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# Extraction, Phytochemical Screening and Anti-Inflammatory Evaluation of *Carica papaya* (Linn) Seed Oil

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**ABSTRACT**

Inflammation is a basic pathological response to the immune system. Lots of the known anti-inflammatory drugs possess serious adverse effects on humans. Natural products, mainly plants, possess bioactive compounds, which have been researched to have none or less side effects. *Carica papaya* seed is of great interest due to its medicinal and pharmacological properties and has a rich chemical composition. The aim of the present research was to screen the phytochemicals present and evaluate the anti-inflammatory activity of the various extracts of *Carica papaya* seed oil. Ultrasonic-assisted-maceration was used for the extraction. The phytochemical screening of the seed oils was performed by adaptation of the standard procedure. The anti-inflammatory activity was investigated using protein denaturation method. The phytochemical screening result showed the absence of phytosterols in hexane and chloroform extracts; saponins in hexane and ethanol; flavonoid, fixed oils in chloroform; steroids in hexane and glycosides; carbohydrates in ethanolic extracts but terpenoids, alkaloids, tannins and phenolic compounds were present in all extracts. Concentrations of 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml of the extracts were tested for anti-inflammatory activity and the result showed a decrease inhibition with increase in concentration.

**Keywords:** Anti-inflammatory, protein denaturation, phytochemicals, ultrasonic-assisted-maceration

## INTRODUCTION

Natural products particularly plants, have been utilised as a medicinal agent since ancient time by different countries. In the past, numerous antimicrobial compounds were found from both synthetic and natural products for the treatment and management of infectious pathogens [1-2]. Nevertheless, just a few of them were accessible to the needy world's market [3]. Plant-derived medicinal products have captivated the attention of scientists around the world for many years because of its minimal negative effects and positive effects on human health. Medicinal plants are thought to be a storehouse of innumerable types of bioactive compounds possessing diverse therapeutic properties.

Anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic properties are among the vast array of therapeutic effects associated with medicinal plants [4].

Plant constituents, also known as secondary metabolites or phytochemicals, are responsible for therapeutic qualities of plants to which they belong. Although their function in the plant is unclear, it might be outside the scope of protection. Plant secondary metabolites are different from primary metabolites. This includes, lipid, glucose, amino acid, and nucleic acid [5] are exceptionally different. Thousands of them have been identified in diverse classes. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are dispersed in various parts of the plants are some of the important secondary metabolites or phytochemicals [6].

Although natural products possess a wide range of intricate chemical structures, the plant secondary metabolites appear to possess greater biological friendliness and drug-likeness than those derived from exclusively synthetic sources. Hence, it is anticipated that the molecules derived from natural source are supposed to be better candidates for further drug development [7].

Paw-paw (*Carica papaya Linn*,) is a member in Caricaceae family allied to the Passifloraceae. It has been used experientially as food or as treatment for kidney stones, hypertension, urinary tract disorders, abdominal pain during menstruation, analgesic, dysentery, diarrhea, fever. It possesses carpain alkaloids, papain enzyme, pseudocarpain, glicoside, carposide, saponins, sucrose, dextrose and levulose [8]. Like other plants, *Carica papaya* have been reported to be used as medication in the treatment of diseases [9-11] such as antimalaria, anti diabetic, anti-cancer, anti-inflammatory etc. All parts of the papaya plant, including its leaves, fruits, seeds, bark, stem, and root, can be processed for nutritional and pharmaceutical goals. They are abundant in antioxidants and mineral ions, and they also contain phytochemicals and vitamins B [12]. Seeds make up to 15–20% of the fruit [13] and can be used as both food and medicine. Papaya (*Carica papaya Linn*.) have been valued for both its nutritional and medicinal value since long time. Papaya is a powerhouse of nutrients and is available as a year-round vegetable. It contains vitamin C, vitamin A, and vitamin E, three potent antioxidants [14] and also contains minerals, magnesium and potassium, folate and fibre.

To obtain oil from papaya seeds, different techniques are used in laboratory conditions such as soxhlet extraction using an organic solvent. The oil content of papaya seeds using soxhlet extraction was found to be in the range of 25.3–0.7 percent [15]. Also, maceration extraction is commonly used. In this case, oil is extracted by diffusing a solvent into oil bearing cells via the plant's cell wall. Samaram *et al* [16] used the supercritical fluid method to extract from papaya seeds; the result shows that the yield was low along with an excessive number of impurities detected in the oil.

*Carica papaya* (Linn) is such a plant, with several therapeutic properties making it unique among other species of family Caricaceae. The anti-malarial action of *C. papaya* has been supported by multiple investigations Papaya is a potential source of glucosinolates, isothiocyanates, and Benzyl isothiocyanates (BITC), all of which have been demonstrated anti-cancer-fighting effects. Studies have it that Benzyl isothiocyanate (BITC) tumour stops growth by causing apoptosis in cancer cells. Some researchers have suggested that the anti-carcinogenic characteristics of isothiocyanates could be related to their ability to activate phase II enzymes, including glutathione S-transferase, nicotinamide, adenosine diphosphate (ADP), and quinine reductase [17]. To avert stomach ulcers, papaya leaves and unripe fruits may be of help in treatment. Rats which receive papaya extracts as a pretreatment had a much lower stomach ulcer index than those that received alcohol and the conventional medications cimetidine and indomethacin respectively [18].

As inflammation is a basic host defence response of the body against external stimuli like an injury or infection caused by pathogen. Inflammation is a critical immunological response that enables the body to survive during an injury [19,20]. Studies on the anti-inflammatory activity of methanolic pulp and seed extracts of C. papaya. Findings from this study showed that the administration of 2 ml of extract decreased levels of serum IL-1 , serum IL-6 and IL-12 levels [21,22]. Several studies show the anti-inflammatory activities have been revealed on the leaf extracts of *Carica papaya* but limited research have been performed to test the anti-inflammatory activities of it seed oil.

Hence this research aimed at extraction, phytochemical analysis and evaluating the anti-inflammatory activity of n-hexane, chloroform and ethanol extracts of *Carica papaya* (*Linn*) seed oil. This will serve as a novel for anti-inflammatory drug discovery.

## MATERIALS AND METHODS

### Solvents/Reagents

Solvents used are ethanol, n-hexane, chloroform, ethyl acetate and methanol. They were made by Honeywell research chemicals. They were of general-purpose grade and were distilled before use. The chemicals used are of analytical grade. They are: Concentrated Hydrochloric acid (HCl), concentrated Sulphuric acid ($H_2SO_4$), NaOH, Benedict's reagents, Ferric chloride ($FeCl_3$), iodine solution, Distilled water, Ammonium solution, Dilute HCl, Glacial acetic acid, Dragendroff's reagent, Mayer's reagent, Ferric chloride, Hager's reagent.

**Plant Sample Collection**

The *Carica papaya* fresh seed was obtained from a farm in Potiskum town of Potiskum local government area of Yobe State, Nigeria, between the months of October and November, 2023 and was identified at the Department of Plant Biology, Federal University, Dutse, and assigned the herbarium accession number FUDHAN:004/25.

**Drying and Pulverization of the Plant Material**

After collection and authentication, the seeds were washed with distilled water to remove dust particles and air-dried in a shade. Then the dried seeds were pulverised to powder form in a mixer grinder.

**Extraction of Plant Material**

Ultrasonic-assisted-maceration method was used according to Soares et al [23] with minor modification. The extraction process of *Carica papaya* seed was performed by successive extraction (n-hexane, chloroform and ethanol) using ultrasonic bath (T-100S, China) planned with 40 KHZ fixed frequency and power density (600 W). temperature of 50-55 $^0$C on 6 hours sonication. The resulting mixture was kept for 72 hours with frequent agitation. The extracts were filtered with Whatman No. 1 filter paper (15 mm) and dried under vacuum at 40 $^0$C using a rotary evaporator (RE-52A, union laboratories England). The dried extracts were refrigerated at 4 $^0$C until further use. The percentage yield was calculated using the formula;

$$\% \text{ yield of extraction} = \frac{\text{Weight of extract} \times 100}{\text{weight of the dry sample}}$$

**Phytochemical Analysis**

The phytochemical screening of the seed extracts of *Carica papaya* plant were performed using an adaptation of the standard procedures outlined by Harborne [24], Abayomi [25] and Trease and Evans [26].

***Test for Alkaloids***

*Dragendorff's Test*: A small amount of the dried crude extracts (0.2 g) are dissolved in 1 ml of methanol in a test tube and then 2 - 3 drops of Dragendorff's reagent are added. The appearance of orange-red precipitate was taken as positive test for the presence of alkaloids.

*Mayer's Test*: Another 0.2 g of the crude extract are taken in 1 ml of methanol and 1 ml of Mayer's reagent added. The development of dull white precipitate indicated the presence of alkaloids.

***Test for Flavonoids***

*Alkali Test*: About 0.2 g of the dried crude extract in 1 ml of methanol is treated with 1 ml of 10% NaOH. The appearance of yellow colouration which disappeared upon addition of dilute acid indicated the presence the presence of flavonoids.

***Test for Saponins***

*Frothing Test*: Small portion of the plant extract are added to distilled water (10 ml) in a 50 ml beaker, boiled and filtered. About 5 ml of the filtrate are shaken vigorously for about 5 minutes. Frothing which persists on warming indicated the presence of saponins

***Test for Cardiac glycosides***

*Keller-killiani's Test:* A mixture of Acetic acid glacial (2 ml) with 2 drops of 2% $FeCl_3$ solution are added to the plant extract and test tube are held at an angle of 45 ˚ and 1 ml of concentrated sulphuric acid are also added carefully down the side. A brown ring produced between the layers indicated the entity of cardiac steroidal glycosides.

***Test for Steroids***

*Chloroform Test*: Exactly 2 ml of chloroform is added to 2 ml extract followed by same quantity (2 ml) of concentrated $H_2SO_4$, Formation of red colour in chloroform layer shows the presence of steroids

***Test for Tannins***

*Ferric chloride Test*: A solution of 0.2 g of the crude extract in 1 ml of methanol is treated with 2 ml of distilled water followed by 2 - 3 drops of fresh 2 % ferric chloride solution. The appearance of blue-green colour was indicative of the presence of tannins.

***Test for Terpenoids***

*Chloroform test:* To 0.2 g of the extract dissolved in 1ml of methanol, 2 ml of chloroform is added followed by few drops of conc. $H_2SO_4$ are added carefully along the side of the tube. The appearance of a reddish-brown colouration at the interface between chloroform and conc. sulphuric acid layers indicated the presence of terpenoids.

***Test for Glycosides***

*Ferric chloride Test*: To 0.2 g of the extract dissolved in 1ml of methanol is added 1ml of ferric chloride solution (prepared by mixing 1 volume of 5% ferric chloride, w/v and 99 volumes of glacial

acetic acid). Then few drops of conc. sulphuric are added carefully. The appearance of greenish blue colour was taken to indicate the presence of glycosides.

***Test for Reducing Sugars***

*Fehling's Test*: Fehling's solutions A and B were prepared fresh according to the methods outlined by Harborne [24] and Abayomi [25]. Equal volumes of Fehling's solutions A and B were mixed when required for use. 2 ml of this mixture is added to 0.2 g of the extract and boiled gently. The appearance of a brick red precipitate was taken to indicate the presence of reducing sugars.

***Test for Volatile Oil***

For volatile oil estimation 50 mg of powdered material (crude) is taken and subjected to hydro-distillation. The distillate is collected in graduate tube of the assembly, wherein the aqueous portion automatically separated out from the volatile oil.

***Test for Anthraquinone***

About 3.0 ml of the extract is shaken with 10.0ml of benzene, the mixture was filtered and 5.0 ml of 10 % v/v $NH_3$ solution was added to the filtrate. The presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxyl anthraquinones [26].

**Anti-Inflammatory Activity (Protein Denaturation Assay)**

The anti-inflammatory activity of *Carica papaya* was studied by using inhibition of albumin denaturation technique which was studied according to [27, 28] followed by minor modifications. The reaction mixture is made up of test extracts, Phosphate Buffer Saline (pH 6.4) and a 1% aqueous solution of bovine albumin fraction. A little amount of 1N HCl was used to alter the reaction mixture's pH. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, the turbidity was measured at 660 nm after cooling the samples (UV-Visible Spectrophotometer Model 721, Elico India Ltd). The experiment was performed in triplicate and the reaction mixture without test extracts was used as the negative control. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) / Abs control X 100

**Statistical Analysis**

Statistical analyses were performed using SPSS version 22 software. Data were analysed by one way analysis of variance (ANOVA) to determine difference between treatment means followed by Dunnett's multiple comparison test and the ranking was obtained using Turkey HSD test. The p-value ($< 0.05$) was considered statistically significant. All data are presented as mean ± standard deviation and each analysis was done in triplicate.

## RESULTS AND DISCUSSION

### Physical Characteristics of the Extracts of *Carica papaya* Seed oil

The physical characteristics of different extracts (n-hexane, chloroform and ethanol) of *Carica papaya* seed oil is shown in Table 1.

Table 1: Physical characteristics of various extracts of the seed *Carica papaya* (*Linn*)

| Plant part | Extracts | Color | Texture |
|---|---|---|---|
| Seed | Ethanol | Reddish brown | Oil |
| | Chloroform | Brownish green | Oil |
| | n-Hexane | Lighter brown | Oil |

### Percentage yield of *Carica papaya* seed oil

The total amount in gram of the dry sample that was subjected for extraction and the percentage yield in w/w of the three solvent is shown in Table 2.

Table 2: Percentage yield of seed of *Carica papaya* (*Linn*)

| Plant Sample | Dry sample(g) | Extracts | Weight (g) | % Yield (w/w) |
|---|---|---|---|---|
| *Carica papaya* Seed | 600 | n-Hexane | 234.38 | 39.00 |
| | | Chloroform | 71.50 | 15.03 |
| | | Ethanol | 47.00 | 10.68 |

### Phytochemical Screening Results

The phytochemical screening of the n-hexane, chloroform and ethanol seed oil are depicted in Table 3. The result reveals the presence of alkaloids, terpenoids, tannins and phenolic compounds in the three extracts (n-hexane, chloroform and ethanol). Saponins and steroids were absent in the n-hexane extract, flavonoids, phytosterols and fixed oils & fats were absent in chloroform extract while tannins & phenolic compounds, saponins, glycosides and carbohydrates were absent in ethanol. The presence of alkaloid, terpenoids, tannins and phenolic compounds in the oils suggests that it could be used as anti-inflammatory drug plant

Table 3: Phytochemical screening result of seed extracts of *Carica papaya* (*Linn*)

| Phytochemical | Test name | Seed | | |
|---|---|---|---|---|
| | | HE | CL | ET |
| Alkaloid | Mayers test | - | + | + |
| | Dragendroff's test | + | + | + |
| | Wagner's test | + | + | + |

| | | | | |
|---|---|---|---|---|
| Tannin & phenolic compounds | General test | + | + | - |
| | Lead acetate test | + | + | + |
| Flavonoids | Shinoda's test | + | - | + |
| | Alkaline reagent test | + | - | + |
| Saponins | Frothing test | - | + | - |
| Glycosides | Salwoski's test | + | + | - |
| | Keller killani test | - | - | - |
| Terpenoids | - | + | + | + |
| Steroids | - | - | + | + |
| Fixed oil & fats | Spot/stain test | + | - | + |
| Phytosterols | Libermann Burchard's test | - | - | + |
| | Salwoski's test | + | - | + |
| Carbohydrate | Test for starch | + | + | - |

KEY: +(positive), -(negative), HE = n-hexane, CL = Chloroform, ET = Ethanol

**Result of anti-inflammatory activity (Protein denaturation assay)**

Protein denaturation can be defined as a process by which proteins lose their tertiary structure and secondary structure by the application of external stress during food processing and post-harvest treatments [29]. Most biological proteins loose their biological function when denatured. The compounds inhibiting denaturation greater than 20% over the range of concentration were considered as having anti-inflammatory properties [30]. The result of the study showed that there was a statistical significance between the test groups and the control groups (standard drugs) (Table 4). In this study, n-hexane, chloroform and ethanolic extracts of *Carica papaya* seed oil, at a concentration of 200 mg/mL, were capable of inhibiting denaturation of albumin by 64%, 59% and 62%, respectively. An increase of sample concentrations resulted in decrease anti-inflammatory property (Table 4), while the controls (prednisolone and diclofenac) show the highest inhibition of denaturation by 63% and 68% at 400 and 800 µg/ml. The results suggest *Carica papaya* seed oil of the varying extracts used may have the anti-inflammatory property approximately by 50% inhibition, if they are used at low concentration.

Table 4: Result of percentage protein inhibition of standard drug

| Treatments | Concentrations (µg/ml) | Absorbance(660nm) | % inhibition | p-value(0<0.05) |
|---|---|---|---|---|
| Control | - | 0.245 | - | |
| Hexane Extracts | 200 | 0.08670±0.000 [a] | 64 | 0.000 |
| | 400 | 0.08767±0.003[a] | 63 | |
| | 600 | 0.09967±0.001[a] | 60 | |
| | 800 | 0.10133±0.012[a] | 59 | |
| | 1000 | 0.10300±0.005[a] | 58 | |
| Chloroform Extracts | 200 | 0.10100±0.005[b] | 59 | 0.000 |
| | 400 | 0.09567±0.001[b] | 61 | |
| | 600 | 0.11400±0.001[b] | 53 | |
| | 800 | 0.12100±0.001[b] | 51 | |
| | 1000 | 0.19267±0.001[b] | 22 | |
| Ethanolic extracts | 200 | 0.09300±0.001[b] | 62 | 0.000 |
| | 400 | 0.10467±0.001[c] | 57 | |
| | 600 | 0.13367±0.008 [c] | 45 | |
| | 800 | 0.14033±0.008[b] | 43 | |
| | 1000 | 0.17033±0.001[c] | 31 | |
| Prednisolone | 200 | 0.09800±0.001[c] | 60 | 0.000 |
| | 400 | 0.09100±0.001[d] | 63 | |
| | 600 | 0.09333±0.008[d] | 62 | |
| | 800 | 0.09500±0.001[b] | 61 | |
| | 1000 | 0.10667±0.001[c] | 57 | |
| Diclofenac Sodium | 200 | 0.10867±0.000 [c] | 56 | 0.000 |
| | 400 | 0.09633±0.000[e] | 61 | |
| | 600 | 0.08300±0.004[e] | 66 | |
| | 800 | 0.07800±0.001[d] | 68 | |
| | 1000 | 0.09000±0.001[d] | 63 | |

Values are Mean ± Standard Deviation, n = 3, values with superscripts a, b, c, d, e, are considered significant ($p < 0.05$). when compared with the standard and depending on the concentration variation.

## CONCLUSION

The resources available on this plant (*Carica papaya seed* oil) show the medicinal value of *Carica papaya* seed oil as it possesses potent anti-inflammatory property. This may be due to the presence of secondary metabolites in the plant as observed in the phytochemical screening. The present study evaluated the anti-inflammatory activity of n-hexane, chloroform, and ethanol extracts of seed of *Carica papaya* using protein denaturation (Bovine serum albumin) method at the concentrations of 200, 400, 600, 800 and 1000 µg/ml. n-Hexane extract of *Carica papaya* seed oil showed maximum percentage inhibition against the test samples than the chloroform and ethanol extracts when compared with the standard drugs (Diclofenac and Prednisolone). The percentage inhibition decreased with an increase in the concentration. The statistical analysis of the test group compared to the standard shows that there is a statistically significant observation between the test group and the standard drugs.

## RECOMMENDATION

Safety level (Toxicity) on the consumption of the *Carica papaya* seed oil should be looked upon. Isolation of the various compounds should be performed whereas their anti-inflammatory efficacy should be tested separately.

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