



Quantitative Analysis of Phytoconstituents of Hydroalcoholic Extract of *Carica Papaya* Leaf

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 29 Oct 2023	<i>Carica papaya</i> (<i>C. papaya</i>) leaves have been used in folk medicine for centuries. Several reports are available for use of <i>Carica papaya</i> leaves in increasing blood platelet count in dengue hemorrhagic fever. Recent studies also reported that <i>Carica papaya</i> leaves exhibits anti-tumor activity and immunomodulatory effects. Papaya leaf extract is reported to contain carbohydrates, amino acids, sterols, saponin glycosides, iridoids, flavonoids, phenolics, anthraquinones, triterpenes and alkaloids. The present investigation demonstrates the concentration of secondary metabolites in the hydroalcoholic extract of <i>Carica papaya</i> leaves. The total phenolics, total alkaloid and total flavonoid content were calculated by Folin-Ciocalteu colorimetric method, Bromocresol green colorimetric method and aluminum chloride colorimetric method respectively.
CC License CC-BY-NC-SA 4.0	Keywords: <i>Carica papaya</i> , total phenolic content, total alkaloid content, total flavonoid content

1. Introduction

Carica papaya Linn. (Caricaceae family) is a well-known tree for its nutritional value worldwide. It is also known as paw-paw or papaya. It has economic value for its medicinal properties. The countries like Sri Lanka, Malaysia and India, uses papaya leaf juice to treat thrombocytopenic conditions in Dengue patients. In India the papaya leaf juice is used to increase the platelet count from ancient times.¹

Different parts of papaya like seeds, fruits, latex and leaves are reported to have immunomodulatory effects along with anti-ulcer properties^{2,3}. The seeds of papaya fruits are effective to treat bleeding piles and enlargement in liver and spleen^{4,5,6}. The stem bark of *C. papaya* is helpful in recovering from hemolysis and jaundice⁵. The papaya leaf extract has shown wound healing and an antisickling property^{7,8,9}.

The countries like Lao, Cambodia, and Vietnam use latex of papaya to treat skin diseases like eczema and psoriasis^{10,11}. It is also reported to have antidiabetic, antimalarial, anticancer and antifungal properties^{12,13,14,15}. Various in vitro studies on different parts of *Carica papaya* exhibit antioxidant activity^{16,17,18,19}.

Carica papaya contains the enzyme papain in the fruits, stem and leaves. The seeds of papaya contain oil rich in flavonoids and fruit contains monoterpenoids. Latex of *C. papaya* contains enzymes like cysteine endopeptidases, chitinase, and glutaminyl cyclase^{20,21}.

Papaya leaf extract is reported to contain carbohydrates, amino acids, sterols, saponin glycosides, iridoids, flavonoids, phenolics, anthraquinones, triterpenes and alkaloids. *C. papaya* fruits contain benzyl glucosinolate and benzyl isothiocyanate²⁰.

The leaves of papaya have been shown to contain many active components such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, phenolic acids (trans-ferulic acid, para-coumaric acid, caffeic acid, vanillic acid), flavonoids (myricetin, kaempferol, quercetin), cyanogenic glucosides and glucosinolates²¹. Several reports are available for use of *Carica papaya* leaves in increasing blood

platelet count in dengue hemorrhagic fever^{22,23}. Recent studies also reported that *Carica papaya* leaves exhibits antisickling, anti-tumor activity and immunomodulatory effects^{24, 25, 26}

2. Materials And Methods

All chemicals used were of analytical grade. Quercetin, Gallic acid, Atropine, anhydrous sodium carbonate (Na_2CO_3), Aluminum chloride (AlCl_3), Folin–Ciocalteu reagent, Sodium nitrite (NaNO_2), Bromocresol green (BCG), sodium phosphate, Dragendorff's reagent, mercuric chloride, potassium iodide, iodine was purchased from Sigma–Aldrich. Ethanol, methanol, citric acid, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH) was purchased from Merck.

Collection and authentication of plant

The leaves of *Carica papaya* were collected from a local area of Dehra, district Kangra (Himachal Pradesh), India in the month of July-August, 2020 depending upon its easy availability. The leaves were authenticated by Dr. Anjula Pandey, principal scientist at ICAR- National Bureau of Plant Genetic Resources, National Herbarium of cultivated plants, New Delhi against a voucher specimen NHCP/NBPGR/2013-24, NHCP/NBPGR/2013-25, NHCP/NBPGR/2013-23. The leaves were thoroughly washed with water to remove the impurities and air dried.

Preparation of sample

The fresh leaves of *Carica papaya* were macerated with hydroalcoholic mixture for 24 hours with occasional shaking. The extracts were then filtered and concentrated to dryness. The resultant extracts were stored for further study.

Phytochemical screening

The hydroalcoholic extract of *Carica papaya* leaves were tested for the presence of alkaloids, steroids, tannins, saponins and glycosides using reported methods²⁷. The qualitative results are expressed as (+) for the presence and (–) for the absence of phytochemicals.

Quantitative analysis

Total phenolic content

Total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method^{28,29,30,31} with some modifications. Standard stock solution of $100\mu\text{g/ml}$ of Gallic acid was prepared by dissolving 10mg of Gallic acid in methanol in 100ml volumetric flask.

Preparation of test solution:

1 ml from prepared *C. papaya* extract was taken in 25ml volumetric flask and 10ml water and 1.5ml of Folin–Ciocalteu reagent (FCR) was added. The above mixture was kept for 5min and then 4ml of 20% Na_2CO_3 was added and volume was made up to 25ml with distilled water and mixture was kept up to 30 min and absorbance of blue color was measured at 739nm.

From the stock solution of standard Gallic acid 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75ml were taken which gave 50, 75, 100, 125, 150, $175\mu\text{g/ml}$ concentration respectively. These solutions were taken in 25ml volumetric flask and 10ml water and 1.5ml of FCR was added. The above mixture was kept for 5min and then 4ml of 20% Na_2CO_3 was added and volume was made up to 25ml with distilled water and mixture was kept up to 30 min and absorbance of blue color was measured at 739nm using UV-visible spectrometer Lab India, UV-3000+. Calibration curve of concentration vs. absorbance was plotted. From the graph total phenolic content were measured.

Total flavonoid content

Total flavonoid content was analyzed using the aluminium chloride colorimetric method^{32,33} with some modifications.

Preparation of Standard solution for Quercetin

Standard stock solution of Quercetin $1000\mu\text{g/ml}$ was prepared by dissolving 10 mg of Quercetin in 10 ml ethanol. From stock standard solution, aliquots were prepared of concentration ranging 20 - $100\mu\text{g/ml}$.

Sample preparation

An aliquot (1 ml) of extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml 5 % NaNO_2 were added. After 5 min, 0.3 ml 10 % AlCl_3 was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm using UV-visible spectrometer Lab India, UV-3000+. The sample was analyzed in triplicates.

Total alkaloid content

Total alkaloid content was quantified by bromocresol green colorimetric method³⁴. Bromocresol green solution was prepared by dissolving 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water and the solution was diluted to 1000 ml with distilled water.

Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na₂HPO₄ in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1 mg of pure Atropine in 10 ml distilled water.

Preparation of standard curve

Accurately measured aliquots from 0.4 to 1.2 ml of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform.

The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-visible spectrometer Lab India, UV-3000+ against the blank prepared as above but without Atropine.

3. Results and Discussion

Phytochemical screening

Table 1: Qualitative analysis of *Carica papaya* leaves

Sr. no.	Tests	Hydroalcoholic extract of <i>C. papaya</i> leaves
1	Carbohydrates	+
2	Proteins	+
3	Alkaloids	+
4	Tannins and Phenolics	+
5	Saponin glycosides	-
6	Cardiac glycosides	-
7	Anthraquinone glycosides	-
8	Flavonoids	+
9	Steroids	-

The phytochemical screening of hydroalcoholic extract of *C. papaya* leaves shows the presence of carbohydrates, proteins, alkaloids, flavonoids, tannins and phenolics.

Total Phenolic content

Estimation was carried out by taking Gallic acid as a standard and absorbance was taken on the UV-visible spectrometer Lab India, UV-3000+.

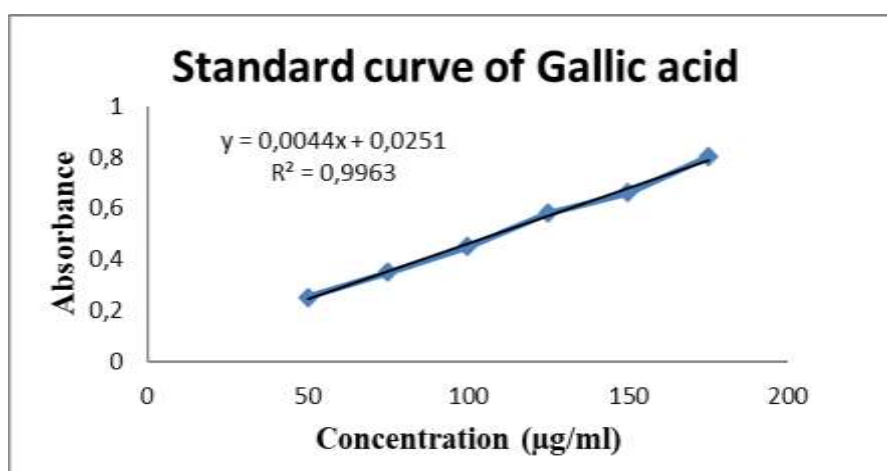


Fig. 1: Standard curve of Gallic acid

Total Phenolic content of hydroalcoholic extracts of *Carica papaya* leaves was found to be 13.06 ± 0.8 % w/w.

Total flavonoid content

Total flavanoid content of hydroalcoholic extract of *Carica papaya* leaves was estimated by AlCl₃ colorimetric method and the results was 0.2 ± 0.4 % w/w.

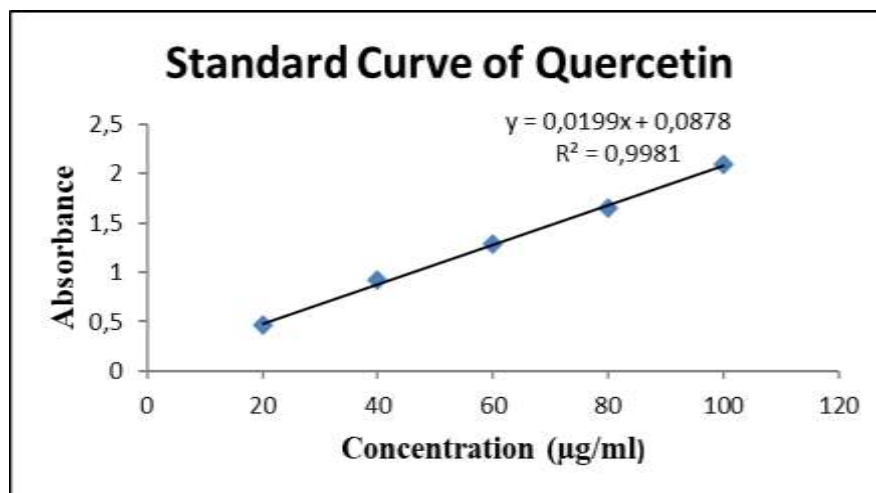


Fig. 2: Standard curve of Quercetin

Total alkaloid content

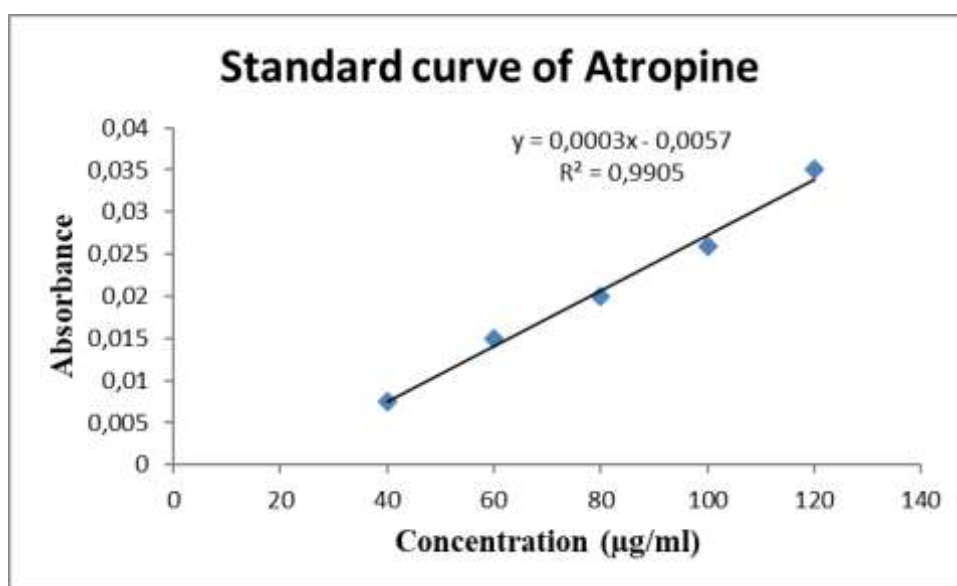


Fig. 3: Standard curve of Atropine

Total alkaloid content of hydroalcoholic extract of *Carica papaya* leaves was estimated by Bromocresol green colorimetric method and the results was 0.073 ± 0.6 % w/w.

Table 2: Quantitative analysis of *Carica papaya* leaves

Quantitative analysis	% w/w
Total Phenolics	13.06 ± 0.8
Total flavonoid	0.2 ± 0.4
Total alkaloid	0.073 ± 0.6

4. Conclusion

The *Carica papaya* is a known drug from the ancient times used for diseases like dengue, liver diseases. The present study explores the phytochemical characteristics of *Carica papaya* leaves. This study was carried over to evaluate its chemical attributes by modern scientific way.

In present study, the hydroalcoholic extract of *Carica papaya* leaves was evaluated for its constituents. The study showed the presence of carbohydrates, tannins, phenolics, alkaloid, proteins and flavonoids. The quantitative analysis showed the 13.06 ± 0.8 % concentration of total phenolics, 0.073 ± 0.6 % concentration of total alkaloid and 0.2 ± 0.4 % concentration of total flavonoids.

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