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In Vitro Phytochemical Screening and Antioxidant Activity of *Carica papaya* Plant Parts Collected from Lahore, Pakistan

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ABSTRACT

Our research focuses on the antioxidant activity and presence of phytoconstituents, qualitatively and quantitatively in water and ethanolic extracts of Carica papaya leave, stem and fruit by using standard protocols. Qualitative phytochemical analysis was done by biochemical testing, quantitative measurements were taken spectrophotometrically and antioxidant activity of extracts was checked by DPPH radical scavenging activity. The quantified results of our study revealed that ethanol is appropriate solvent system that contains high quantity of bioactive compounds like phenols, tannins, carotenoids, steroids and flavonoids than water solvent system. Statistical analysis for antioxidant activity disclosed that ethanolic leave, fruit and stem extracts remove free radicals 95%, 68% and 26%, respectively in comparison with water extracts of leave, fruit and stem that kills 7.11%, 3.06% and 2.26%, respectively. Ascorbic acid was taken as standard that kills 98% free radical species. To conclude, extracts in ethanol solvent system exhibits prominent antioxidant activity and also contains the high amount of phytochemicals.

Keywords: *Carica papaya*, Phytochemicals, Antioxidant activity, Statistical analysis

INTRODUCTION

Carica papaya has admirable restorative properties for treatment of various diseases. The distinctive parts of *Carica papaya* plant including leaves, seeds, latex and natural product shown to have therapeutic worth [1]. *Carica papaya* is related to the family of caricaceae and generally termed as paw-paw [1]. It is cultivated in distinct georgic locations of the world, inclusively hot zone of America and Europe, India and also in sub-tropical regions of the world. Essentially it is fugitive Indian tree [2]. It is extensively matured in Punjab and Sindh, Pakistan.

It holds many useful compounds that impart countless health benefits [3]. Several distinct secondary metabolites like alkaloids, steroids, terpenoids and phenolic components are enclosed by *Carica papaya* [4]. They have shown various pharmacological actions such as wound healing, diuretics, antitumor, anti-amoebic, antibacterial, anti-inflammatory and anti-hypersensitivity and many more [5].

There are three types of efficacious antioxidants are present in enormous amount in papaya namely vitamin C, vitamin A and vitamin E [5]. Proteolytic chemicals like papain and chymopapain that shows antiviral, antifungal and antibacterial properties, along with these antioxidant agents diminish the harshness of the inflammatory conditions like asthma, osteoarthritis, and rheumatoid joint pain [1]. Several distinct secondary metabolites like alkaloids, steroids, terpenoids and phenolic revealed the antioxidant activity that have displayed the repressive response towards the creation of volatile species, appeared as a result of regular uptake and breakdown of various compounds due to cell metabolism [4].

OBJECTIVE

The purpose of present research was to examine the phyto constituents present in different parts of *Carica papaya* qualitatively and quantitatively and to investigate the antioxidant activity.

MATERIALS AND METHODS

Collection of plant material

Natural and aseptic leaves, fruit and young stem of *Carica papaya* were collected very carefully from Lahore, Pakistan in October 2016. Collected plant parts were cleaned properly.

Preparation of extracts

Six extracts were prepared through cold maceration method in two solvent systems namely water and ethanol. All the six obtained crude extracts were named as CP.L.W (*Carica papaya* leave in water), CP.L.E (*Carica papaya* leave in ethanol), CP.F.W (*Carica papaya* fruit in water), CP.F.E (*Carica papaya* fruit in ethanol), CP.S.W (*Carica papaya* stem in water) and CP.S.E (*Carica papaya* stem in ethanol) respectively. 95% ethanol was used in ethanolic fractions of CP.L, CP.F and CP.S containing 30 g, 40 g and 60 g of fresh sample providing 1.2 g, 2.3 g and 1.06 g of dry extracts respectively. Likewise water fractions of CP.L, CP.F and CP.S were 40 g, 120 g and 75 g providing 1.8 g, 1.2 g and 1.4 g, respectively.

Phytochemical screening

Qualitative analysis

Different biochemical tests performed with the extracts of leaves, fruit and young stem of *Carica papaya* to determine the primary and secondary metabolites by the help of standard protocols [6].

Tests for carbohydrates

A small fraction of crude extract was taken and mixed with 2 ml of Molisch's reagent. The mixture was rattled well. Hereafter, 2 ml of concentrated H_2SO_4 was added carefully on the walls of test tube. Purple coloured ring appeared at the interphase indicated the presence of carbohydrates.

Test for phenol and tannins

A fraction of crude extract was taken and mixed with 2 ml of 2% solution of $FeCl_3$. Presence of phenol and tannins was indicated by the appearance of blue-green colour.

Test for flavonoids

A fraction of crude extract was taken in a test tube and mixed with 2 ml of 2% solution of NaOH. A deep yellow colour was appeared which became colourless when few drops of diluted acid was added. This test indicates the presence of flavonoids.

Test for saponins

A fraction of crude extract was mixed with 5 ml of distilled water in a test tube and rattled quickly. A stable foam was formed in a test tube indicates the presence of saponins. All the extracts manifested the presence of saponins except CP.L.E and CP.F.E.

Test for cardiac glycosides

A small fraction of crude extract was taken and mixed with 2 ml of glacial acetic acid in a test tube containing 1-2 drops of 2% solution of $FeCl_3$. Another test tube was taken and 2 ml of concentrated H_2SO_4 was added into it. The former test tube containing the mixture was poured into it carefully. Presence of glycosides was indicated by the appearance of a brown ring at interphase.

Test for steroids

A fraction of crude extract was taken in a test tube and mixed with 2 ml of chloroform. Then along the sides of the test tube 2 ml of concentrated H_2SO_4 was added. Presence of steroid was indicated by the appearance of red colour in the lower chloroform layer.

Test for terpenoids

A small fraction of crude extract was taken in a test tube. Then 2 ml of chloroform was added in it and evaporated

to dryness. Hereafter, 2 ml of concentrated H_2SO_4 was poured into it and heated carefully for 2 min. Appearance of greyish colour in the test tube indicated the presence of terpenoids.

Test for alkaloids

A fraction of crude extract was taken and 2 ml of 1% HCL was added in it and then heated mildly. Few drops of Wagner's reagent were added in the mixture. Presence of alkaloids was indicated by the turbidity of emerging precipitates.

Test for proteins

A fraction of crude extract was taken in a test tube and 2 ml of 0.2% solution of ninhydrin was added in it. It was heated until boiling. Presence of amino acids and proteins was determined by the appearance of violet colour.

Quantitative analysis

Quantity of bioactive metabolites in ethanolic and aqueous extracts of *Carica papaya* leaves, fruit and stem was measured in triplicate with the help of standard procedures.

Determination of tannins

20 mg of *Carica papaya* crude water and ethanolic extracts of leaves, stem and fruit was dissolved in 1 ml of ethanol. From this concentration 0.1 ml of each ethanolic extract was taken and 1 ml of distilled water, 0.5 ml Folin-Denis reagent, 1 ml of sodium bicarbonate solution and 7.4 ml of distilled water was added in each sample to make the solution of 10 ml. After that, absorbance was taken at 755 nm within 30 min. on spectrophotometer. Standard curve was drawn by taking tannic acid as standard [7].

Determination of phenols

0.25 ml of each ethanolic and water extract was taken and 0.25 ml of Folin-Ciocalteu reagent and 2 ml of sodium bicarbonate solution was pipetted step by step in each extract separately. The reaction mixture was heated for 5 min in incubator at 50°C. Then centrifuged for 10 min and absorbance was taken at 765 nm. Gallic acid was taken as standard [6].

Determination of alkaloids

0.1 mg of crude ethanolic and water extract of each sample was taken and 1 ml DMSO (dimethyl sulphoxide), 1 ml of HCl, 1 ml of BCG (Bromocresol green) solution, 5 ml of phosphate buffer and 1ml of chloroform was pipetted with micropipette one by one in each sample successively. The reaction mixture was shaken vigorously to dissolve the reagents and pH was maintained at 7.0. After centrifugation of 10 min absorbance was taken at 470 nm. Atropine was taken as standard [8].

Determination of flavonoids

0.25 ml of ethanolic and water extract of each sample was taken and 0.75 ml of ethanol, 0.05 ml of aluminium chloride and potassium acetate and 1.4 ml of distilled water was added in each sample subsequently. The reaction mixture was kept at room temperature for 30 min and then centrifuged for 10 min. After centrifugation absorbance was measured at 415 nm. Quercetin was used as standard [9].

Determination of steroids

1ml of ethanolic and water extract of each sample was taken and 2 ml of sulphuric acid, 2 ml of 0.5% solution of ferric chloride and 0.5 ml of 0.5% solution of potassium iodide were added one by one in all samples, respectively. Then the reaction mixture was heated in a water bath controlled at 70 ± 20°C for 30 min with unfrequently shaking then absorbance was measured at 780 nm on spectrophotometer.

Determination of carotenoids

1 ml of ethanolic and water extract of each sample was taken in centrifuge tubes and centrifuged for 10 min. Absorbance was taken at the wavelength of 470 nm, 649 nm and 665 nm on spectrophotometer. Final readings were calculated by using standard formulas designed for the determination of carotenoids that are given below:

$C_a=13.95A_{665}-6.88A_{649}$

$C_b=24.96A_{649}-7.32A_{665}$

$$C_{x+c}=\frac{1000A_{470}-2.05C_a-114.8C_b}{245}$$

Antioxidant activity of Carica papaya

1 ml of ethanolic and water extract of each sample was taken and 1 ml of DPPH was pipetted in all the samples very carefully. The reaction mixture was kept in dark for 24 h. After incubation period, absorbance was measured at 517 nm. Standard curve was drawn by taking ascorbic acid as standard [10].

RESULTS

Qualitative phytochemical analysis

Current study unfolded the presence of primary and secondary phytoconstituents in both water and ethanolic extracts of *Carica papaya* leave, stem and fruit. Some phytochemicals are present in both solvent systems; some compounds are present in water while others are present in ethanolic solvent system.

Table 1: Phytoconstituents in water and ethanolic extracts of *Carica papaya* leaves, stem and fruit

Phytochemicals	CP.L.W	CP.L.E	CP.S.W	CP.S.E	CP.F.W	CP.F.E
Carbohydrates	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	+ve	-ve	+ve	+ve	+ve	-ve
Proteins	+ve	-ve	+ve	+ve	+ve	+ve
Phenol	-ve	+ve	+ve	-ve	-ve	-ve
Tannins	-ve	+ve	+ve	-ve	-ve	-ve
Glycosides	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	-ve	-ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve
Steroids	+ve	-ve	+ve	-ve	+ve	+ve
Terpenoids	-ve	+ve	+ve	-ve	+ve	+ve

CP.L.W: *Carica papaya* Leaves in Water; CP.L.E: *Carica papaya* Leaves in Ethanol; CP.S.W: *Carica papaya* Stem in Water; CP.S.E: *Carica papaya* Stem in Ethanol; CP.F.W: *Carica papaya* Fruit in Water; CP.F.E: *Carica papaya* Fruit in Ethanol; positive result (+ve); negative result (-ve)

Quantitative phytochemical analysis

This study revealed that bioactive compounds are present in high quantity in ethanolic solvent system rather than water solvent system as shown in Table 2. These compounds have great medicinal importance as they act as antibacterial, antifungal, anti-inflammatory, anti-malarial, anti-cancerous and detoxifying agents.

Table 2: Phytochemical characterization of *Carica papaya*

Solvent extraction mg/g of dry extract	Phenols	Tannins	Alkaloids	Flavonoids	Carotenoids	Steroids
CP.L.E	35.29 ± 4.28	23.26 ± 2.25	61.25 ± 7.58	70.67 ± 9.56	25.26 ± 1.99	5.26 ± 1.06
CP.L.W	1.29 ± 0.28	5.26 ± 1.29	8.26 ± 1.28	3.09 ± 0.98	1.26 ± 0.29	1.08 ± 0.095
CP.F.E	17.26 ± 2.08	45.16 ± 1.26	7.29 ± 1.09	61.98 ± 4.45	27.29 ± 2.29	17.28 ± 2.26
CP.F.W	8.29 ± 1.11	6.25 ± 0.25	2.06 ± 0.14	3.06 ± 1.09	1.26 ± 0.009	0.26 ± 0.085
CP.S.E	37.29 ± 3.09	28.29 ± 2.35	16.28 ± 2.28	17.26 ± 1.28	7.16 ± 1.09	1.05 ± 0.005
CP.S.W	0.9 ± 0.25	4.26 ± 1.28	0.66 ± 0.09	1.29 ± 0.09	2.29 ± 0.25	6.29 ± 1.20

Note: Each value is the average of three analyses (Mean) ± standard deviation (SD)

CP.L.W: *Carica papaya* Leaves in Water; CP.L.E: *Carica papaya* Leaves in Ethanol; CP.S.W: *Carica papaya* Stem in Water; CP.S.E: *Carica papaya* Stem in Ethanol; CP.F.W: *Carica papaya* Fruit in Water; CP.F.E: *Carica papaya* Fruit in Ethanol

Antioxidant activity of Carica papaya

By the help of DPPH scavenging activity, it is revealed that ethanolic leave, fruit and water extracts have shown prominent antioxidant activity than water extracts that is clearly visible in Table 3 and also in graph.

Table 3: DPPH scavenging activity of *Carica papaya* (%)

Plant Parts and Solvent System	Percentage (%)
CP.L.E	95.26 ± 5.26
CP.L.W	7.11 ± 2.29
CP.F.E	68.36 ± 1.26
CP.F.W	3.06 ± 0.86
CP.S.E	26.29 ± 1.26
CP.S.W	2.26 ± 0.26
Ascorbic Acid	98.29 ± 5.26

CP.L.W: *Carica papaya* Leaves in Water; CP.L.E: *Carica papaya* Leaves in Ethanol; CP.S.W: *Carica papaya* Stem in Water; CP.S.E: *Carica papaya* Stem in Ethanol; CP.F.W: *Carica papaya* Fruit in Water; CP.F.E: *Carica papaya* Fruit in Ethanol

DISCUSSION

Tannins are present in chloroform extract, saponins, glycosides, flavonoids and carbohydrates in ethanolic extract, anthraquinnon and alkaloids in ethanolic and chloroform extracts of *Carica papaya* fruit and seed [11]. Not all the phytochemicals are present in all solvent systems. Present consideration revealed that some phytochemicals are present in both water and ethanolic solvent systems, some compounds are present in water while others are present in ethanolic solvent system.

Phytochemical quantification of *Carica papaya* methanolic leaf extracts showed that phenolics are present in remarkable amount than flavonoids and alkaloids respectively [12]. Our study of spectrophotometric analysis to quantify the phytochemicals highlighted that phenols, tannins, carotenoids, steroids and flavonoids are present in great amount in ethanolic extracts in comparison to water extracts.

Notable antioxidant activity is displayed by methanolic extract of young leaves of *Carica papaya* as compared to other parts [10]. Present research unfolded that ethanolic extract possess the maximum antioxidant activity than water extracts. The array of distribution of antioxidant activity was ethanolic leave extract>ethanolic fruit extract>ethanolic stem extract>leave extract with water>fruit extract with water>stem extract with water.

CONCLUSION

Carica papaya leave, fruit and stem extracts in ethanolic solvent system exhibits prominent antioxidant activity and also contains the high amount of phytochemicals than water solvent system. Quantified results showed that some bioactive compounds are also present in crude extracts of *Carica papaya* in average amount that were not identified by the qualitative phytochemical screening.

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