# Phytochemical Screening, Antioxidant and Antidiabetic Activities of Extracts of Leaves and Seeds of *Carica papaya*

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## ABSTRACT

The phytochemicals, phenolic, flavonoid contents, and antibacterial, antioxidant and antidiabetic activities of leaves and seeds extract of *Carica papaya* via Soxhlet extraction were investigated. The phytochemical screening indicated different bioactive compounds such as saponins, alkaloids, glycosides, tannins, polyphenols, steroids, and flavonoids in different extracts. The total phenolic content (TPC) was determined spectrophotometrically by Folin–Ciocalteu reagent method and aluminium chloride reagent to measure total flavonoid content (TFC). TPC was found more in leaf methanol extract (LME) (93.18±0.40 mg GAE/g) than that in seed methanol extract (SME) (89.14±0.45 mg GAE/g). Comparatively, the flavonoid content was found more in LME (18.85±0.061 mgQE/g) than in SME (16.64±0.065 mgQE/g). The antioxidant activity was assessed for LME and SME by DPPH radical scavenging activity and $IC_{50}$ values. The $IC_{50}$ values of LME and SME were 43.54±0.007 and 15.48±0.13µg/mL, respectively. Thus, seeds methanol extract showed better antioxidant activity than leaves methanol extract. The antidiabetic assay was performed via α- amylase inhibition method. Among the selected parts (seeds and leaves), methanol seed extract exhibited high α-amylase inhibition with an $IC_{50}$ value of 46.99±0.018 µg/mL. The study explored the potential value of *Carica papaya* in medicinal applications.

**Keywords:** α-Amylase inhibition, $IC_{50}$, Total flavonoid content, Total phenolic content

## 1. INTRODUCTION

*Carica papaya*, locally known as Pawpaw belongs to Caricaceae family, is an evergreen shrub. It is a large tree with a solo stem growing in height upto 10 m tall, with spirally arranged leaves confined to the trunk's top. The lower trunk contains leaves and fruits. The leaves are large, 50-70

cm in diameter (Aravind *et al.* 2013). *Carica papaya* is a nutraceutical plant having a wide range of pharmacological activities (Peter *et al.* 2014). Its fruit is a rich source of nutrients but papaya leaves and seeds also contain various phytochemicals, including phenols. The edible black seeds of the papaya with sharp and spicy tests are ground and used as a substitute for black pepper (Nna *et al.* 2019). However, the seeds seem to have more potent medicinal values than the flesh (Peter *et al.* 2014; Nna *et al.* 2019). *Carica papaya* seeds possess various pharmacological activities such as antifertility, contraceptive, anthelmintic, anti-inflammatory, analgesic and antimicrobial property (Agarwal *et al.* 2016). Other pharmacological uses of papaya seeds include carminative, emmenagogue, abortifacient, counterirritant, paste in psoriasis, ringworm disease, and an antifertility agent in males (Peter *et al.* 2014). Water extract of Papaya seeds protects fibroblasts from $H_2O_2$, induced stress due to the antioxidant activity (Panzarini *et al.* 2014). *Carica papaya* seeds are also used in the treatment of hypertension, diabetes mellitus and hypercholesterolemia. The vegetative parts of the papaya plant have enormous medicinal values (Asghar *et al.* 2016). Papaya leaves are made into tea as a treatment for malaria. The antimalarial and antiplasmodial activity has been noted due to the presence of Karpain compound, which kills microorganisms that often interfere with the digestive function (Udoh *et al.* 2005, Kousika & Priyadharshini 2020). The leaves of *Carica papaya* are also used traditionally to cure various ailments like malaria, dengue, jaundice, immunomodulatory, and antiviral activity. Other diseases that can be controlled by *Carica papaya* include; abdominal discomfort, pain, diabetes, obesity, infection, and oral drug poisonings. It also possesses therapeutic, anti-inflammatory, and sugar reducing tendency (Ogundele *et al.* 2017). Therefore, the present study was carried out for the comparative study extracts of seeds and leaf's phytoconstituents (quantitative & qualitative analysis) and their biological activity, i.e. antibacterial, antioxidant and antidiabetic.

## 2. METHODOLOGY

### 2.1 Collection of Plant Materials and Preparation of Seed and Leaves Extracts

The leaves and fruits of *Carica papaya* were collected from Suklagandaki-3, Tanahun district Gandaki zone of Nepal at the beginning of April 2019. The dried and powdered form (50 g) of leaves and seeds were extracted successively with hexane (350 mL), chloroform (300 mL), ethylacetate (250 mL), methanol (200 mL) by Soxhlet method. The remaining residue after extraction with methanol was refluxed with water (500 mL) for 3h. The extracts were filtered and concentrated using Rota vapour to get solid or semi-solid mass and kept in the freezer for further analysis.

### 2.2 Phytochemical Screening

Preliminary phytochemical screening of different extracts of leaves and seeds were done using the standard procedure put forward by Ciulei I (Vongsak *et al.* 2013).

### 2.3 Quantitative Analysis of Extracts of Leaves and Seeds of *Carica papaya*

#### 2.3.1 *Total Phenolic Content (TPC)*

The total phenolic content in plant extracts was determined using the Folin-Ciocalteu colourimetric method (Waterhouse 2002). Various gallic acid solutions in methanol (10, 25, 50, and 100 µg/mL) were prepared. In a 20 mL test tube, 1 mL of gallic acid of each concentration was added to that 5 mL of 10% FCR (1:10 v/v), and 4 mL of 7% $Na_2CO_3$ were added to get a total volume of 10 mL. The blue coloured mixture was shaken well and incubated for 30 minutes at 40 °C in a water bath. Then, the absorbance was measured at 760 nm. Similarly, the absorbance was measured against a blank containing all reagents except gallic acid. All experiments were carried out in triplicate. The average absorbance values obtained at different concentration was used to plot the calibration curve.

Absorbance for various extracts (10, 25, 50, 80 and 100 µg/mL) was recorded by following the standard procedure. Total phenolic content in the extracts of leaves and seeds was calculated as mg gallic acid equivalents (GAE) per gram dry extract (mg/g).

### 2.3.2 *Total Flavonoid Content (TFC)*

The total flavonoid present in the different plants' methanolic extract was estimated by the aluminium chloride colourimetric assay involving quercetin as standard (Pallab *et al.* 2011; Pandey & Rajbhandari 2014). The absorbance values for different concentration of each extract were recorded at 510 nm. The total flavonoid content in different extract was calculated from the calibration curve using regression equation y = 0.0113x-0.0837, $R^2$ = 0.988 followed by the formula C = cV/m expressed as mg quercetin equivalents per gram of extract in dry weight (mg QE/g) (Ariffin *et al.* 2021). The various standard quercetin concentrations (10, 25, 50, 100 and 125 µg/mL) were prepared. 1.0 mL of quercetin was taken from each concentration, and 2.0 mL of methanol and 4.0 mL of distilled water were added. At the zero time, 0.3 mL of 5 % sodium nitrite solution was added, and 0.3 mL of 10% $AlCl_3$ was added after 5 min, and 2.0 mL of 1M NaOH solution was added after 6 min to the mixture. Immediately, the total volume of the mixture was made 10.0 mL by adding the distilled water. Finally, pink colour mixture was recorded at 510 nm against a blank containing all reagents except quercetin. The average absorbance values obtained at different concentrations of quercetin were used to plot the calibration curve. Various concentration of seeds and leaves extract (10, 25, 50, 100 and 125 µg/mL) were prepared. Following the procedure described for standard quercetin, absorbance for each concentration of the extract was measured. Total Flavonoid content in extract was expressed as mg quercetin equivalent (QE) per gram of dry extract (mg/g).

Total phenolic and flavonoid content were calculated by using the formula given by Zuhair *et al.* (2013): C = cV/m where, C = total phenolic/flavonoid compound in mg QE/g dry extract, c = concentration of gallic acid/quercetin established from calibration curve in mg/mL, V = volume of extract in mL, m = weight of extract in gram (Khanal *et al.* 2020). Data were recorded as a mean±standard deviation of three determination of Absorbance for each concentration, from which the linear correlation coefficient ($R^2$) value was calculated using MS Office Excel 2007. The linear regression equation for a straight line is, y = mx + c where, y = absorbance of extract, m = slope of the calibration curve, x = concentration of extract, c=intercept. Using this regression equation, concentrations of each extract, the phenolics and flavonoid content were calculated.

## 2.4 Biological Screening

### 2.4.1 *Antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay*

Antioxidant activity of the selected extracts was assessed using DPPH free radical (Jamuna *et al.* 2012; Pokhrel & Neupane 2021). DPPH solution (0.2 mM) was prepared by dissolving 7.8 mg of DPPH in 100 mL methanol. Different concentrations (20, 40, 60, 80 and 100 µg/mL) of methanolic solutions of each extract were prepared by serial dilution of the stock solution of the respective extract. To each 0.5 mL extract solution, 2.5 mL DPPH (0.2 mM) solution was added. A control was prepared by mixing 0.5 mL distilled water and 2.5 mL DPPH (0.2 mM) methanolic solution. These samples were shaken well, incubated at 37 °C for half an hour, and the absorbance was recorded at 517 nm by UV- Vis spectrophotometer (Hitachi U2800). The radical scavenging activity was expressed as the radical scavenging percentage using the following formula (Mishra *et al.* 2012; Dhital 2017):

DPPH Inhibition (%) = [1-A1/A0] × 100

Where, A1 = Absorbance of the sample, and A0 = Absorbance of control

The assay was replicated thrice, and the result was taken as mean±standard deviation. The $IC_{50}$ (50% inhibitory concentration) value is the effective concentration of the sample required to scavenge 50% of the DPPH free radicals. It was calculated using the inhibition curve by plotting extract concentration versus the corresponding scavenging effect (Pokhrel & Neupane 2021).

### 2.4.2 *α-Amylase Antidiabetic Assay*

α-amylase inhibition assay was carried out to determine the antidiabetic potential of plants. The undigested starch due to enzyme inhibition was detected through the blue starch iodine complex detected at 630 nm. The starch solution

was prepared by dissolving 0.2 g starch in 25 mL of NaOH (0.4 M), and pH was maintained to 7.0, and the final volume made up to 100 mL by adding distilled water. Acarbose was used as a standard. 400 µL of starch was incubated at 37 ℃ for 5 min with 200 µL of acarbose or plant extract at different concentrations (40, 60, 80, 160, 320 and 640 µg/mL), followed by 200 µL of α-amylase (20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9), and incubation at 37 ℃ for 15 min. Termination of the reaction was done by adding 800 µL of HCl (0.1 M). Then, 1000 µL of iodine reagent (2.5 mM) was added, and absorbance was measured at 630 nm. The percentage of enzyme inhibition was calculated by using the following equation (Chakrabarti *et al.* 2014);

% inhibition = [1-(Abs2 - Abs1/Abs4 - Abs3)] ×100

Where, Abs1 = Absorbance of an incubated mixture containing plant extract, starch and amylase, Abs2 = Absorbance of an incubated mixture containing plant extract and starch. Abs3= Absorbance of an incubated mixture containing starch and amylase Abs4 = Absorbance of an incubated mixture containing starch only. A graph was plotted by taking the concentration on the X-axis and % inhibition on the Y-axis. From this graph, each extract's $IC_{50}$ value was calculated, and the values of different extracts were compared. The extract having the lowest $IC_{50}$ is considered to have the best inhibition property.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Screening

The results of the phytochemical screening of *Carica papaya* leaves and seeds extracts are shown in Table 1. Phytochemical screening indicated alkaloids, phenols, glycoside, quinones, saponins, tannins, and steroids in *Carica papaya* seeds and leaves' extracts. Flavonoids are present only in leaves extract. Among phytochemicals, alkaloids are significant for the protection and survival of plants because they ensure their survival against microorganisms (antibacterial activities). Phenolic compounds are a large and complex group of chemical constituents found in the plant (de Beer *et al.* 2002). They have an essential role as defense compound. The best-described property of flavonoids is their capacity to act as antioxidants to protect the human body from free radicals and reactive oxygen species. Recently, tannins have attracted scientific interest due to increased AIDS and various cancers (Saxena *et al.* 2013). The phytochemical analysis revealed the presence of many phytoconstituents (Peter *et al.* 2014; Dwivedi *et al.* 2020) which indicated that it has some medicinal potential (Pokhrel & Chaulagain 2019). Phytochemical screening revealed that the methanol extract of *Carica papaya* flowers contains alkaloids, flavonoids, saponins, and tannins as major components; saponins and tannins were present in chloroform and n-hexane extracts; however, steroids and flavonoids were additionally found in n-hexane extract (Dwivedi *et al.* 2020).

Table 1. Qualitative screening of *Carica papaya* leaves and seeds

| | | ***Leaves Extract (LE)*** | | | | |
|---|---|---|---|---|---|---|
| **Phytochemicals** | **Method** | **Hexane** | **Ethylacetate** | **Chloroform** | **Methanol** | **Water** |
| | Mayer's Test | - | - | - | + | + |
| **Alkaloids** | Dragendorff's Test | - | - | - | + | + |
| Phenols | | - | - | - | + | + |
| Polyphenols | $FeCl_3$ Test | - | - | - | + | + |
| Flavonoids | Alkaline Reagent Test | + | + | + | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| Reducing sugar | Fehling's Test | + | + | + | + | + |
| Glycosides | Molisch's Test | + | + | + | + | + |
| Quinones | - | - | - | + | + | + |
| Saponins | Chloroform and $H_2SO_4$ Test | + | + | + | + | + |
| Tannins | Lead Acetate Test | + | + | + | + | + |
| Steroids | Liebermann-Burchard Test | + | + | + | + | + |
| | Salkowski Test | + | + | + | + | + |

***Seeds Extract (SE)***

| **Phytochemicals** | **Method** | **Hexane** | **Ethyl-acetate** | **Chloroform** | **Methanol** | **Water** |
|---|---|---|---|---|---|---|
| | Mayer's Test | + | + | + | + | + |
| **Alkaloids** | Dragendorff's Test | + | + | + | + | + |
| **Phenols** | | + | + | + | + | + |
| **Polyphenols** | $FeCl_3$ Test | + | + | + | + | + |
| **Flavonoids** | Alkaline Reagent Test | + | + | - | + | - |
| | Lead Acetate Test | + | + | - | + | - |
| **Reducing sugar** | Fehling's Test | - | - | + | + | + |
| **Glycosides** | Molisch's Test | + | + | - | + | - |
| **Quinones** | - | - | - | + | + | + |
| **Saponins** | Chloroform and $H_2SO_4$ Test | + | + | + | + | + |
| **Tannins** | Lead Acetate Test | + | + | + | + | + |
| | Liebermann Burchard Test | - | - | + | + | + |
| **Steroids** | Salkowski Test | - | - | + | + | + |

## 3.2 Quantitative Analysis

### 3.2.1 *Total Phenolic Content (TPC)*

The quantitative assessment of total phenol was done using Folin-Ciocalteu reagent (FCR) in terms of gallic acid equivalent. The absorbance values obtained at 760 nm for different concentrations of gallic acid were used to construct the calibration curve. The obtained TPC values of methanol leaves and seeds extracts are shown in Table 2. TPC contents of methanol leaf and seed extract showed 93.18±0.40 and 84.14±0.45 mgGAE/g,

respectively. So, the leaf extract has high phenolic content than that of seed extract. However, Asghar *et al.* (2016) reported the phenolic content of methanol extract of leaves and the seeds was 54.28 ±0.10 and 38.86±0.82 mgGAE/g respectively whereas, Vuong *et al.* (2013) and Gogna *et al.* (2015) reported 63.59±0.62 and 97.08±2.8 mgGAE/g TPC content in leaves and seed extracts of *C. papaya,* respectively. The total phenolic content in our sample is relatively different when compared with other studies. This variation is due to different extraction parameters, including temperature and extraction duration (Vuong *et al.* 2013). The result also indicated that the leaves contain high phenolic content that may provide an excellent dietary antioxidant source. The phenolic compounds are the main micro constituents contributing to the antioxidant property of different parts of papaya (Addai *et al.* 2013).

Table 2.Total phenolic contents (TPC) in methanol extract of *Carica papaya* leaves and seeds (LME and SME)

| SN. | Conc. (µg/mL) | Methanol leaves extract (LME) | | | Methanol seeds extract (SME) | | |
|---|---|---|---|---|---|---|---|
| | | Abs. | TPC (mgGAE/g) | Mean TPC | Abs | TPC (mgGAE/g) | Mean TPC |
| 1 | 10 | 0.812 | 54.63 | | 0.612 | 41.93 | |
| | | | 74.31 | | | 76.14 | |
| 2 | 25 | 1.095 | 79.13 | 93.18±0.40 | 1.091 | 81.52 | 89.14±0.45 |
| | | | 126.04 | | | 119.76 | |
| 3 | 50 | 1.165 | 131.78 | | 1.166 | 126.36 | |
| 4 | 100 | 1.74 | | | 1.75 | | |
| 5 | 125 | 1.867 | | | 1.843 | | |

### 3.2.2 *Total Flavonoid Content (TFC)*

The TFC of methanol leaf and seed extract of *Carica papaya* presented in Table 3. The results show that the total flavonoid content (TFC) was comparatively higher in leaf extract (18.85±0.061) than methanol seed extract (16.64±0.065). Similarly, Asghar *et al.* (2016) also reported higher TFC in leaves (15.54 ± 0.12) mg catechin equivalent (CE)/g than in seeds (08.62 ± 0.16) mg CE/g ethanol extract. Therefore, *Carica papaya* leaves and seeds are good sources of flavonoid content. Dwivedi *et al.* (2020) also reported that *Carica papaya* flowers are good sources of total flavonoid content (1.53 ± 0.10 mg QE/g dry weight).

| S.N | Concentration (µg/mL) | Leaves methanol extract (LME) | | | Seeds methanol extract (SME) | | |
|---|---|---|---|---|---|---|---|
| | | Abs. | TFC (mgQE/g) | Mean TFC | Abs. | TFC (mgQE/g) | Mean TFC |
| 1 | 10 | 0.038 | 10.76 | | 0.012 | 8.54 | |
| 2 | 25 | 0.106 | 17.18 | 18.85±0.061 | 0.109 | 16.69 | 16.64±0.065 |
| 3 | 50 | 0.149 | 21.09 | | 0.12 | 18.02 | |
| 4 | 100 | 0.207 | 26.36 | | 0.178 | 23.15 | |

## 3.3 Biological Screening

### 3.3.1 *DPPH Free Radical Scavenging Potential for Antioxidant Activity*

The recorded absorbance with different concentration of ascorbic acid is shown in Figure 1. The DPPH free radical scavenging assay results showed a significant difference in scavenging activity among seeds and leaves of *Carica papaya*. Free radicals act as triggers to several degenerative diseases; therefore, samples having free radical scavenging activity can be of potent medicinal importance.

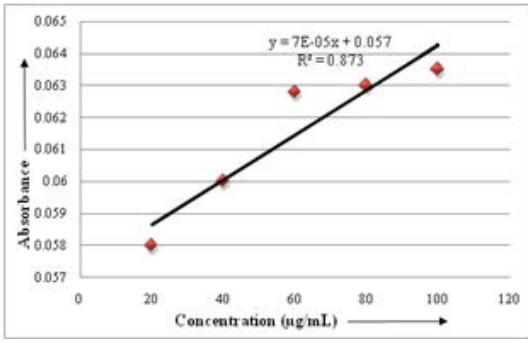


Fig. 1. Absorbance vs concentration of ascorbic acid.

The % radical scavenging activity of methanol extract of *Carica papaya* seeds and standard ascorbic acid was found almost similar and better than leaves extract (LME). The antioxidant potential has an inverse relation with $IC_{50}$ value, lower value of $IC_{50}$ represents high antioxidant ability. The $IC_{50}$ value for seed methanolic extract (SME) was 15.48±0.013 µg/mL which was comparatively lower than the $IC_{50}$ (43.54±0.007 µg/mL) of methanolic leaves extract (LME), showed that SME of *Carica papaya* is more effective as antioxidant compared to LME. The seed methanol extracts were observed as good antioxidants as their $IC_{50}$ values were close to the standard ascorbic acid. Kumawat *et al.* (2012) reported 21.23 µg/mL $IC_{50}$ values for ascorbic acid. Flavones and flavanols are the major compounds responsible for the antioxidant activity, which depends on the presence of free OH groups (Dwivedi *et al.* 2020). The present finding suggests that methanolic seed extract of *Carica papaya* could be a potential natural source of antioxidants and could have greater importance as a therapeutic agent in preventing oxidative stress-related degenerative diseases (Kumawat *et al.* 2012). Dwivedi *et al.* (2020) studied the antioxidant and antibacterial properties of phytochemical extracts of *Carica papaya* flowers. It's leaves methanolic extract showed the presence of more phytochemicals and have phenolic, flavonoid content, and exhibited strongest antioxidant properties which can effectively scavenge reactive oxygen species compared to n-hexane, ethylacetate and ethanol solvent extracts (Nandini *et al.* 2020).

Here, the effective results were obtained from methanol extract of seeds of *Carica papaya* which showed the strongest DPPH radical scavenging activity as its $IC_{50}$ value was near to standard ascorbic acid. So, this result show that the plant used for this study can play a significant role in the field of medicine based on the antioxidant property.

Table 4. Comparison of $IC_{50}$ values of leaf and seed extracts with standard ascorbic acid.

| S.N. | Sample | $IC_{50}$ value (µg/mL) |
|---|---|---|
| 1 | Standard ascorbic acid | 15.38±0.013 |
| 2 | Seed extract | 15.48±0.013 |
| 3 | Leaf extract | 43.54±0.007 |

### 3.3.2 *Antidiabetic Activity using α-amylase Inhibition Assay*

Among five different extracts, the antidiabetic activity of methanol extracts of seeds and leaves *Carica papaya* were assessed using Acarbose as standard. An α-amylase inhibition assay was carried out to assess the antidiabetic activity of selected samples. The methanol extract of *Carica papaya* seed showed a lower $IC_{50}$ value than leaf extract. The $IC_{50}$ value of standard Acarbose was found as 56.12±0.013 µg/mL. The $IC_{50}$ value of seed extract was 46.99±0.018 µg/mL, which showed higher antidiabetic activity than leaf extract. However, Cowan (1999) showed that the methanolic extract of *Carica papaya* leaves also possesses significant antidiabetic activity, which shows that leaves can develop drugs in combating drugs. The results provide useful information on pharmacological activities of the plant associated with people with diabetes (Gawli & Lakshmidevi 2015).

## 4. CONCLUSION

This study revealed that all extracts of leaves and seeds of *Carica papaya* consist of pharmacological substances. The leaf methanol extract contains more phenolic and flavonoid contents than seed methanol extract. These seeds methanol extracts exhibited good antioxidant and antidiabetic activity, whereas leaf methanol extract showed more antibacterial property in comparison to leaf chloroform extracts. The study thus reveals the potential value of *Carica papaya* in medicinal applications. However, further investigations are needed to identify the active compounds to confirm the antioxidant and antidiabetic seed papaya extract activity.

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