In-vitro Phytochemical Screening And Antioxidant Potential of Ethanolic Extract of Carica Papaya Leaf

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Abstract

Phytochemicals, which are biologically active compounds and a powerful group of plant chemicals, are thought to stimulate the immune system, along with antioxidants, which are molecules that prevent the oxidation of other molecules by inhibiting or generating oxidising chain reactions and thus prevent diseases. The total alkaloid content (TAC) and total flavonoid content (TFC) were evaluated using the Atropine technique, and antioxidant activity was determined using the Free radical scavenging activity by stable DPPH radical, Hydrogen peroxide scavenging method, and Superoxide radical scavenging method.The presence of bioactive substances such as alkaloids, carbohydrates, amino acids, and TAC and TFC of ethanolic extract were examined and showed the maximum quantity of phytochemicals, TPC and TFC, and antioxidants when compared to other solvents.After the isolation and purification, certain bioactive compounds can be used to develop natural and promising medications for drug development.

Keywords:*Carica papaya Linn*, Phytochemicals, Antioxidants, DPPH, Hydrogen peroxide scavenging, Superoxide radical scavenging.

1 Introduction

Medicinal plants have been shown to have antioxidant, antibacterial, and antimicrobial properties, which have been attributed to the presence of secondary metabolites such as alkaloids, flavanols, flavones, tannins, saponins, steroids and so on.(1) Many studies have also found that medicinal plants contain a wide range of free radical scavenging molecules that are effective against bacterial diseases. According to current estimates, approximately 80 million people worldwide still rely on plants for their health, and approximately 95 percent of modern drugs have been isolated from traditional medicinal plants.(2) According to the World Health Organization (WHO), approximately 80% of rural patients seek alternative treatment options in many countries and rely on various medicinal plants to cure various diseases.

Carica papaya are herbaceous plants that belong to the family Caricaceae.(3) It is a dicotyledonous, polygamous, and diploid species with 31 species in four genera, three of which are native to the United States (Carica, Jacaritia, and Jarilla) and one from equatorial Africa (Cylicomorpha). It is a fast growing herb with a short life span, and all parts of the plant, including the fruit, root, stem, seed, leaves, and flower, are used extensively in the treatment of various diseases. Papaya flowers are tiny, yellow, funnel-shaped, and can be solitary or clustered in the leaf axils. It has been used for centuries to treat coughs, bronchitis, chest asthma, and colds.(4) *C. papaya* plants have medicinal value because of the presence of natural metabolites found in the leaves, bark, and twigs that have anti-tumor and pesticidal properties. (5)The entire plant is being used to generate plant biomass, which will be used in the development of anticancer drugs.(6)(7) The plant is also used as a natural pesticide, and approval from the Food and Drug Administration is pending. (8)The plant's high concentration of natural self-defence compounds makes it highly resistant to insect and disease infestation. Traditionally, the flowers of *C. papaya* are used as a fresh vegetable to supplement our society's diet and support higher levels of individual growth. *C. papaya* flowers have medicinal properties that can help prevent cancer, improve digestion and appetite, and identify heart problems.(9) The tannins, flavonoids, and antioxidants found in papaya flowers have been shown to remove free radicals from the body. Consumption of papaya flowers assists the body in neutralizing free radicals and modulating the immune system, thereby increasing disease susceptibility.

An antioxidant is a substance that, even at low concentrations, significantly delays or prevents the oxidation of a substrate. Antioxidants have been linked to decreased cellular damage and cell malignant transformation.(10) Many plants have antioxidants that may contribute to the total antioxidant activity of plant materials, such as carotenoids, polyphenols, and traditional antioxidant vitamins C and E.(11) The major bioactive phytochemicals found in plants that have human health benefits are phenolic compounds.(12)

The current study is a preliminary phytochemical investigation of *C. papaya* leaves. Total alkaloid and total flavonoid contents of *C. papaya* leaves were determined, as well as antioxidant activity of different leaves extracts, in methanolic, chloroform, n-hexane, and aqueous extracts of *C. papaya*.

2 Materials and Methods

2.1 Chemicals and Reagents

Petroleum ether and Ethanol were obtained from Avantor Performance Materials (RANKEM) Pvt. Ltd, Gurgaon, India. Follinciocalteu's reagent, Ascorbic acid and Rutin were obtained from Merck, Pvt. Ltd. Sodium carbonate were obtained from Sd fine chemicals, Pvt. Ltd. Ethanol used were of Analytical grade. All other reagents, solvents and chemicals used were of laboratory reagent grade.

2.2 Plant Material

Plant material was collected from beganda of dhar district of nimar, Madhya Pradesh, India.

2.3 Extraction

2.3.1 Cold Maceration

C.papaya leaves was extracted using the cold maceration method; plant samples were properly collected, washed, and dried. Dried plant powder was extracted using different organic solvents with different polarities, namely petroleum ether and ethanol, and allowed to stand for 4-5 days in each. All unextractable matter was removed. Excess moisture was removed from the extract and collected in an airtight container after being transferred to a beaker and evaporated.(13) The extraction yield of all extracts was calculated.

2.4 Qualitative Phytochemical Estimation of Extracts

To determine the presence of phytochemicals such as carbohydrates, alkaloids, flavonoids, glycosides, proteins and amino acids, saponins, triterpenoids and steroids, tannins and other phenolic compounds, extracts of the leaves of the C. papaya (obtained in solvents such as Pet. Ether, and Ethanol) were screened.Specific qualitative phytochemical tests were carried out to identify the constituents both plant extracts.

2.5 Quantitative Phytochemical estimation

2.5.1 Total Alkaloids contents Estimation

2.5.1.1 Preparation of solutions

Bromocresol green solution was made by dissolving 69.8 mg bromocresol green in 1 ml of 0.1 N NaOH and 5 ml of distilled water and diluting the solution to 1000 ml with distilled water. The pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) was adjusted to 4.7 with 0.2 M citric acid to make phosphate buffer solution (pH 4.7). (42.02 g citric acid in 1 L distilled water). 1 mg pure atropine was dissolved in 10 ml distilled water to make an atropine standard solution. (14)

2.5.1.2 Preparation of standard curve

Transfer aliquots of atropine standard solution (20, 40, 60, 80, and 100 g / ml) to different separatory funnels. After that, add 5 mL of pH 4.7 phosphate buffer and 5 mL of BCG solution and shake with 1, 2, 3, and 4 mL of chloroform. The extracts were collected in a 10-ml volumetric flask and diluted with chloroform to adjust volume. The complex's absorbance in chloroform was measured at 470 nm in comparison to a blank prepared in the same manner but without atropine.(14)

2.5.2 Total Flavonoid Content Estimation

The total flavonoids content of different extracts was determined using rutintrihydrate (standard) and aluminum chloride in this method. The total flavonoid content was expressed as mg RE (rutintrihydrate equivalents)/g. Rutin concentrations of 20, 40, 60, 80, and 100 g/ml were prepared. Deionized water (2 mL) was added to the standard and extract (0.5 mL), followed by 75ul of sodium nitrite solution (5 % w/v). After 6 minutes, 0.5 mL sodium hydroxide (4% w/v) and 0.15 mL aluminium chloride (10% w/v) were added to the entire mixture solution. Finally, the absorbance of a UV-Visible spectrophotometer (510 nm) was measured. The total flavonoid content of various samples was determined versus a prepared water blank and expressed as mg/gm or g/mg of rutintrihydrate equivalents (RE) / g. (15)

2.6 Estimation of antioxidant potential

The DPPH radical scavenging method, Hydrogen peroxide assay (H_2O_2) and Superoxide scavenging assay method were used to determine the biological potential i.e. antioxidant activities of various crude extracts of plant material.

2.6.1 Free radical scavenging activity by stable DPPH radical

Based on electron-transfer, 2,2-Diphenyl-1-picryl-hydrazyl-hydrate free radical method used by Athavale et al. (2012) was followed. For the preparation of DPPH reagent, 0.1mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared. After that freshly prepared 1 mg/ml methanol solution of Ascorbic acid was used as standard. 1 mg of test sample (extracts) of /standard was taken with methanol to make 1mg/ml stock solution. Different volumes of extract/standard (20 – 100µl) were taken from stock solution in a set of test tubes and methanol was added to make the volume upto 1 ml. To this, 2 ml of 0.1 mM DPPH reagent was added and mixed thoroughly. After that absorbance was recorded at 517 nm after 10 minutes' incubation in dark at room temperature and for the preparation of control, 1 ml of methanol was mixed with 2 ml of 0.1mM DPPH solution and incubated for 10 min at room temperature in dark condition. Absorbance of the control was taken against methanol (as blank) at 517 nm.(16)

2.6.2 Hydrogen peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined using Ruch et al's method (1989). In phosphate buffer (pH 7.4), a solution of hydrogen peroxide (4 mmol/L) was prepared. The concentration of hydrogen peroxide was measured spectrophotometrically at 230nm. A hydrogen peroxide solution (0.6ml, 40 mmol/L) was mixed with sample extracts and a standard (4 ml). After 10 minutes, the absorbance at 230nm was measured in comparison to a blank solution containing phosphate

buffer but no hydrogen peroxide. The percentage of [H_2O_2] inhibition was calculated as (A_0-A_1)/A_0)100, where A_0 was the absorbance of the control and A_1 was the absorbance of the extracts and standard. (17)

2.6.3 Superoxide radical scavenging activity

Superoxide radical is generated by a non-enzymatic system produced by Nitro-blue tetrazolium (NBT) into purple colored formazan which is measured spectrophotometrically

Superoxide radical scavenging activity was determined by the reaction mixture containing 0.1 ml of NBT (1mg/ml in DMSO) and 0.3 ml of the extracts *Carica papaya* (10 µg/ml – 100 µg/ml) and standard in DMSO. 1 ml of alkaline DMSO was added to a final volume and the absorbance was measured at 560 nm. DMSO was used as blank and the reactant mixture without extract was used as control. The percentage of super oxide radical scavenging by the extracts and standard was calculated by following formula:(18)

$$\textbf{Superoxide radical scavenging activity (\%)} = [(A_0 - A_1)/A_0 \times 100],$$

where A_0 is the absorbance of the control and A_1 is the absorbance of NJE or the standard sample.

3 Results and Discussion

3.1 Percentage Yield

The dried powder of leaves of C. papaya were extracted, using two solvents of increasing polarities order. The percentage yield crude extracts was 0.464% for pet. Ether extract and 2.46 % for ethanol extract, depends on nature of the solvent. Result of percentage yields of the materials with different solvents were compared, it was noted that ethanol extract provided the better yield (2.46%).

3.2 Qualitative analysis of phytochemicals

Phytochemical analysis was done to analyze the chemical constituents present in the pet. Ether and ethanol extracts of fresh leaves (Table 2).Terpenoids and Saponins were present in the pet. Ether extract, but carbohydrates, alkaloids, flavonoids, tannins and phenolic compounds, protein and amino acids and glycosides were absent (Table 2). Ethanol extract was found to contain carbohydrates, alkaloids, flavonoids, and tannins and amino acids butterpenoids, saponins, protein and amino acids and glycosides were absent. (Table 1).

Table 1 Results of qualitative phytochemical analysis of various extracts acquired from *C. papaya*

Chemical Constituent	Pet. Ether Extract	Ethanol Extract
Carbohydrates	-	+
Alkaloids	-	+
Terpenoids	+	-
Flavonoids	-	+
Tannins and Phenolic Compounds	-	+
Saponins	+	-
Protein and Amino acids	-	-
Glycosides	-	-

Cold Maceration extraction method, + and – indicates the presence and absence of corresponding SMs in the test extract

3.3 Quantitative Phytochemical Analysis

3.3.1 Total Alkaloids contents Estimation

Atropine was used to evaluate the total alkaloid content of ethanolic extract, TAC yield of ethanolic extracts is 107.4 mg/ 100 gm of extract. The results shown in **[Table 2]** have been calculated from linear equation.

$$Y= 0.005X +0.026$$

This linear equation has been obtained from the standard curve of atropine, where Y is the absorbance of the sample obtained from UV-visible spectrophotometer.

Table 3 Results of Total Phenolic and Total Flavanoids contents

Extracts	Total Alkaloids Content(mg/gm equivalent to atropine)	Total Flavanoids contents(mg/gm equivalent to rutin
Ethanolic extract	107.4	25.29

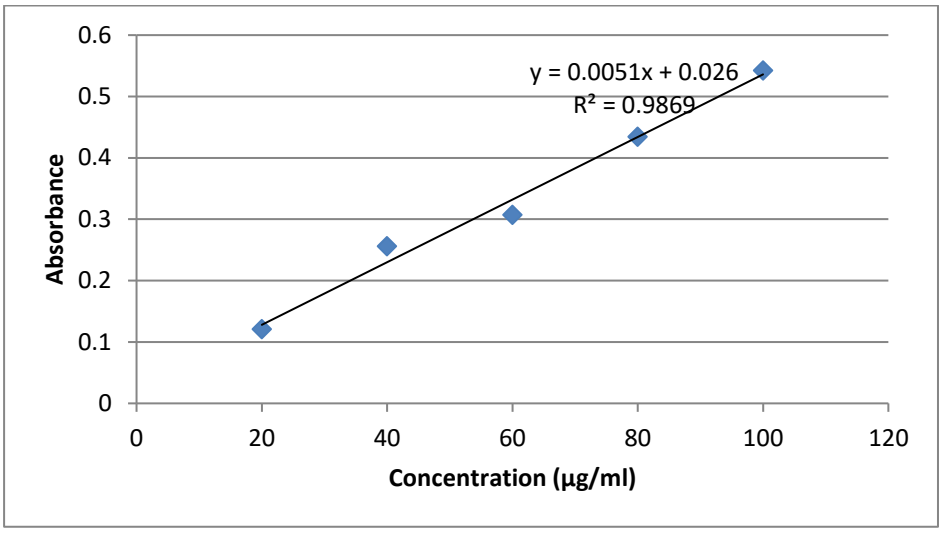

Figure 1: Graph represent standard curve of Atropine

3.3.2 Total Flavonoids Content

The flavonoids content was determined quantatively using aluminium chloride method with the help of UV-Spectrophotometer. The TFC yield of of ethanolic extracts was 25.29 mg of RE/g . **[Table 2]**. The results mentioned have been calculated (using same method as used for total alkaloid content) from the linear equation derived by standard curve of Rutin at different concentration.

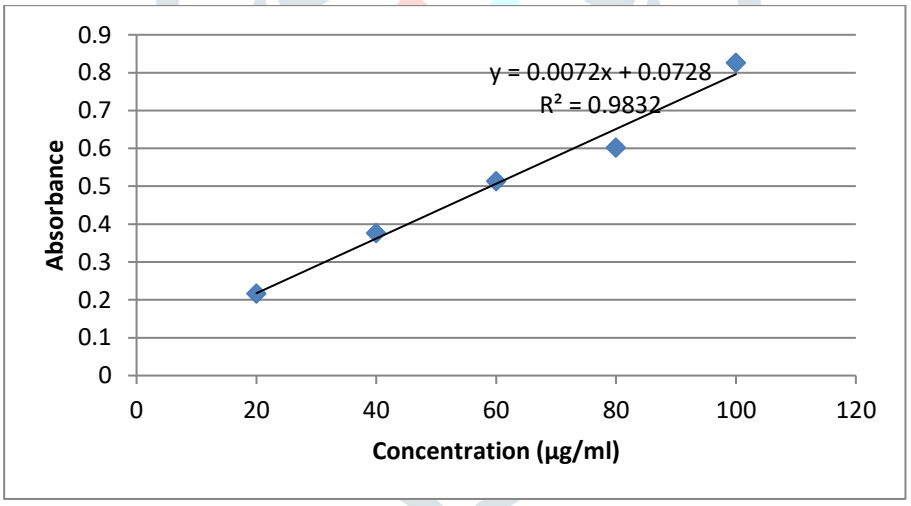

Figure 2: Graph represent standard curve of Rutin

3.4 Antioxidant Potential

3.4.1 DPPH radical scavenging activity

In this method, the DPPH free radical scavenging activity was performed with ethanolic extract of *C. papaya*. The percent of inhibition at each concentration of ethanolic extract of *Carica papaya* was calculated. According to the observations it was found that as the concentration of ascorbic acid increases, the DPPH radical scavenging activity also increases. Similarly, as the concentration of plant extract increases, the percent inhibition also increases. The highest percent of inhibition (84.25%) was found at 30µg/ml concentration in ascorbic acid. The DPPH activity of ethanolic extract of ***Caricapapaya*** was 72.03 %.[Figure 3]

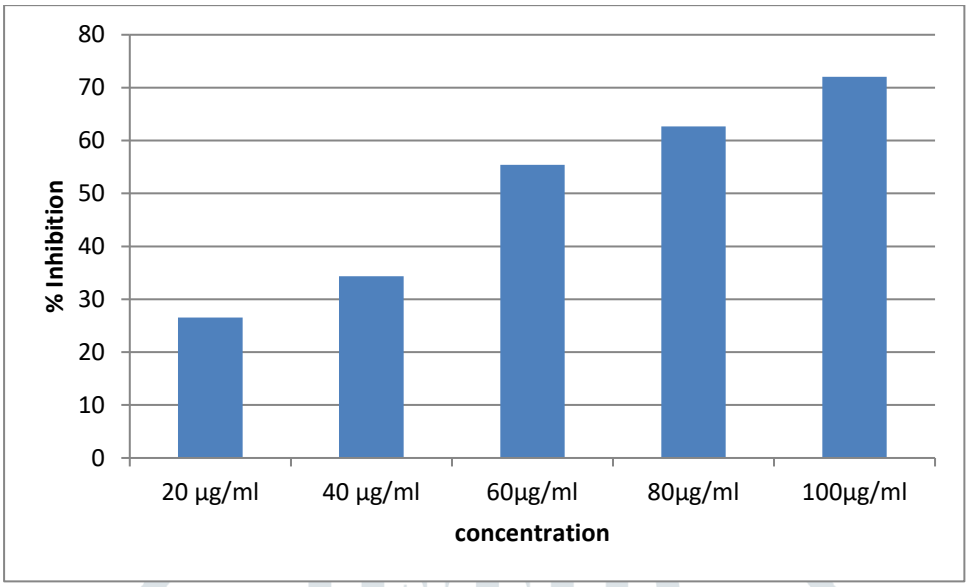

Figure 3:DPPH radical scavenging activity of *Carica papaya*

3.4.2 Hydrogen peroxide scavenging activity

Table 5&6 showing hydrogen peroxide scavenging activity of the methanol extract and standard.The extracts of *Carica papaya* caused a dose-dependent inhibition of hydrogen peroxide. The IC50 values for the extract of *Carica papaya* was70.32 compared to standard ascorbic acid 12.54.[Figure 4]

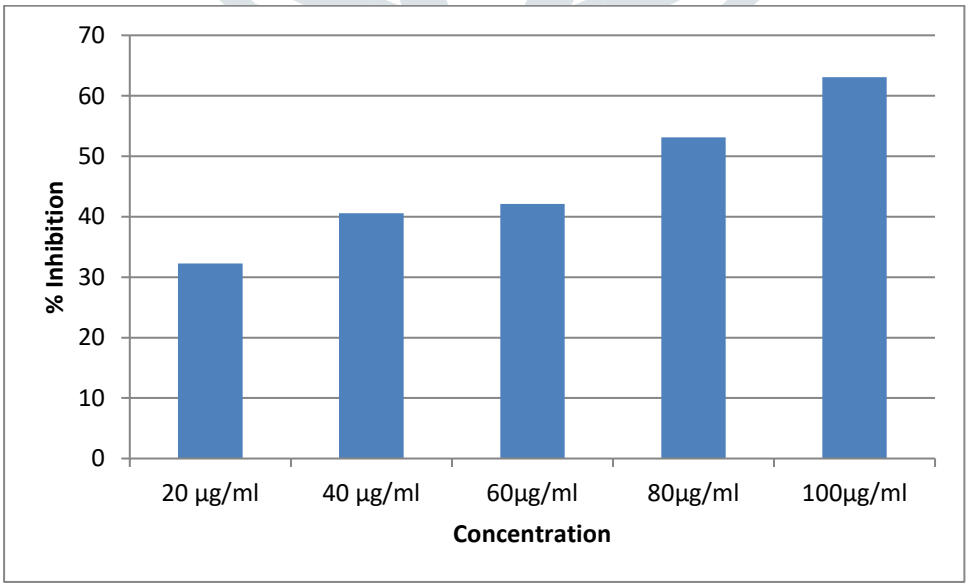

Figure 4:H_2O_2radical scavenging activity of *Carica papaya*

3.4.3 Superoxide scavenging activity

The PMS-NADH method was used to measure the superoxide anion radical scavenging capabilities of extracts. The % inhibition of superoxide radical formation by extracts is shown in the graph, along with a comparison to ascorbic acid. The percentage suppression of superoxide generation by *Carica papaya* at 100g/ml concentrations was determined and was found to be 57.39 %. Ascorbic acid, on the other hand, inhibits the superoxide radical by 80.22 percent.**[Figure 5]**

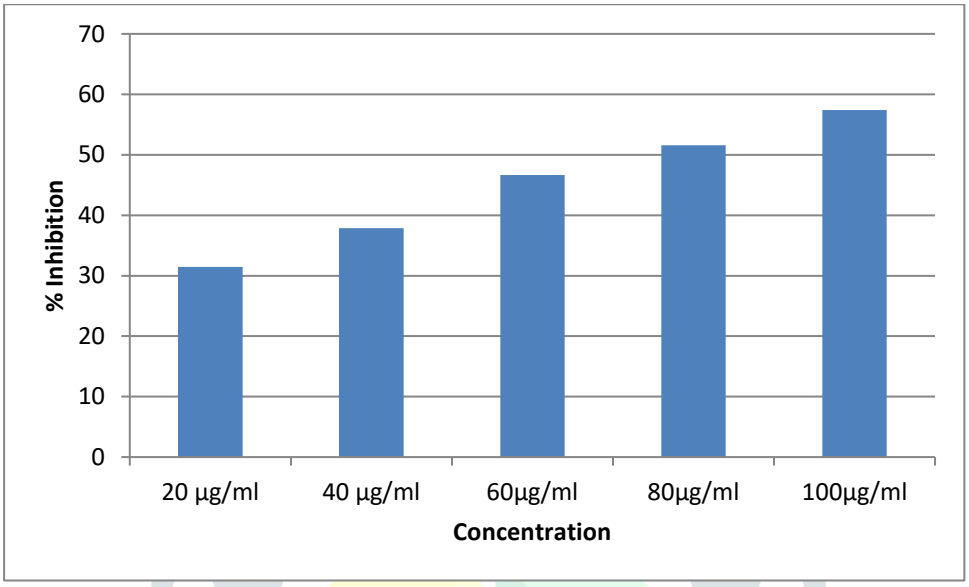

Figure 5: Superoxide radical scavenging activity of *Carica papaya*

4 Conclusion

The current study concluded that *Carica papaya*is richsource of phytochemicals.Results showed the presence of alkaloids, amino acids,carbohydrates, flavonoids, phlobatannins and tannins in the leaves of *Carica papaya*. The quantative phytochemical analysis reveled that it contained 25.29mg/100g of TFC and 107.4 mg/100g of TAC. Results of DPPHassay, H_2O_2 radical scavenging and Superoxide scavenging assay showed that *Carica papaya*(100 µg/mL) has about 72.03%, 63.05% and 57.39% scavenging activity respectively. Therefore, it is concluded from this preliminary study that *Carica papaya* can beused for isolation of important compounds with medicinal and pharmacological importance.

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