ANTI-INFLAMMATORY ACTIVITY OF SEED EXTRACT OF CARICA PAPAYA : AN IN-VITRO STUDY

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ABSTRACT:
The most frequently used non-steroidal anti-inflammatory drugs (NSAIDs) to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease. Moreover, long-term use of NSAIDs leads to serious side effects. Hence, the current study was designed with the main purpose to evaluate the anti-inflammatory activity of methanolic seed extract of *Carica papaya*. Seeds of *C. papaya* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with methanol. The *in-vitro* anti-inflammatory activity of methanolic seed extract of *C. papaya* was determined using albumin denaturation method. Qualitative analysis revealed major phytochemicals such as alkaloids, saponins, phenolic compounds, and tannins in methanolic seed extract of *C. papaya*. Quantitative estimation revealed 182.45 mg GAE/g and 24.12 mg GAE/g quantities of total phenolic compounds and tannins in methanolic seed extract of *C. papaya*. There was dose dependent inhibition (%) was observed in standard as well as methanolic seed extract of *C. papaya*. Furthermore, the inhibition (%) of methanolic seed extract of *C. papaya* at the concentration of 500 µg/mL was at par with that of standard drug i.e., Aspirin. In conclusion, methanolic seed extract of *C. papaya* could be explored in the development natural anti-inflammatory drugs.

INTRODUCTION

The growing incidence of cardiovascular, immunosuppressive and chronic inflammatory diseases due to the rapid pace of industrialization poses a serious threat for the well-being of mankind.[1] Several disorders such as cancer, diseases of the heart, gut, and central nervous system, diabetes, and many others have been treated through herbal medicine for centuries.[2-5] Recently, natural remedies and herbal medicines have attracted the world's attention because of the chemical hazards in the food industry.[6-10]

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants,[11] and it is often associated with pain and involves occurrences such as increased vascular permeability, enhanced protein denaturation, and rearrangement of the membrane.[12] When cells in the body are damaged by microbes, physical agents, or chemical agents, the injury is in the form of stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat, and swelling and loss of function in the injured area. Loss of function depends on the area and severity of the condition occurring. Since inflammation is one of the non-specific intelligences which is based on mechanisms of the body, a tissue response to an unintentional cut is similar to that of other forms of tissue damage caused by heat, radiation, bacterial, or viral.[12]

An organism or tissue elicits the inflammatory responses as a defensive mechanism; however, prolonged inflammation can result in undesired health effects as a result of interplaying various biomolecules that are secreted during the inflammation phase.

Inflammation in many diseases has been documented including cancer.[13] Chronic pain induced by inflammatory processes is a major clinical problem worldwide.[14] Non-steroidal anti-inflammatory drugs (NSAIDs) that are mainly used in the treatment of pain and inflammation related to a large variety of pathologies have been prepared and marketed.[1] NSAIDs, such as diclofenac, aspirin, and indometacin, block the biosynthesis pathway of prostaglandins by inhibiting the cyclooxygenase (COX) enzymes, producing anti-inflammatory, analgesic, and antipyretic effects.[14] Also, their long-term uses are associated with several serious adverse effects such as gastrointestinal disorders, water retention, renal failure, bronchospasm, and hypersensitivity reactions.[15] Hence, the discovery of new and safe analgesic and anti-inflammatory drug is needed.[16]

Screening of bioactive compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases. Papaya (*Carica papaya* L.) is a member of the family Caricaceae. This plant family has four genera including Jarilla, Cylicomorpha, and Carica. *C. papaya* L. is common papaya and extensively grown over the world. The plant is herbaceous, soft tissue and fast-growing (Figure 1).[17] It is known for its plethora folkloric used and pharmacological activities. It contains two major bioactive compounds, Papain and Chymopapain, which are sued in brewing, wine making, textile and tanning industries.[18] It also contains alkaloids, flavonoids and other phenolic compounds. It is used by the natives in treating malarial fever, diabetes mellitus, bacterial infections, as de-wormer and as an ecbolic agent.[19,20]

Figure 1. Showing *C. papaya* plant

The dry seeds of *C. papaya* are chewed to alleviate nagging headache and in reducing swollen wounds and reducing high blood pressure. The central nervous and cardiovascular effects of the methanol leaf extract of *C. papaya* have been documented.[21] The fruits can be directly applied topically to skin sours.[22] Papaya seeds extract is being currently marketed as a nutritional supplement with purported ability to rejuvenate the body condition and to increase energy. The product claims to improve immunity against common infections and body functioning. This provides the evidence for its immunomodulatory and its anti-inflammatory actions. Recently, the methanol extract of *C. papaya* seeds has been reported to have anti-nociceptive and anti-inflammatory activity in mice and rats.[23] With this background, in the present study we aimed to assess the anti-inflammatory activity of seed parts of *C. papaya*.

MATERIALS AND METHODS

Collection Seeds of *C. papaya*

C. papaya fruits (Figure 2) were collected from local fruit market in Bengaluru urban district, Karnataka, India. The papaya fruits were washed in tap water and then rinsed in sterile distilled water. The seeds were removed and shade dried at room temperature for one week. The dried seeds of *C. papaya* were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Figure 2. Showing *C. papaya* fruits and seeds

Extraction

Approximately 50 g of dried and coarsely powdered seeds of *C. papaya* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 500 mL of methanol. The extracts were concentrated by distilling the solvents in a rotary flash evaporator and dried at 40ºC. The extract was preserved in airtight containers and stored at room temperature until further use.

Qualitative Analysis

Qualitative analysis of methanolic seed extract of *C. papaya* was carried out through phytochemical screening using standard procedures to detect phytoconstituents as described by sofora,[24] Trease and Evans[25] and Harborne.[26]

Quantitative Analysis

Total phenolics

The concentration of total phenolics in methanolic seed extract of *C. papaya* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[27] The phenolic content of the extracts was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in methanol solution and was expressed in mg gallic acid equivalent (GAE)/g of extract powder (mg GAE/g).

Tannins

The tannin concentration was determined in methanolic seed extract of *C. papaya* following a modified version of the vanillin-HCl method.[28]

Assay of *In-vitro* Anti-inflammatory Activity

The *in-vitro* anti-inflammatory activity of methanolic seed extract of *C. papaya* was determined using albumin denaturation method. Control, Standard (Aspirin), and different concentrations of methanolic seed extract of *C. papaya* (i.e., 100-500 µg/mL) were prepared as follows;

Control: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and final volume was made up to 50 ml with double distilled water.

Standard (Aspirin): 2 ml of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations (100-500 µg/mL) of standard drug (Asprin) were taken and final volume was made up to 50 mL.

Methanolic seed extract of C. papaya: 2 mL of egg albumin, 28 mL of phosphate buffer (pH 6.4) and different concentrations of methanolic seed extract of *C. papaya* (i.e., 100-500 µg/mL) were taken and final volume was made up to 50 mL.

The reaction mixtures of control, standard (Aspirin), and different concentrations of methanolic seed extract of *C. papaya* (i.e., 100-500 µg/mL) were incubated at 37ºC for 15 minutes and heated at 70ºC for 5 minutes. After cooling the turbidity was measured at 660nm. Percentage inhibition of albumin denaturation was calculated using the following formula;

$$Inhibition\ (\%) = (1 - A2/A1)\ X\ 100$$

Where,
A1 = Absorption of the control sample; A2 = Absorption of the test sample

RESULTS

The major phytochemicals found in methanolic seed extract of *C. papaya* were found to be alkaloids, saponins, phenolic compounds, and tannins. Whereas, phytochemicals flavonoids and terpenoids were found to be absent in methanolic seed extract of *C. papaya* (Table 1).

Table 1: Qualitative analysis of methanolic seed extract of *C. papaya*

Phytochemical Components	**Methanolic Seed Extract of *C. papaya***
Alkaloids	+
Flavonoids	-
Saponins	+
Phenolic compounds	+
Tannins	+
Terpenoids	-

+: Present; -: Absent;

The results of quantitative estimation of phytochemicals in methanolic seed extract of *C. papaya* was represented in Table 2. Results revealed that the quantities of total phenolics and tannin was found to be 182.45 mg GAE/g and 24.12 mg GAE/g of extract respectively.

Table 2: Quantitative estimation of phytochemicals in methanolic seed extract of *C. papaya*

Phytochemical Components	**Methanolic Seed Extract of *C. papaya***
Total phenolics	182.45 mg GAE/g
Tannins	24.12 mg GAE/g

Values were expressed as mean; n=3

The results of *in-vitro* anti-inflammatory activities of standard and methanolic extract of *C. papaya* was represented in Table 3. Results depicted that the mean inhibition (%) exhibited by standard was found to be 36.91, 55.85, 67.14, 75.34, and 81.26 at the concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL respectively. The mean inhibition (%) exhibited by methanolic extract of *C. papaya* was found to be 23.26, 42.20, 53.49, 61.69, and 67.61 at the concentrations of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL respectively. These findings implied that there was dose dependent inhibition (%) was observed in standard as well as methanolic extract of *C. papaya*. Furthermore, the inhibition (%) of methanolic extract of *C. papaya* at the concentration of 500 µg/mL was at par with that of standard drug i.e., Aspirin.

Table 3. Assay of *in-vitro* anti-inflammatory activity of methanolic seed extract of *C. papaya*

Concentration (µg/mL)	**Standard**	**Methanolic Seed Extract of *C. papaya***
100	36.91	23.26
200	55.85	42.20
300	67.14	53.49
400	75.34	61.69
500	81.26	67.61

Values were expressed as mean; n=3

DISCUSSION

Ancient and traditional medicine widely used plants and their parts in treating several diseases and maintaining health, and this would be attributed to the fact that these plants contain large quantities of pharmacologically useful compounds in them.[29] Moreover, the most frequently used NSAIDs used to treat inflammatory conditions only alter the inflammatory response to the diseases, not the underlying cause of the disease.[30,31] Market demand exists for orally active molecules that are more effective than currently available medications at treating the underlying causes of inflammatory disease as opposed to just the symptoms.[11] Furthermore, Ethnobotanicals are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds.[32] Hence, the current study was designed with the main purpose to evaluate the anti-inflammatory activity of methanolic seed extract of *C. papaya.*

Chemokines, proinflammatory cytokines, C reactive Protein, vascular adhesion molecules, proinflammatory transcription factors and other neuropeptides regulate and contribute to the inflammation. TNF-α which is secreted by monocytes and macrophages plays a prominent role in the inflammatory process by induction of proinflammatory mediators such as IL-6, and IFN. Agents and compounds that reduced TNF-α were considered to possess anti-inflammatory properties.[33-35]

Protein denaturation is one of the important known causes of certain anti-inflammatory diseases where electrostatic, hydrogen and disulphide bonding were altered in denaturation mechanism. Results of our study delineated that methanolic seed extracts of *C. papaya* demonstrated anti-inflammatory activity at par with standard drug Aspirin. These findings were comparable with literature findings reports by various other research investigators. An *in-vitro* study reported that 1 mg/ml ethanolic papaya leaf extract demonstrated significant inhibition of TNF-α in dendritic cells which were treated with Lipopolysaccharide.[36] Aqueous extract of *C. papaya* seeds significantly reduced NO radical by 69.4% in a cell free assay *in-vitro*. Meanwhile, the aqueous extract at a concentration of 150 g/mL inhibited the release of lysosomal enzymes and stabilized human red blood cell membrane by 22.7%.[37]

Literature reports evidenced that *C. papaya* contains alkaloids, flavonoids and polyphenolic compounds,[38] and alkaloids, flavonoids and saponins have been found in other natural products with analgesic and anti-inflammatory properties.[39] Moreover, anti-inflammatory potential of methanolic fruit extract of Ashwagandha (W. somnifera) was ascribed to phenolic and flavonoid compounds present in the extract.[32] Therefore the anti-inflammatory activity of *C. papaya* extract may be due to the presence of alkaloids, flavonoids and other polyphenols.[40] In concurrence with literature findings in our study also methanolic seed extract

of *C. papaya* contains considerable quantities of phenolic compounds and tannins, and anti-inflammatory activity exhibited by methanolic seed extract of *C. papaya* could be accredited to secondary metabolites like phenolic compounds and tannins.

CONCLUSION

In conclusion, our study findings demonstrated anti-inflammatory potential of methanolic seed extract of *C. papaya*, and it is ascribed to secondary metabolites like phenolic compounds and tannins present in it. Therefore, methanolic seed extract of *C. papaya* could be explored in the development natural anti-inflammatory drugs.

REFERENCES

1. Keshamma E. Evaluation of Anti-inflammatory Activity *Solanum lycopersicum* (Tomato). International Journal of Food and Nutritional Sciences. 2022;11(11):2560-71.
2. Aziz T, Qadir R, Anwar F, Naz S, Nazir N, Nabi G, Haiying C, Lin L, Alharbi M, Alasmari AF. Optimal enzyme-assisted extraction of phenolics from leaves of *Pongamia pinnata* via response surface methodology and artificial neural networking. Applied Biochemistry and Biotechnology. 2024:1-8.
3. Rahim G, Qureshi R, Hazrat A, Ahmad B, Ali Khan A, Aziz T, Alharbi M, Alshammari A. Phytochemical, antimicrobial, radical scavenging and in-vitro biological activities of *Teucrium stocksianum* leaves. Journal of the Chilean Chemical Society. 2023;68(1):5748-54.
4. Shah SW, Siddique Afridi M, Ur-Rehman M, Hayat A, Sarwar A, Aziz T, Alharbi M, Alshammari A, Alasmari AF. In-vitro evaluation of phytochemicals, heavy metals and antimicrobial activities of leaf, stem and roots extracts of Caltha palustris var. alba. Journal of the Chilean Chemical Society. 2023;68(1):5807-12.
5. Aziz T, Ihsan F, Khan AA, ur Rahman S, Zamani GY, Alharbi M, Alshammari A, Alasmari AF. Assessing the pharmacological and biochemical effects of *Salvia hispanica* (Chia seed) against oxidized *Helianthus annuus* (sunflower) oil in selected animals. Acta Biochimica Polonica. 2023;70(1):211-8.
6. Ejaz U, Afzal M, Naveed M, Amin ZS, Atta A, Aziz T, Kainat G, Mehmood N, Alharbi M, Alasmari AF. Pharmacological evaluation and phytochemical profiling of butanol extract of *L. edodes* with in-silico virtual screening. Scientific Reports. 2024;14(1):5751.
7. Ahmad E, Jahangeer M, Akhtar ZM, Aziz T, Alharbi M, Alshammari A, Alasmari AF, Bukhari NI. Characterization and gastroprotective effects of *Rosa brunonii* Lindl. fruit on gastric mucosal injury in experimental rats–a preliminary study. Acta Biochimica Polonica. 2023;70(3):633-41.
8. Ahmad B, Muhammad Yousafzai A, Maria H, Khan AA, Aziz T, Alharbi M, Alsahammari A, Alasmari AF. Curative effects of *Dianthus orientalis* against paracetamol triggered oxidative stress, hepatic and renal injuries in rabbit as an experimental model. Separations. 2023;10(3):182.
9. Ahmad E, Jahangir M, Bukhari NI, Sarwar A, Aziz T, Alharbi M, Alsahammari A, Alasmari AF. Isolation, structure elucidation & antidiabetic potential of *Rosa brunonii* L. fruit–fight diabetes with natural remedies. Journal of the Chilean Chemical Society. 2023;68(2):5887-94.
10. Rather LJ, Mohammad F. *Acacia nilotica* (L.): A review of its traditional uses, phytochemistry, and pharmacology. Sustainable Chemistry and Pharmacy. 2015; 2:12-30.
11. Keshamma E. A Study on Assessment of In-Vitro Anti-Inflammatory Activity of Garlic (*Allium sativum*) Extract Using Albumin Denaturation Method. Natural volatiles & essential oils. 2018:167-72.
12. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clin Exp Immunol. 2007;147(2):227-35.

13. Medzhitov R. Inflammation 2010: New adventures of an old flame. Cell. 2010 Mar 19;140(6):771-6.
14. De Almeida Barros TA, De Freitas LA, Filho JM, Nunes XP, Giulietti AM, De Souza GE, Dos Santos RR, Soares MB, Villarreal CF. Antinociceptive and anti-inflammatory properties of 7-hydroxycoumarin in experimental animal models: potential therapeutic for the control of inflammatory chronic pain. Journal of Pharmacy and Pharmacology. 2010;62(2):205-13.
15. Hawkey CJ, Langman MJ. Non-steroidal anti-inflammatory drugs: overall risks and management. Complementary roles for COX-2 inhibitors and proton pump inhibitors. Gut. 2003;52(4):600-8.
16. Khan RA, Siddiqui SA, Azhar I, Ahmed SP. Preliminary screening of methanol and butanol extracts of *Tamarindus indica* for anti-emetic activity. Journal of basic and applied sciences. 2005; 1:2-5.
17. Singh PG, Madhu SB, Shailasreesekhar GT, Basalingappa KM, Sushma BV. In vitro antioxidant, anti-inflammatory and anti-microbial activity of *Carica papaya* seeds. Global Journal of Medical Research. 2020; 20:19-38.
18. Brocklehurst K, Salih E, McKee R, Smith H. Fresh non-fruit latex of *Carica papaya* contains papain, multiple forms of chymopapain A and papaya proteinase omega. Biochemical Journal. 1985;228(2):525.
19. Lohiya NK, Goyal RB, Jayaprakash D, Sharma S, Kumar M, Ansari AS. Induction of reversible antifertility with a crude ethanol extract of *Carica papaya* seeds in albino male rats. International journal of pharmacognosy. 1992;30(4):308-20.
20. Chinoy NJ, Dilip T, Harsha J. Effect of *Carica papaya* seed extract on female rat ovaries and uteri. Phytotherapy Research. 1995;9(3):169-75.
21. Gupta A, Wambebe CO, Parsons DL. Central and cardiovascular effects of the alcoholic extract of the leaves of *Carica papaya*. International Journal of Crude Drug Research. 1990;28(4):257-66.
22. Chinoy NJ, Padman P. Antifertility investigations on the benzene extract of *Carica papaya* seeds in male albino rats. J Aromatic Plant Sci. 1996; 18:489.
23. Anaga AO, Onehi EV. Antinociceptive and anti-inflammatory effects of the methanol seed extract of *Carica papaya* in mice and rats. African journal of pharmacy and pharmacology. 2010;4(4):140-4.
24. Sofora A. Medicinal plants and Traditional Medicine in Afric. John Wiley Son Ltd. 1993:150-3.
25. Trease GE, Evans WC. Pharmaccology, 11th Edtn. London: Brailliar Tiridel and Macmillian Publishers; 1989.
26. Herborne JB. Phytochemical methods. 3rd ed D.E. and Hall Ltd. London; 1973. p. 135-203.
27. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer L. Methods in Enzymology. 1999; 299:152-78.
28. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food chemistry. 2005;92(3):491-7.
29. Thomas E, Vandebroek I, Sanca S, Van Damme P. Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. Journal of ethnopharmacology. 2009;122(1):60-7.
30. O'Byrne KJ, Dalgleish AG, Browning MJ, Steward WP, Harris AL. The relationship between angiogenesis and the immune response in carcinogenesis and the progression of malignant disease. European journal of cancer. 2000;36(2):151-69.
31. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. British journal of cancer. 2001;85(4):473-83.

32. Akhila A and Keshamma E. Assessment of In-vivo Anti-inflammatory Potential of Fruit Extract of Ashwagandha (*Withania somnifera*) Using Carrageenan Induced Paw Edema Rat Model Study. Journal of Advanced Zoology. 2023;44(2): 3570-9.
33. Pratheebha C, Gayathri R, Veeraraghava VP, Kavitha S. Knowledge, awareness, and perception on root canal treatment among South Indian population–A survey. Journal of Advanced Pharmaceutical Technology & Research. 2022;13(Suppl 1):S302-7.
34. Kulkarni S, Wahane K, Daokar S, Patil K, Patel K, Thorat T. An assessment of the efficacy of a rotary and a reciprocating retreatment file system for removal of gutta-percha from root canals: An in vitro cone-beam computed tomography study. Endodontology. 2021;33(1):20.
35. Divya S, Jeevanandan G, Sujatha S, Subramanian EM, Ravindran V. Comparison of quality of obturation and post-operative pain using manual vs rotary files in primary teeth-A randomised clinical trial. Indian Journal of Dental Research. 2019;30(6):904-8.
36. Arun N, Ramesh S, Shankar A. Comparative Evaluation of Anti-Inflammatory and Antioxidant Property of *Carica Papaya* Leaf and Seed Extract - An Invitro Study. J Popul Ther Clin Pharmacol. 2023;30(14):e11-18.
37. Wijesooriya AA, Deraniyagala SA, Hettiarachchi CM. Antioxidant, anti-inflammatory and antibacterial activities of the seeds of a Sri Lankan variety of *Carica papaya*. Biomedical and Pharmacology Journal. 2019;12(2):539-47.
38. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of ethnopharmacology. 1998;61(1):57-65.
39. Fernanda LB, Victor AK, Amelia TH, Elisabetsky E. Analgesic properties of Umbellatine from *Psychotria umbellate*. Pharmaceutical Biol. 2002; 44:54-6.
40. Ahmed MZ, Ramabhimalah S. Anti-inflammatory activity of aqueous extract of *Carica papaya* seeds in albino rats. Biomedical and Pharmacology Journal. 2015;5(1):173-7.