introduction

The majority emergent countryside, medicinal plants, and their products are making use of the essential healing agent to treat various infectious diseases [1]. The papaya, *Carica papaya* L., is a member of the small family Caricaceae related to the Passifloraceae [2]. The papaya leaves contain the secondary metabolites which are bitter alkaloids; Carpaine and pseudocarpaine, which take action on the cardiovascular and respiration like digitalis but are dented by heat. During in the year of 1979, the University of Hawaii report to facilitate the two metabolites in the past discovery shows that major D1-piperidine alkaloids, dehydrocarpaine I and II, more effective than Carpaine found to possess antitumor activity [2, 3, 4]. The country like Asian and Indonesian markets and in New Guinea are sold the sprays of male flowers for boiling with several changes of water to remove bitterness and then eating as a vegetable and it's also used for infectious illness therapeutics [5, 6]. In Little is still known in relation to the endogenous protease of plant Cystatins targeted in the leaves of Papaya contain a wide variety of flavonoids and various Cystatin proteins, consumption of which has been associated with the reduced risk of chronic diseases [7]. The family of Cystatins is cysteine proteinase inhibitors, which also play a vital role in all cells, serve various essential and decisive physiological functions. A previous report assessing their inhibitory specificities and expression profiles over the last decades upholds, generally, the intention of multiple roles in Planta for the regulation of endogenous proteolytic growth, or the Planta for the inhibition of Cys proteases concealed by herbivores and pathogens to digest or penetrate plant tissues [8, 9]. Plant Cystatins has the capacity to restrain papain-like Cys proteases, their protein tertiary structure was highly conserved by helix loop, hydrophobic bond interactions, and protease inhibitor motifs, and their universal distribution among plant taxa, suggest an essential protease regulatory function of the protein moieties take part predominantly in the leaves of plants. The plant cystatin is characterized as the inhibitors of papain C1A family complexes with Cys proteases in from different plant species particularly in maize [7, 8, 9].

Additionally, papaya leaves include huge amounts of secondary metabolites like alkaloids, Carpaine, Dehydrocarpines, and Pseudocarpine which generate positive effects on the arterial wall as well as on accumulation in the lungs [2, 3, 4, 10]. The extract of *Carica papaya* leaves is well known as an anti-tumor, antioxidant and anti hypoglycemic agent [11]. The papaya fruit, as well as the entire parts of the plant, have been a milky juice in which an enzymatic protein has

a digestive function known as papain. On the other hand, papaya leaves, seed, and fruits have the nutritive value more and more for curing the dyspepsia, inflammation diseases like dengue and kindred ailments, technologically it has been utilized for the revelation of fermentation [12]. Papaya leaves have been shown various active components that can raise the total antioxidant power in the blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates [13]. In this paper, phytochemical metabolites and the permeability of Ascorbic acid concentration has been carried out using different methods of solvent fraction obtained from *Carica papaya* leaves. Therefore, the leaves of papaya and these secondary metabolites compounds might be used as natural antioxidants.

Materials and Methods

The leaves of *Carica papaya* were collected from the semi-arid region of Perambalur district and sun-dried for further analysis [14]. The papaya leaf was authenticated in Department of Botany, Periyar University, Salem, Tamil Nadu, India [14, 15]

Taxonomic classification

Kingdom:	Plantae – Plants
Subkingdom:	Tracheobionta – Vascular plants
Superdivision:	Spermatophyta – Seed plants
Division:	Magnoliophyta – Flowering plants
Class:	Magnoliopsida – Dicotyledons
Subclass:	Dilleniidae
Order:	Violales
Family:	Caricaceae – Papaya family
Genus:	*Carica* L.
Species:	*Carica papaya* L. – papaya

Preparation of total plant extract

Take 100 g of air dried powder from each plant under investigation was *Carica papaya* L. separately extracted by refluxing in 200 mL methanol 70% three times for 3 hours, filtered off, and concentrated using rotary evaporator to obtain 50 g from each plants. The residue attaining from each plant was separately suspended in 200 mL deionised water followed by filtering over a piece of Whatman paper No: 1 or cotton. The filtered aqueous layers were successively fractionated using several solvents such as methanol, ethyl acetate, n-hexane and aqueous extracts were dried using anhydrous sodium sulfate, followed by concentrations to obtain 50, 100, 150, 200, 250 and 300 g for methanol, ethyl acetate, n-hexane and aqueous extracts respectively [14, 15].

For the preparation of total leaf extract, seedling leaves were frozen in liquid N_2, ground to powder, and mixed with one volume of Methanol and distilled water and glass beads. Samples were boiled for 5 min and vortexed for 15 min, twice. Phenols, flavonoids, saponins and Cystatin concentration in the sample supernatant was determined using phytochemical analysis [14].

Detection of phenols

a. Test of ferric chloride

Papaya leaf extracts were treated with 3-4 drops to test with ferric chloride solution. The appearance of a bluish-black color which indicates the presence of phenols [16, 17, 18].

Detection of flavonoids

a. Test for alkaline reagent

Papaya leaf extracts were treated with a few drops of NaOH solution. Observation shows the formation of an intense yellow color, which turn into colorless on an addition with dilute acid, show the presence of flavonoids [19].

b. Test for Lead acetate

Papaya leaf extracts were added with little drops of lead acetate solution. Presence of yellow color precipitates which indicates the formation of flavonoids.

Detection of saponins

a. Froth Test for Saponins

Papaya leaf extracts were diluted with ionized water to 20ml and this was shaking thoroughly within a graduated cylinder for 15 minutes. Presence of foam into 1 cm layer which indicates the presence of saponins [20, 21, 22].

b. Foam Test for Saponins: 0.5 gm of the papaya leaf extract was shaking thoroughly by adding 2 ml of water. Due to the formation of foam is produced which is persist for ten minutes it indicates the presence of saponins.

Plant Cystatin - A typical Protein in papaya leaves

a. **Xanthoproteic Test:** Papaya leaves extracts were treated with little drops of conc. HNO_3. Presence of yellow colour indicates the presence of proteins
b. **Biuret Test:** Add 5 ml of biuret Reagent is an aqueous solution of potassium sodium tartrate treated with cupric sulfate and sodium hydroxide mixed with papaya leaves extract of 5 ml. The presence of peptide bonds (protein), this blue solution will change color to pink-purple.
c. Protein concentration in the sample supernatant was determined by the Bradford method quantification. Plant cystatin also characterized by Mass spectrometry analysis followed by N- terminal protein sequence method [21, 22].

Measurement the total ascorbic acid content of the papaya leaf extract

According to Bolin and Book (1947) [23, 24] proposed the use of 2, 6-dichlorophenolindophenol as an oxidant for the estimation of total ascorbic acid. The above method has, conversely, been set up to be most suitable because of its trouble-free process and by this method, one can evaluate both the reduced ascorbic acid as well as total ascorbic acid concurrently in the same solution [23].

It is based on the reduction of the 2, 6-dichlorophenolindophenol by an ascorbic acid solution. In the absence of interfering substances, the capacity of the extract of the sample to reduce a standard solution of the dye which has been standardized against pure ascorbic acid solution is directly proportional to the ascorbic acid content.

Ascorbic acid standard solution

Take 100 mg of ascorbic acid was taken in a 500 ml volumetric flask and the volume made to the mark with 10% metaphosphoric acid. 1 ml of this solution contained 0.2 mg of ascorbic acid. It was freshly prepared immediately before use for the standardization of indophenol dye [24].

Preparation of Dye solution - 2, 6-Dichlorophenolindophenol

Take 100 mg of 2, 6-dichlorophenolindophenol was taken in a beaker. To it, 250 ml of glass distilled water was added, warmed gently until dissolved and then added 42 mg of $NaHCO_3$. The solution was cooled, filtered and made to 200 ml with glass distilled water. Titrametrically, 2, 6-

dichlorophenolindophenol is a dye that is • blue in alkali and pink in acid. It is reduced by ascorbic acid to the decolorize formation and potency of the dye was calculated.

Ascorbic acid + 2, 6 Dichlorophenol Indophenol → Dehydroascorbic acid (Oxidized form) + reduced 2, 6 Dichlorophenol Indophenol (Colourless)

Sample preparation

Take 5 grams of papaya leaf sample were homogenized with 25 mL of metaphosphoric acid in addition to adding acetic acid solution, which is transferred into a 50 mL volumetric flask and shaken gently to homogenize solution. Then it was diluted up to the mark by the metaphosphoric acid - acetic acid solution. The obtained answer is filtered and centrifuged at 4000 revolutions per minute for quarter-hour when what the supernatant answer is employed for spectrophotometric determination (UV-1800. Shimadzu. Spectrophotometer) of Ascorbic acid content in 6 different concentrations of papaya leaf extract samples were determined [23. 24].

Estimation of ascorbic acid in papaya leaf extract

Take 0.25 mL of 3% bromine water were added into 5 mL of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0.15 mL of 10% thiourea to remove the excess of bromine. Then 2 ml of 2, 4-DNPH solution was added to form osazone. All standards, samples and blank solution were kept at 37°C temperature for 3 hours in a thermostatic bath. After it all were cooled in ice bath for 30 minutes and treated with 5 mL chilled 85% H_2SO_4, with constant stirring. As a result, a colored solution's absorbance was taken at 521 nm [25].

Statistical analysis

The experimental results were subjected to analysis of variance using Statistics Software, USA. All differences among means were declared as significant at $p \leq 0.05$ level.

Results and Discussion

The result of phytochemical analysis showing of all the extracts was given in the Fig-1 and it evidently explicates the most of the secondary metabolites constituents are present in methanol and ethyl acetate compare to all other extracts. The present study reveals that all the extracts have the Plant Cystatin, Saponins, flavonoid and Phenol contents rather than others. In this trial (Fig, 2), we find quadrupole testing shows predominantly contains Saponins, flavanoids and 7.5% of phenol contents present especially in methanolic extracts of papaya leaves. A newly cys protease moieties of plant cystatin was remarkably crop up in all the solvents. Particularly methanolic extract of plant cystatin show significantly high ($p \leq 0.05$). The comparison with solvent extraction such as ethyl acetate, methanol, n-hexane and aqueous contains ascorbic acid content are highly significant ($p \leq 0.05$) in methanol extract shows 77% gradient [35, 36, 37, 38]. Ethyl acetate also extremely rises in ascorbic acid content on extracted papaya leaves exceeds up to 65% this is because of phenol, saponins and flavanoid content has the metabolic function [37, 38, 41]. Ascorbic acid has the antioxidant properties which protect cellular components from free radical damage in the aqueous phases of cells and the circulatory system [25, 27, 32, 33]. In papaya leaves dried and green extract has the prediction of reducing the oxidative damage and lowering the risk of certain chronic of infectious diseases [39, 40]. Hence, the secondary metabolites of the plant as phenol, plant cystatin, saponins and flavonoids act an important role in the defense against free radicals.

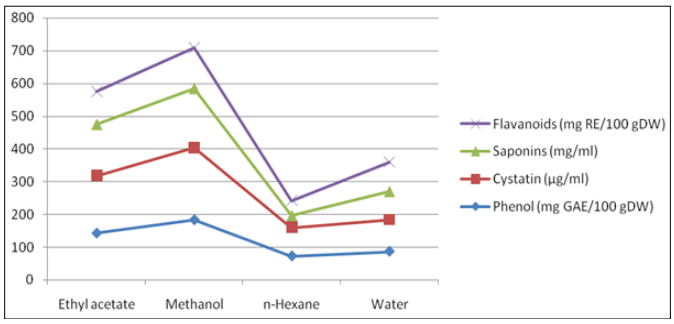

Fig 1: Phytochemical analysis of papaya leaves using different solvent extraction methods (n=6)

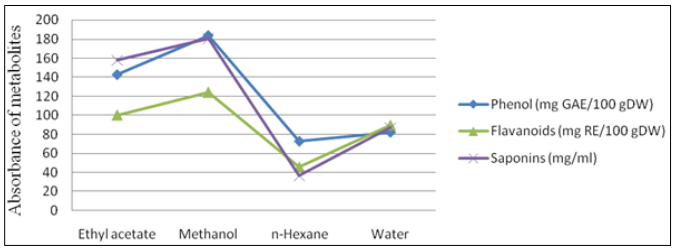

Fig 2: Assay of phytochemical analysis of *carica papaya* L. form different solvent extraction

Quantification of Ascorbic acid content in papaya leaves using different solvent extractions

The secondary metabolites of flavanoid, Plant cystatin, saponins, and phenol content was present in methanol, ethyl acetate extract of papaya leaves were determined and the results were presented in Fig. 1. The ascorbic acid concentration of the linear curve shows Y= 0.326x+21.13-R2=0.964 in the papaya leaves extraction of ethyl acetate are presumably raise while comparing to another plant extract [25, 26, 27]. Dried leaf powdered extract of papaya possess antioxidant activity in methanolic solvent contains Y= 0.237x+8.33-R2=0.980 which showing the permeability of ascorbic acid contents is highly significant at 5% level while comparing with the other solvent process [26, 27]. Papaya leaves make conform to the expression of replicase polyproteins [28, 29, 30]. It also has a strong digestive enzyme and efficient in severe digestive ailments such as bloating and chronic indigestion [31, 32, 33, 34]. The papaya leaves also extracted with n-hexane and an aqueous solution of ascorbic acid concentration shows slopes, intercept radically vary the correlation coefficient determine as Y= 0.203x+3.4-R2=0.988 and Y= 0.230x+0.2-R2=0.985. The consistency of this method is defensible by the calculation of the% of standard deviation and it was found to be varied within the range of 12-78% (Fig. 2). Papaya leaf extract was analyzing intake of 1.0 g mL-1 of ascorbic acid for 6 days checked the accuracy of the method. The standard deviation and relative standard deviation were found to be 0.0078 -1.78%, respectively. Papaya leaves extracts constitutes the detection limit (amount of ascorbic acid causing an absorbance more than thrice of std. dev.) was found to be 77 g mL-1. Since the methanol and ethyl acetate extraction has the maximum plant Cystatin [3, 4, 5], saponins [20] and flavanoid [22] content might be evidenced for an alternative source of herbal formulation for treating Skin degeneration and gastrointestinal inflammation [41, 42].

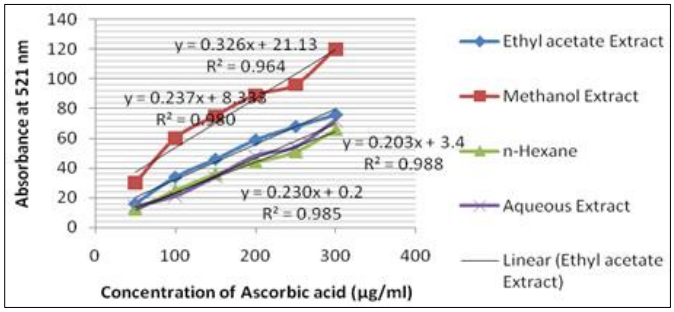

Fig 3: Ascorbic acid content in papaya leaves extract by Spectrophotometric method

Conclusion

Papaya leaves have the phytochemical property of Cystatin, saponins and flavanoid content play a pivotal role in traditional herbal medicine particularly in therapeutic the Skin degeneration, gastrointestinal inflammation and melanoma for chemoprevention. The chemical constituent of papaya leaves contains a metabolite Papain, chymopapain, pectin, carotenoids, carposide, and carpaine associated substrate as pseudocarpaine, dehydrocarpines. Papaya leaves have a prospective on antioxidants, anti-inflammatory, anti-ulcerative and anticarcinogenic effects. Ascorbic acid and its antioxidant scavenging substances, synthesis and precursor of oxygen radicals such as a nitric oxide (NO), making it important in the regulation of blood pressure and hypoglycemic effect which elicit the blood sugar levels. Plant flavonoids, saponins, Cystatin are frequently dug out with methanol and ethyl acetate solvent extraction of *Carica papaya* L. and the plant Cystatin, flavonoid content highly extract with methanol. *Carica papaya* L. infusion used for repairing the cell which is also having the pharmacological and a physiological in papaya leaves has the catalytic action of antioxidant, tumor suppression, hepatoprotective and antimicrobial. Papain is undergone the lysis mechanism to form plant-based protein which also alters the fraction of it into arginine control the production of the human growth hormone, cell division, wound healing, confiscate ammonia from the body and immune function.

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