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Comparative Evaluation of Anti-Inflammatory and Antioxidant Property Of Carica Papaya Leaf And Seed Extract - An Invitro Study

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ABSTRACT

Introduction: Carica papaya is widely known for its medicinal properties. Various parts of papaya such as the seeds, leaves, fruit, and latex are used worldwide therapeutically for maintaining health and also treatment of diseases. Literature has also reported the prominent anti-inflammatory and antioxidant properties of C.papaya. The aim of the study is to comparatively evaluate the anti-inflammatory and antioxidant properties of c.papaya leaf and seed extracts.

Materials and Method: The C.papaya seed and leaf extracts were prepared by adding 300mg of the shade dried powder to 300mL of ethanol and placing it in a shaker for 24 hours. The Ant-inflammatory activity was determined using Albumin Denaturation Assay. The Antioxidant activity was determined using DPPH Assay.

Results: C.papaya leaf extract showed higher anti-inflammatory and Antioxidant properties at 100,200 and 300µL compared to standard and at all concentrations compared to C.papaya seed extract.

Conclusion: C.papaya leaf extract showed superior anti-inflammatory and antioxidant properties compared to C.papaya seed extract at all concentrations. The concentration, the method of extraction and solvent used plays an important role in the activity of the phytochemicals present in extract. Further cell line and animal studies are necessary to validate the clinical use of these phytochemicals present in C.papaya extract.

Keywords: *Carica papaya, papaya seed extract, papaya leaf extract, anti-inflammatory property, antioxidant property, medical, health*

INTRODUCTION

The age-old tradition of using plants and their parts for the prevention and treatment of various disease conditions has been recently rekindled. This could be due to the lesser adverse effects associated with these medications compared to their synthetic counterparts(1). These plant derived medications have also shown significant efficiency similar or superior to the synthetic drugs in treating various diseases. Pawpaw is one of the most economical and noteworthy fruits in the caricaceae family. Papaya contains various valuable phytochemicals including polysaccharides,

vitamins, minerals, enzymes, alkaloids, flavonoids, sterols and other important derivatives(2)(3,4)(2). The various phytochemicals found in papaya have shown to reduce the inflammatory conditions and related adverse effects by modulating the inflammatory mediators(5)(6–11)(5).

Inflammation is the first line response of tissue to injury. This response is non-specific(12)(13)(12). This inflammatory response can be acute or chronic. In inflammation there is an increase in permeability of the blood vessels which will lead to increased migration of inflammatory mediators to the site of injury along with fluid accumulation. Inflammation is a coordinated local and systemic response with mobilisation and release of immune, neurological and endocrine mediators(14)(15)(14).

Substances that prevent oxidation of readily oxidisable materials are known as anti-oxidants(16)(17–20)(16). Previously published studies have well documented the usage of Carica papaya leaf, seed, fruit and peel extracts owing to its antioxidant properties(21)(22–25)(21).

The aim of this study is to determine the anti-inflammatory and antioxidant properties of Carica papaya leaf and seed extract.

MATERIALS AND METHOD

Preparation of Carica papaya Leaf and Seed Extract

Seeds and leaves of Carica papaya were collected from Agriculture Research Farm, SHIATS, Naini, UP. The seeds and leaves were dried at room temperature for 15 days. After that the seeds and leaves were ground. 300mg of the seed and leaf powder were separately added to 2 shakers containing 500mL of ethanol. These beakers were placed in the shaker for 24 hours. The extract was purified and boiled for 10 minutes at 25 celsius.

Assessment of Anti Inflammatory Activity

Protein Denaturation Assay

The anti-inflammatory property of C.papaya leaf and seed extract was assessed using Albumin Denaturation assay. The papaya leaf and seed extract of various fixations (100, 200, 300, 400, 500µg/mL) were added and mixed to 0.45 mL of bovine serum albumin (1% aqueous solution). The pH of this mixture was adjusted to 6.3 using 1N hydrochloric acid. The samples were then incubated for 20 minutes at room temperature followed by heating at 55°C for 30 minutes in the water bath. Later, the samples were cooled following which the absorbance was estimated at 660 nm spectrophotometrically. Diclofenac Sodium was used as standard in this study.

Percentage of protein denaturation was determined utilising the following equation.

*% Anti-Denaturation Activity = Absorbance of control – Absorbance of sample x 100 Absorbance of control.

Assessment of Antioxidant activity

DPPH Assay

2,2-diphenylpicrylhydrazyl (DPPH) assay was used to test the antioxidant activity of the C.papaya leaf and seed extract. Diverse concentration (100-500µg/mL) of the leaf and seed extract was added and mixed with 1mL of 0.1mM DPPH in methanol and 450µL of 50mM Tris HCL buffer (pH 7.4). This mixture was incubated for 30 minutes. 517 nm wavelength was used to determine the reduction in DPPH free radicals based on the absorbance at the specific wavelength. BHT was used as control. The percentage of inhibition of DPPH free radical was determined from the equation,

*% Antioxidant Activity = Absorbance of control – Absorbance of sample x 100 Absorbance of control.

RESULTS

The antioxidant property of papaya leaf and seed extract which was analysed using DPPH assay showed that the antioxidant property of papaya leaf extract to be significantly higher than standard and papaya seed extract at 100, 200 and 300µg/mL, while papaya seed extract showed inferior antioxidant properties to papaya leaf extract and standard at all concentrations. At 400 and 500µg/mL concentrations standard showed

better antioxidant properties than papaya leaf extract (Table 1) (Figure 1).

The anti-inflammatory property of Papaya leaf and seed extract was analysed using Protein (Albumin) Denaturation Assay. Papaya leaf extract showed significantly superior anti-inflammatory properties at 100, 200 and 300µg/mL with the peak activity at 300µg/mL compared to standard and papaya seed extract. While papaya seed extract showed inferior anti-inflammatory activity to standard and papaya leaf extract at all concentrations. At 400 and 500µg/mL, the standard showed better anti-inflammatory properties than papaya leaf extract (Table 2) (Figure 2).

DISCUSSION

Ancient and traditional medicine widely used plants and their parts in treating several diseases and maintaining health, this would be attributed to the fact that these plants contain large quantities of pharmacologically useful compounds in them(26). The anti-inflammatory and antioxidant properties of these medicinal plants can be credited to the presence of phytochemicals like volatile oils, phenolic compounds, essential oils, tannins, phenolic compounds, phytosterols, tannins and flavonoids(27). The leaves of these medicinal important plants have been reported to have appreciable quantities of tannins and alkaloids(28)(29,30)(28) (31)(32) (33,34)(31).

Chemokines, Proinflammatory cytokines , C reactive Protein, vascular adhesion molecules, proinflammatory transcription factors and other neuropeptides regulate and contribute to the inflammation.

TNFα which is secreted by monocytes and macrophages plays a prominent role in the inflammatory process by induction of proinflammatory mediators such as IL-6,1, IFN. Agents and compounds that reduced TNFα were considered to possess anti-inflammatory properties(35)(6,36)(37–40)(35). A previous in vitro study reported that 1 mg/ml ethanolic papaya leaf extract demonstrated significant inhibition of TNFα in dendritic cells which were treated with Lipopolysaccharide(LPS). The same extract also showed antioxidant properties by protecting DNA damage in Lymphocytes.

The proteolytic enzymes such as papain and chymopapain present in C.papaya have been reported to have anti-inflammatory and antioxidant properties.(41)(42–45)(41). TGFβ plays an important role in Anti-inflammation but over synthesis of TGFβ can lead to chronic inflammation(46)(47,48)(46). Desser et al in his study reported that TGFβ levels were significantly reduced in patients with herpes zoster, osteomyelofibrosis and rheumatoid arthritis by trypsin and chymotrypsin present in C.papaya extract. Alkaloids such as choline and nicotine present in papaya also demonstrated potential anti-inflammatory properties(49,50)(51,52)(53,54)(49,50). Papaya leaf and seed extract have shown to have potent anti-inflammatory and antioxidant properties.

CONCLUSION

C.papaya leaf extract showed superior anti-inflammatory and antioxidant properties compared to C.papaya seed extract at all concentrations. The concentration, the method of extraction and solvent used plays an important role in the activity of the phytochemicals present in extract. Further cell line and animal studies are necessary to validate the clinical use of these phytochemicals present in C.papaya extract.

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TABLE 1: Antioxidant Property Of C.Papaya Leaf And Seed Extract

Concentration (µg/mL)	Standard	C.Papaya Leaf	C.Papaya Seed
100	9.97±0.70	20.28±0.96	$15.13\pm0.60_{1}$
200	24.12±0.45	34.83 ± 0.85^{a}	$27.23\pm0.35_{12}$
300	36.10±0.46	52.30 ± 0.60^{ab}	$31.33\pm0.61_{123}{}^{ab}$
400	73.13±0.42	68.93 ± 0.95^{ac}	$45.60\pm0.40_{1234}{}^{ab}$
500	78.70±0.36	72.50 ± 0.50^{abc}	$60.60\pm0.53_{1234}{}^{abc}$

Percentage of Inhibition of DPPH by Standard, C.papaya leaf and seed extracts. Significance at P <0.05, ‘a’ Significantly different compared with 100µg/mL,‘b’ significantly different compared with 200µg/mL, ‘c’ significantly different compared with 300µg/mL of extracts. ‘1’ significantly different compared with 100µg/mL of papaya leaf extract, ‘2’ significantly different compared with 200µg/mL of papaya leaf extract, ‘3’ significantly different

compared with 300µg/mL of papaya leaf extract, '4' significantly different compared with 400µg/mL of papaya leaf extract.

TABLE 2: Anti-Inflammatory Property Of C.Papaya Leaf And Seed Extract

Concentration (µg/mL)	Standard	C.Papaya Leaf	C.Papaya Seed
100	46.98±0.47	57.10±0.40	$42.13±0.37_{1}$
200	60.03±0.30	$62.63±0.62^{a}$	$47.73±0.42_{12}$
300	71.97±0.60	$100.00±0.35^{ab}$	$58.97±0.30_{123}^{ab}$
400	77.93±0.26	$76.33±0.80^{ac}$	$60.73±0.65_{1234}^{ab}$
500	84.10±0.40	$80.67±0.73^{abc}$	$70.17±0.42_{1234}^{abc}$

Percentage of Inhibition of Albumin Denaturation by Standard, C.papaya leaf and seed extracts. Significance at P <0.05, 'a' Significantly different compared with 100µg/mL,'b' significantly different compared with 200µg/mL, 'c' significantly different compared with 300µg/mL of extracts. '1' significantly different compared with 100µg/mL of papaya leaf extract, '2' significantly different compared with 200µg/mL of papaya leaf extract, '3' significantly different compared with 300µg/mL of papaya leaf extract, '4' significantly different compared with 400µg/mL of papaya leaf extract.

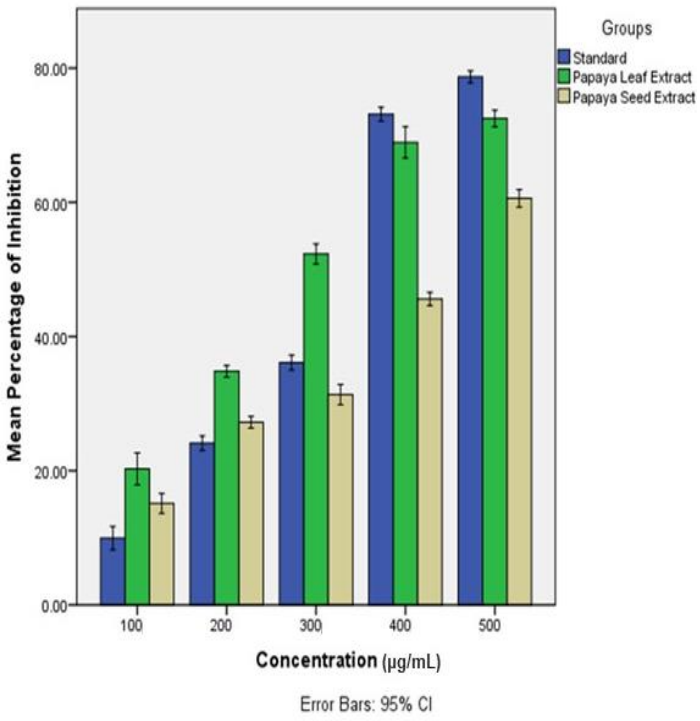

FIGURE 1: Graph showing the percentage of Inhibition of DPPH by Standard, C.papaya leaf and seed extracts at 100, 200, 300, 400 and 500 µg/mL concentrations observed by absorbance at 517 nm.

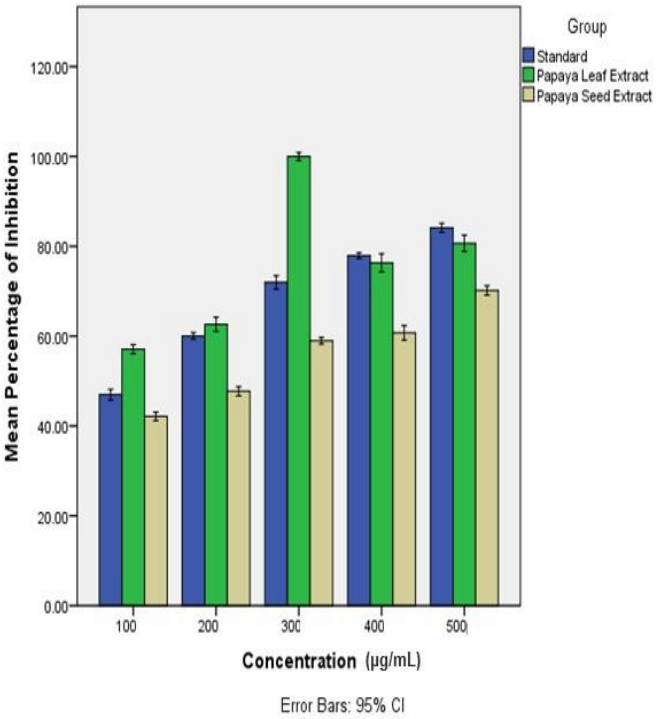

FIGURE 2: Graph showing percentage of Inhibition of Albumin Denaturation by Standard, C.papaya leaf and seed extracts at 100, 200, 300, 400 and 500 µg/mL concentrations observed by absorbance at 660 nm.