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Preliminary phytochemical and biochemical analysis of *Carica papaya* Linn. (Seed)

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Abstract
The present investigation was carried to find out the phytochemical and biochemical constituents present in *Carica papaya*. The ethanolic extract of *Carica papaya* seed contains carbohydrate, protein, flavonoids, alkaloids, saponin, steroids, and glycosides. The chloroform extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, alkaloid, tannin, and saponin. The acetone extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, and alkaloid. The moisture content in dry seeds of *Carica papaya* is 9.5% and ash content 15%. The physicochemical parameters such as ash value and determination of moisture loss was used to determine the quality and purity of a crude drug. The biochemical analysis on *Carica papaya* revealed 18.75 mg of carbohydrates, 7.28 mg protein and 16.87 mg starch.
The extracts of *Carica papaya* seeds have great medicinal and nutrient values which could be used for treating many ailments such as ringworm, psoriasis, vermifuge, liver cirrhosis, abortifacient and maintenance of the body. All the nutrients of papaya improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. *Carica papaya* is a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health.

Keywords: *Carica papaya*, caricaceae, phytochemical analysis, biochemical analysis, physico chemical analysis

Introduction
India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plant. Plants contain natural substances that can promote health and alleviate illness. Natural drugs play an important role in the modern medicine. *Carica papaya* has grown in all home gardens. Papaya believed native to tropical America. *Carica papaya* is known with many other common names such as papaya, pappali, parangi, pappayi, and papaw. It may cultivate for its young leaves, shoots and fruits which are cooked as a vegetable. The papaya seed is currently a waste product as it is often discarded after eaten the papaya fruits. The seed are recently gaining importance due to its medicinal value. The seed had recently been linked to curing sickle cell diseases, poisoning related renal disorder, and as an antihelminthes. All the nutrients of papaya improve cardiovascular system, protect againt heart diseases, heart attacks, strokes and prevent colon cancer (Adebiyi *et al.*, (2003) [1]. Preliminary phytochemical screening were conducted to identify the presence of various phytochemicals, such as carbohydrate, protein, fats, oils, alkaloids, tannins, saponins, steroids, glycosides and flavonoids. (Suresh *et al.*, 2008) [15] Biochemical analysis refers to set of methods, assays and procedures that enable to analyse the substances found in living organisms and the chemical reactions.

Materials and Method
Study Area
Carica papaya seeds were collected from Nilambur, Malappuram district of Kerala. Nilambur gets an average rainfall of about 2400 mm and situated at a height of approximately 183 feet above sea level.

Sample collection
Fresh seeds of the selected plant materials were collected during September. Shade dried and ground to fine powder and stored in air tight container for further analysis.

Habit and fresh seeds of *Carica papaya*

Fig 1

Physicochemical Analysis
The seed was evaluated for its physicochemical parameters like total ash, determination of moisture loss using standard procedure (Pulok Mukherjee, 2010; Anonymous, 2007, Kokate *et al*., 1994)

Moisture content

$$\text{Moisture content \%} = \frac{\text{Fresh wt – Dry wt x 100}}{\text{Fresh wt}}$$

Total ash

$$\text{Ash content \%} = \frac{\text{Fresh wt – Dry wt}}{\text{Fresh weight}} \text{ x } 100$$

Preliminary phytochemical analysis

(Raaman, 2006; Karpagam *et al*., 2008; Kokate *et al*., 2001) [13, 6, 7]

Extraction

The powdered seeds were collected and 15g of it were measured and introduced into 100ml of ethanol, chloroform and acetone. Extration is carried out by shaker system for 48hrs.The nature and yield of the extract were noted. The extracts were stored in a refrigerator at 4˚c for further studies.

The aqueous extracts of the selected plant seed were tested for carbohydrates, proteins, starch, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins, saponins, terpenoids, and resins. This phytochemical screening of the extract was carried out by standard methods. (Raaman, 2006; Karpagam *et al*., 2008: Kokate *et al*., 2001) [13, 6, 7]

Test for carbohydrates

To 2 ml test solution add 2 drops of the molish reagent. The solution is then poured slowly into test tube containing 2ml of concentrated sulphuric acid. So that two layers form. The formation of a purple product at the interface of the 2 layers indicated the presence of carbohydrates.

Test for protein

It is used to determine the presence of peptide bounds in protein. To 3ml of test sample add 3% sodium hydroxide and few drops of 1% copper sulphate. The solution turns from blue to violet (purple) indicated the presence of protein.

Test for starch

Mix 3ml test solutions. A few drops of dilute iodine solutions. Blue colour appears. It disappears on boiling and reappears on cooling indicated the presence of starch.

Test for steroids

To 2ml of extract add 2ml chloroform and add 2ml concentrated sulphuric acid. Shake well; chloroform layer appear red and acid layer show greenish yellow fluroscence which indicated the presence of steroid.

Test for amino acid

To 5ml of test sample solution add a few drops of 40% sodium hydroxide &10% lead acetate boiled the solution formation of black precipitate indicated the presence of amino acid.

Test for glycosides

To the extract add Glacial acetic acid, few drops of 5% ferric chloride and concentrated sulphuric acid are added and observed for a reddish brown coloration at the junction of the two layers, and bluish colour in the upper layer which indicated the presence of glycoside

Test for flavonoid

To 2ml of extract add few drops of ammonia solution. A yellow coloration was indicated for the presence of flavonoid

Test for alkaloid

To 0.5g of each extract add 5ml of 1% aqueous hydrochloric acid and kept in water bath, 1ml of filtrate is to be treated with Mayer's reagent. Formation of a yellow coloured precipitate indicated the presence of alkaloids

Test for tannin

To 0.5ml of extract 1ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannin and green black for catecholic tannin.

Test for saponin

To 1ml extract add 2ml distilled water and shake it persistent foam indicated the presence of saponin

Test for terpenoid

2ml of extract was mixed with 2ml chloroform in a test tube. To this 3ml concentrated sulphuric acid was carefully added alone the wall of the test tube, an interface with reddish brown colouration confirmed the presence of terpenoid

Test for gums

To 1ml of extract add 3ml of dil.hydrochloric acid, fehlings solution is added drop by drop, till red coloration visualize the presence of gums.

Biochemical Analysis

The biochemical analysis is performed on *Carica papaya*, Linn. The powder of selected plant seed where tested for estimation of carbohydrate (Anthrone method), protein (Lowry's method) by Sadasivam and Manikam (2008) [14] and starch.

Estimation of carbohydrate by anthorne method

100mg of dried powdered seed was hydrolysed in a boiling water bath for 30 minutes with 80%ethanol in water and centrifuge 8000g for 15 minutes. Preserved 4ml of supernatant. From it 1ml of the supernatant dried and dissolved in 50 ml distilled H2O. Anthrone reagent is prepared by mixing 300gm anthrone with 150ml ice cold H2SO4.0.2ml of sample made up to 1ml with distilled H2O add 4ml of anthrone reagent and rapidly cooled in ice bath. OD values which was read at 630nm using Bovine Serum Albumin (BSA).

Protein estimation lowry's method, 1951

1gm of the sample weighed and grained well with a pestle and motor in 1ml of the buffer. Add 5% of TCA and kept in cooled for 1hour. Centrifuge at 3500rpm for 20 minutes. Dissolved precipitated protein in 0.1N NaOH (Reagent A). 0.5% Cuso4 in 1% potassium sodium tartarate (Reagent B). 50ml of reagent A and B was mixed prior to use and reagent C was obtained. Which was immediately added in to the test

tube was mixed well and allowed to stand for 10minutes. 0.5ml of reagent D (folin ciocaltau reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes to develop blue colour. OD values read at 660 nm using glucose as standard and calculate the amount of protein.

Estimation of starch by hedge and hotreiter, 1962

The total soluble carbohydrates from the selected sample were extract and estimated by the Anthrone reagent method. Using glucose as standard at 620nm in spectrophotometer the values were expressed as mg/100gm on dry weight basis.

Result

The present investigation was carried to find out the phytochemical and biochemical constituents present in *Carica papaya* Linn. Phytochemical analysis in ethanolic extract of *Carica papaya* seed showed positive result for alkaloids, flavonoids, carbohydrate, protein, steroids, saponin, terpenoids and glycosides. The chloroform extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, alkaloid, tannin, and saponin. The acetone extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, and alkaloids. Physicochemical analysis of *Carica papaya* shows the moisture content is 9.5% and ash content is 15%. Biochemical analysis on *Carica papaya* revealed 18.75 mg of carbohydrates, 7.28 mg of protein and 16.87mg of starch.

Phytochemical screening of *Carica papaya*

Table 1

Phytochemicals	Ethanol extract	Chloroform extract	Acetone extract
Alkaloids	+	+	+
Flavonoids	+	_	_
Carbohydrates	+	+	+
Proteins	+	+	+
Starch	+	+	_
Amino acids	_	+	+
Steroids	+	_	_
Tannins	_	+	_
Saponins	+	+	_
Terpenoids	+	_	_
Glycosides	+	_	_
Gum	_	_	_
+ indicates= Present; - indicates= Absent			

Physicochemical analysis in dry seeds of *Carica papaya*

Table 2

Sl. No	Parameter analysed	*Carica papaya* %
1	Moisture content	9.5%
2	Total ash	15%

Carbohydrates, proteins and starch content in the seeds of *Carica papaya*

Table 3

Sl. No	Biochemical constituent	*Carica papaya*
1	Carbohydrate	18.75 mg/g
2	Protein	7.28 mg/g
3	Starch	16.87 mg/g

Composition of carbohydrate, protein, and starch in *Carica papaya*

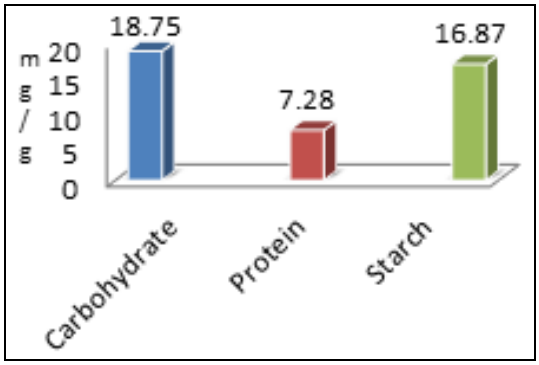

Fig 1

Discussion

Medicinal plants are a rich source of bioactive phytochemicals. Phytochemicals accumulate in different parts of the plant such as in the roots, stems, leaves, flowers, fruits and seeds (Costa *et al*., 1999). Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines, pyrimidines, nucleic acids, and chlorophylls. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, saponins, phenolics, flavonoids and glucosides. (Hahn, 1998) [4].

The ethanolic extract of *Carica papaya* seed contain carbohydrate, protein, alkaloid, flavonoid, starch and saponin. Similar result were reported by E. I Okoye (2011) and Delphin *et al*., (2014) [2]. Carbohydrate are a common source of enery in living organism.

Plant steroids constitute a diverse group of natural products. Steroids have been reported to have antimicrobial properties, analgesic properties and act on central nervous activities. In the present study the ethanolic extract of *Carica papaya* seed contain steroids. L Lohidas *et al*., (2015) [8] reports the steroids are absent in ethanolic extract of *Carica papaya*.

Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, antibacterial, and anti hyperglycemic activities (Wink *et al*., 1998) [16]. Alkaloids are present in ethanol, chloroform and acetone extract. The presence of alkaloids in seed show that this plants is effective to malaria, since alkaloid consists of quinine which is anti-malaria. (Eke *et al*., 2014) [3]

Acetone extract of *Carica papaya* seed contain carbohydrate, protein, starch, amino acid, alkaloids, tannin, saponin, terpenoids, and glycosides. Lohidas *et al*., (2015) [8] reports the saponin and tannins are absent in acetone extract of *Carica papaya*.

Chloroform extract of *Carica papaya* seed contain alkaloids and tannin. Similar result were reported by O N Eke *et al*., (2014) [3] and J Lohidas *et al.,* (2015) [8]. In my study proteins are present in chloroform extract. J Lohidas *et al*., (2015) [8] reported that proteins are absent in chloroform extract.

The biological functions of flavonoids include protection against allergies, inflammations, ulcer, vineses and tumours (Okwu and Okwu, 2004). Flavonoids represent the common and widely distributed group of plant phenolics. Ethanolic extract of *Carica papaya* contain flavonoid.

Tannins have astringent properties which hasten the healing of wounds and inflamed mucous membrane. The presence of tannins in the seed of *Carica papaya* can support its strong use for healing of wounds, ulcers, hemorrhoids, frost-bites and burns in herbal medicine (Igboko *et al*., 1983) [5]. Chloroform extract of *Carica paya* seed contain tannin.

Eke *et al*., (2014) [3] reported that saponin is absent in chloroform extract. In my study saponin present in it. The saponins constituents are responsible for the possession of haemolytic property.

Glycosides are medicines for treating heart failure and certain irregular heartbeats. Ethanolic extract of *Carica papaya* seed contain glycosides. Similar result were reported by Rasha saad *et al*., (2014). Physicochemical analysis of *Carica papaya* showed the moisture content (9.5%). It was higher than the same plant species seed (6.39%) reported by Olorode *et al*., (2014) [11]. *Carica papaya* contain total ash content (15%). It was higher than the same plant species seed (9.56%) of ash content (Olorode *et al.,* 2014) [11].

The biochemical analysis on *Carica papaya* revealed 18.75 mg of carbohydrates, 7.28 mg of protein and 16.87 mg of starch. Carbohydrate is important in our diet as fibre which helps to maintain a healthy digestive system. Protein is essential for the maintenance and building of body tissues and muscles. Starch is the most important carbohydrate in the human diet. Starch molecule consists of a number of glucose units. Starch plays an important role in production of antibiotics, vitamins, dialysis solution, and enteric nutrition.

Summary

The present investigation was carried to find out the phytochemical and biochemical constituents present in *Carica papaya*. The ethanolic extract of *Carica papaya* seed contains carbohydrate, protein, flavonoids, alkaloids, saponin, steroids, and glycosides. The chloroform extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, alkaloid, tannin, and saponin. The acetone extract of *Carica papaya* seed contain carbohydrate, protein, amino acid, and alkaloid.

The moisture content in dry seeds of *Carica papaya* is 9.5% and ash content 15%. The physicochemical parameters such as ash value and determination of moisture loss was used to determine the quality and purity of a crude drug. The biochemical analysis on *Carica papaya* revealed 18.75 mg of carbohydrates, 7.28 mg protein and 16.87 mg starch.

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