

Mineral composition, Antioxidant and Anti-inflammatory Activities of the Crude Extract of Leaves of *Carica papaya* L.

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Carica papaya L. (family *Caricaceae*) commonly known as papaya is a well-known fruit crop also having under-utilized medicinal potential. Mineral analysis shows that a significant level of potassium (207.10 ± 0.40 mg/100g) and phosphorous (65.60 ± 0.59 mg/100g) are present. Antioxidant activity of hexane, chloroform, ethyl acetate and ethanol crude leaf extracts were evaluated. IC_{50} hexane fraction (321.82 ± 0.78) demonstrated the highest ABTS radical scavenging activity and IC_{50} of the chloroform fraction (623.23 ± 1.32) showed the strongest DPPH free radical scavenging activity and their activities were stronger than that of ascorbic acid. Ethanolic extract IC_{50} shows significant anti-inflammatory activity (173.66 ± 1.24) when compared with other extract fractions. The present study signifies that the leaves of *C. papaya* are rich in minerals and known to possess significant antioxidant and anti-inflammatory activities. This shall be further explored to identify novel bioactive substances with pharmaceutical applications.

Key words: *Carica papaya* L., antioxidant, anti inflammatory, mineral composition

Medicinal plants, both wild and cultivated, have been used since the beginning of civilization as food and medicine. These plants produce a wide range of secondary metabolites as defence chemicals and they have found various therapeutic uses in medicine from time immemorial (Dilipkumar Pal *et al.*, 2011). Papaya is a well-known plant for its edible fruit and it belongs to the family *Caricaceae*. They are tree-like large plant with 5-10 m tall stem and the leaves (50-70 cm diameter) are confined strictly to the top. The flowers emerge on the axils of the leaves and develop into large fruit. Fruits are large and big in size, fleshy orange in colour and seeds are numerous, black in colour, round in shape and they are covered with the gelatinous aril (Mahendra and Nikhil, 2016).

Carica papaya is commonly known as paw paw which is a rich source of carotenoids, ascorbic acid, thiamin, riboflavin, niacin, pyridoxine and vitamin K (Adetuyi *et al.*, 2008 and Bari *et al.*, 2006). The well riped fruits are popularly used as dessert or it can be made into a puree, wine and jam, while the unripe ones are used for cooking as raw vegetable (Matsuura *et al.*, 2004 and Ahmed *et al.*, 2002). Papaya is a good source of vitamin as mentioned and the amount varies between the maturation stages and varieties (Hernandez *et al.*, 2006 and Wall 2006).

The entire plant of *Carica papaya* is economically valued in the various field, especially, has excellent medicinal properties. Investigation reports show that it helps to overcome heart-disease and also reduce high cholesterol levels. Papaya efficiently treats all types of abdominal, digestive problems such as dyspepsia, hyperacidity, dysentery and constipation. It is a potential source of beta-carotene that prevents the cause of some forms of cancer due to the activity of free radicals. Flowers are consumed orally to treat cough, bronchitis, asthma and chest cold. Leaves and fruits of papaya contain several proteins and alkaloids with the importance of industrial and pharmaceutical applications. Carapine, an alkaloid present in papaya is used as a heart depressant and for gastrointestinal ailment (Aravind *et al.*, 2013).

Some of the significant medicinal properties which include inhibition of cancer cell growth, helping to cure

dengue fever, facilitate digestion, acne medicine, increase appetite, ease menstrual pain, meat tenderizer and relieve nausea. (Aravind *et al.*, 2013). Regular consumption of ripen papayas overcomes habitual constipation and prevents premature ageing (Orwa *et al.*, 2009). The unripe papaya fruits produce a kind of proteolytic enzyme called papain that helps in the digestion of proteins so it is used as an aid for indigestion (Ezekiel and Florence, 2012).

Biomolecules that inhibit the activity of free radicals and delay the oxidative damage of protein, lipids etc., are known as antioxidants. (Lim *et al.*, 2006). Some secondary metabolites in the papaya were related to the antioxidant activity. Previous studies on the DPPH free-radical scavenging activity using water extract of flesh seeds of papaya indicated the most potent antioxidant activity (Kothari and Seshadri, 2010). Inflammation is a problematic biological comeback of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Many medicinal plants have shown to exhibit potent anti-inflammatory effects in the treatment of inflammation (Deepa and Renuka, 2014). Bamidele *et al.* (2008) reported the anti-inflammatory activities of ethanolic extract of leaves of papaya in rats using carrageenan-induced paw edema and formaldehyde induced arthritis models. The present study is envisaged to estimate the mineral content, antioxidant and anti-inflammatory activities of the crude extract of papaya leaf powder.

MATERIALS AND METHODS

Source of the Material

Leaves of *Carica papaya* L. were collected from in and around Tambaram, Tamil Nadu. The species was authenticated in the Centre for Floristic Research and Herbarium, Department of Botany, Madras Christian College, Tambaram, Tamil Nadu (Livingstone and Henry, 1994).

Mineral Analysis

One hundred gms of fresh leaves were taken and shade-dried for seven days. After that, it was grinded, sieved and the finely powdered leaf sample was kept in an air-tight container and used for further investigation. Minerals were determined by digesting the leaf powder

in 3M Hydrochloric acid. The five inorganic elements calcium, iron, magnesium, phosphorus and potassium were determined according to the methods of AOAC (2003).

Preparation of the Leaf extract by Soxhlet extraction procedure

3gms of leaf samples were placed in the filter paper cartridge (No 89) in a standard Soxhlet apparatus and extracted with 170 ml of different solvents such as hexane, chloroform, ethyl acetate and ethanol for 2 hours. Extracts were cooled to room temperature and used at different dilutions for antioxidant and anti-inflammatory activity determination.

Antioxidant Activity

ABTS Radical Scavenging Assay

Radical scavenging activity using ABTS was carried out according to the procedure followed by Delgado-Andrade *et al.* (2005). ABTS was obtained by mixing 7mM ABTS solution and 2.45 mM $K_2S_2O_8$ (Potassium persulfate). Then the mixture was left undisturbed in the dark for 12 - 16 h before use. The ABTS solution further diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. After the addition of 1 mL of plant extract solution of varying concentrations (10-60 $\mu\text{g/mL}$) to 1 mL of diluted ABTS⁺ solution, the absorbance was measured after 30 min. The free-radical scavenging activity of the samples by ABTS was calculated by,

$$\% = [(A_0 - A_1)/A_0] \times 100$$

DPPH Free-Radical Scavenging Assay

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) assay by Raaman (2015) with slight modification was used to determine free radical scavenging activity of the plant extract. Using methanol the solution of 1 mL of 0.1mM DPPH was prepared. Various concentration of 0.1 ml of the extract was reacted with 1.9 ml of DPPH solution. The reaction content was mixed carefully and left in the dark (room temperature) for 30 mins. The absorbance reading of the extract was recorded at 517 nm wavelength. Ascorbic acid (vitamin C) is a great antioxidant agent was used as the standard reference. The capacity of the sample to scavenge DPPH radical inhibition was calculated from the following formula:

$$\% \text{DPPH inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

In-vitro Anti-inflammatory Activity (Anti-denaturation assay)

Anti-inflammatory assay was carried out according to Gnana Ruby Priya *et al.* (2011) with minor modifications. The standard drug and leaf extracts with different solvents were dissolved in a least amount of DMS (Dimethyl Formamide) and the extract is further diluted with phosphate buffer (0.2 M) with a pH of 7.4. The concluding amount of DMF in all solutions was less than 2.5%. Test Solution (4ml) containing various concentrations of the drug was mixed with 1 ml of 1mM albumin solution diluted in phosphate buffer and incubated at 37°C in the incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 15 min. At 600nm, the turbidity was noted and measured. Inhibition percentage was measured from reference sample (control). The diclofenac sodium which is a strong anti-inflammatory drug was used as standard reference. The percentage of inhibition was calculated using the following formula.

$$\text{Percentage of inhibition} = 100 \times (A_t - A_c) / A_t$$

A_t = O.D. of test solution

A_c = O.D. of control

RESULTS AND DISCUSSION

Carica papaya has been used as a nutritious and medicinal plant over many centuries.

Generally, leaves, fruits and seeds of this plant are used as ethnomedicine. Because of its medicinal uses, we attempted to determine its antioxidant and anti-inflammatory activities. It was also screened for its mineral content. According to Otsuki *et al.* (2010), numerous studies were conducted related to papaya fruits and seeds mentioning about its macro and micro mineral contents.

Mineral analysis

This study made an effort to investigate the mineral composition of papaya leaves which includes potassium, calcium, magnesium, phosphorus and iron (Table 1).

The results revealed that leaves of papaya contain notable amount of potassium (207.10 ± 0.40 mg/100g), phosphorus (65.66 ± 0.59 mg/100g), calcium (23.10 ± 0.99 mg/100g), magnesium (13.83 ± 0.73 mg/100g) and iron (4.67 ± 0.69 mg/100g). The mineral profile of *Peperomia pellucida* whole plant by Der-Jiun *et al.* (2012) shows similar results to our results of mineral composition.

Potassium is an essential mineral to both cellular and electrical functions in the body. Na and K are mainly involved in maintaining the ionic balance of the human body and tissue excitability (Indrayan, 2005). Because of the solubility of both the salts, Na plays a promising role in the transport of metabolites. Potassium salt is of great significance as a diuretic. Makanjuola and Makanjuola (2018) reported that potassium level was high both in the papaya seeds of 720.83 mg/100g and 504.33 mg/100g in the skin. The third most rich element present is calcium (Indrayan, 2005). Cells use calcium to activate certain enzymes and help to transport ion across the cellular membrane, which also plays a vital role in maintaining the regular heartbeat. Therefore Papaya is recognised as an essential source of Ca which is needed for the regulation of muscle contraction, glandular secretion and for mediating vascular contraction (Straub, 2007). Magnesium is an essential mineral for energy activity like calcium and chloride plays a role in regulating the acid-alkaline balance in the body (Bouanga Kalou *et al.*, 2011). In our present study, magnesium is present comparatively in low amount (13.88 ± 0.73 mg/100g). Akpanabiater *et al.* (1998) reported that magnesium is an important cofactor in many enzymatic reactions and in many enzyme-catalysed reactions.

Among all the micronutrients, iron has the best-described history. It is a vital micro mineral which plays an important role in the metabolism of almost all living organisms. Iron is found to be in relatively low amount (4.67 ± 0.69 mg/100g) in papaya leaf. In humans, Iron serves to be a main constituent in a number of proteins and enzymes. (Beard *et al.*, 1997 and Fairbanks, 1999). Deficiency of iron is a frequent nutritional problem affecting many people all over the world. It is an important proportion in the diet of pregnant women, nursing mothers, infants, convulsing patients and elderly

people to prevent anemia and other related diseases (Oluyemi *et al.*, 2006). Similar results were obtained by Makanjuola and Makanjuola (2018) for papaya seeds 4.21 mg/100g and 2.73 mg/100g for skin respectively.

Antioxidant Activity

The scavenging of the stable DPPH radical and ABTS radical is used widely to estimate the antioxidant activity of fruits, vegetables, cereal grains, wine, etc., from the phenolic compounds present in them. It is depended on the quantity of the reducing ability of antioxidants toward DPPH and ABTS. The results of the ABTS antioxidant analysis is shown in Fig. 1. The results indicate that there is significant decolourization in the ethanol and hexane extracts at 500 μ g and 1000 μ g respectively. Fig. 2 indicates the maximum inhibition percentage in the 500 μ g and 1000 μ g concentrations of all the extracts used in this study. The IC₅₀ value of the ethanolic fraction indicates the strongest antioxidant activity (43.48 ± 0.89) followed by ethyl acetate fraction (68.13 ± 0.80). Kaibing Zhou *et al.* (2011) found that ABTS radical scavenging activity of the *n*-butanol fraction was significantly stronger than that of the other extracts in the seeds of papaya. Yuhahara and Iswahyuni (2018) revealed that the extracts from papaya seeds especially ethyl acetate and *n*-butanol contained free radical scavengers, possibly acting as principal antioxidants.

The DPPH is a stable radical with maximum absorption at 517 nm that can readily undergo scavenging by antioxidant (Lu and Yeap Foo, 2001). Porto *et al.* (2000) and Soares *et al.* (1997) found that it has been widely used to test the ability of compounds as free radical scavengers by hydrogen donors and to evaluate the antioxidant capacity. The antioxidant activity performed with the DPPH free radical scavenging method result (Fig 3) indicates there is significant decolouration in ethanol extract at 500 μ g and 1000 μ g concentrations. Fig. 4 demonstrates maximum inhibition percentage in ethanol and ethyl acetate extract at 500 μ g and 1000 μ g concentrations. According to Hanani *et al.* (2005) a substance can be expressed to be a strong antioxidant if it has an IC₅₀ value <200 ppm, that has antioxidant capabilities such as those associated with phytochemical screening results that

include flavonoids. Flavonoids have been studied to have various activities such as antioxidants and antibacterials (Mohan *et al.*, 2008).

The DPPH free radical-scavenging activities of the samples were estimated by comparing the IC₅₀ of the extract fractions and ascorbic acid. Concentration of the sample necessary to decrease the initial concentration of DPPH by 50% (IC₅₀) under the experimental condition was calculated. Therefore, a lower IC₅₀ value indicates a higher antioxidant activity. According to the IC₅₀ values, the ability to scavenge DPPH free radicals of the sample could be ranked as ascorbic acid>ethanol>ethyl acetate>hexane>chloroform fraction (Table 2). The IC₅₀ value of the ethanolic fraction indicates the strongest antioxidant activity (127.41±0.85) followed by ethyl acetate fraction (182.76±0.82) which had stronger antioxidant activity too. Yuhahara and Iswahyuni (2018) reported a similar results that ethanolic extracts of papaya leaf have a strong antioxidant activity with IC₅₀ value of 100.0±0.07 ppm.

Mahattanatawee *et al.* (2006) found that the antioxidant activity is mainly due to the presence of phenolic compounds which are the important constituents of fruit because they inactivate the lipid free radicals or prevent the decomposition of hydroperoxides into free radicals. Kaibing Zhou *et al.* (2011) investigated the DPPH free radical-scavenging activities of all extracts from seeds of papaya which is related to the amount of antioxidant constituents extracted from seeds of papaya by various solvents. Compounds such as ascorbic acid, β-carotene, α-carotene and different xanthophylls have been detected in banana and may have contributed to the antioxidant activity of the extracts (Kondo *et al.*, 2005). The scavenging activity on DPPH radical was related to the ripening stage, the activity increased as a result of increasing ripening stage for each stage. These results imply that antioxidative compounds other than phenolics and flavonoids were also involved in inhibiting the DPPH radicals.

Anti-inflammatory activity

Inflammation is the immune response of body tissue to injury or infection (Bamidele *et al.*, 2008). It is an essential component for natural immunity. The inflammatory route involves a difficult biological fall of

cellular signals that modify physiological responses and eventually resulting in the familiar clinical symptoms of pain, swelling, heat and redness (Comalada *et al.*, 2005). The role of inflammation is to destroy invading microorganisms, inactivate toxins and to achieve healing and repair. However, it may be potentially harmful, causing life threatening hypersensitivity reactions and progressive organ damage. Due to the enormous medicinal potential of the *Carica papaya*, the present study made an effort to evaluate the *in vitro* anti-denaturation activity from leaf powder of papaya.

The results of the different solvent extracts namely chloroform, ethanol, ethyl acetate and hexane towards anti-denaturation activity are indicted in Fig. 5. Maximum inhibition is shown in the hexane extract at 1000µg and 2000µg concentration (Fig 6). The results of IC₅₀ values (Table 3) have demonstrated that the chloroform extract have good anti-denaturation activity 40.94±0.82 followed by ethyl acetate 64.65±0.85 when compared to the other extracts. Saurabh *et al.* (2016) reported significant anti-inflammatory activity of papaya leaf juice (at 0.72 ml/ 100 g body weight) against carrageenan-induced rat paw edema and impaired *in vivo* vascular permeability with a percentage of 82.0 while inducing a percentage of 10 membrane-stabilizing activity of rat RBC. Papaya seeds (methanol and aqueous extract) have also been examined and found to display anti-inflammatory activity in *in vivo* study (Amazu *et al.*, 2010; Umana *et al.*, 2014). Unfortunately, none of the studies in *in vivo* investigated the anti-inflammatory or immunomodulatory effects of any part of the papaya plant in the non-polar extracts.

Many promising bioactive molecules have been isolated from papaya and investigated for their medicinal applications. The proved medicinal activity of papaya includes antioxidant, anti-inflammatory, antimicrobial, anti-fertility, antimalarial and immune modulatory etc. (Krishna *et al.*, 2008; Tatyasaheb *et al.*, 2014; Vij and Prashar, 2015). The leaves of *C. papaya* is an excellent source to treat dengue fever and it was proved to increase platelet and total white blood cell count (Ahmad *et al.*, 2011; Sarala and Paknikar, 2014). The leaves of papaya are rich in several minerals and many studies reported that these minerals content may balance the

deficiency caused by the virus and strengthen the immune system (Sharma *et al.*, 2013). So in this study we made a great attempt to analyse the dried crude leaf extract of papaya to investigate the mineral profile,

antioxidant and anti inflammatory activities which serve as a preliminary source and it needs much more clinical trial on this.

Table 1 Mineral analysis of the leaves of Papaya - Macronutrients

Minerals	mg/100g of leaf powder
Potassium	207.10±0.40
Calcium	23.10±0.99
Magnesium	13.83±0.73
Phosphorus	65.66±0.59
Iron	4.67±0.69

Values are represented as mean ± standard deviation (n=3).

Table 2 Antioxidant activity of the leaves of papaya by ABTS and DPPH free radical scavenging assay

Leaf extract	ABTS assay (IC ₅₀ µg)	DPPH assay (IC ₅₀ µg)
Hexane	321.82±0.78	274.60±0.75
Chloroform	263.06±0.82	623.23±1.32
Ethyl acetate	68.13±0.80	182.76±0.82
Ethanol	43.48±0.89	127.41±0.85
Ascorbic acid	7.42±0.69	11.11±0.81

Values are represented as mean ± standard deviation (n=3).

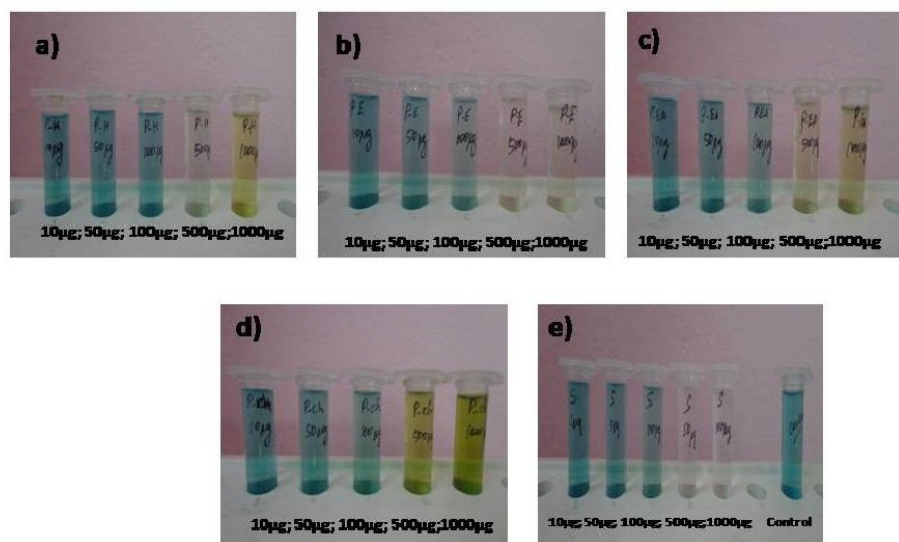


Fig. 1 Antioxidant assay for papaya leaves using ABTS method

a) Hexane; b) Chloroform; c) Ethyl acetate; d) Ethanol; e) Ascorbic acid (Standard)

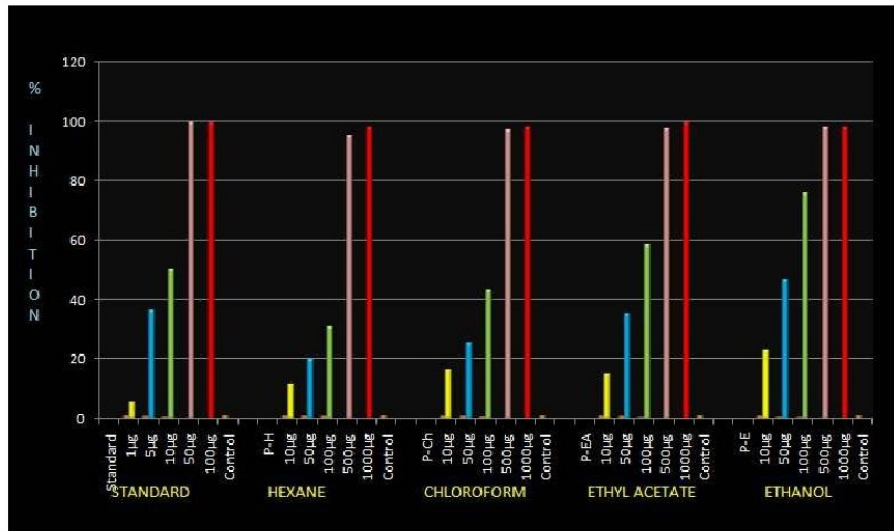


Fig 2 Antioxidant assay by ABTS radical scavenging activity using papaya leaves

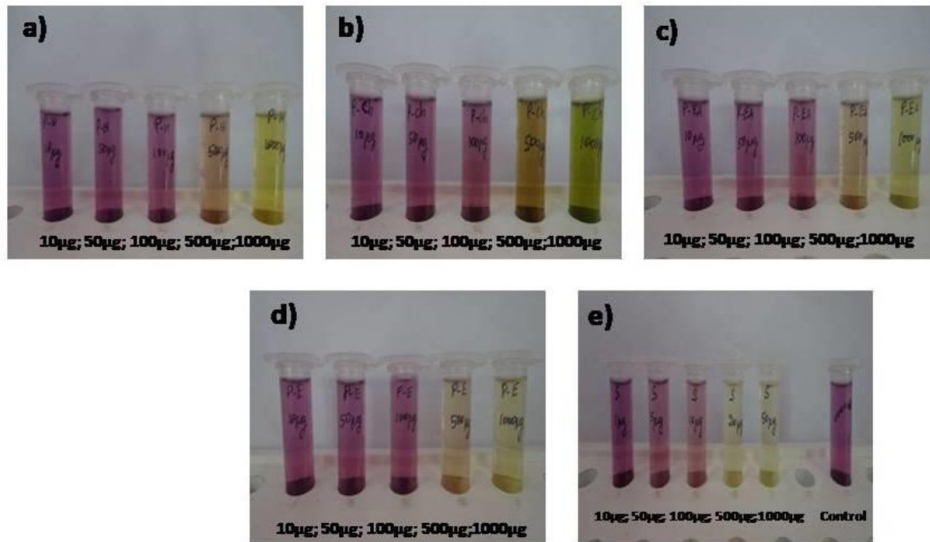


Fig 3 Antioxidant assay of papaya leaves using DPPH method

a) Hexane; b) Chloroform; c) Ethyl acetate; d) Ethanol; e) Ascorbic acid (Standard)

Table 3 Anti-inflammatory activity from the leaves of papaya

Leaf extract	IC ₅₀ µg
Hexane	68.61±0.74
Chloroform	40.94±0.82
Ethyl acetate	64.65±0.85
Ethanol	172.32±0.77
Diclofenac Na	41.07±0.81

Values are represented as mean ± standard deviation (n=3).

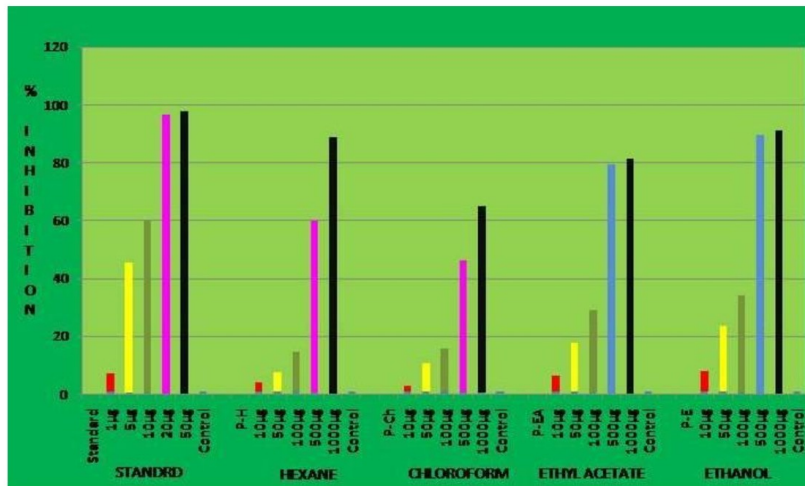


Fig 4 Antioxidant assay by DPPH radical scavenging activity using papaya leaves

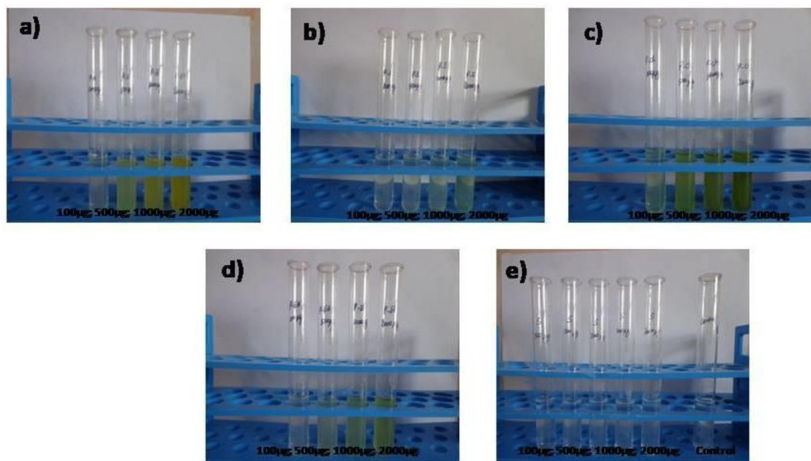


Fig 5 Anti-inflammatory assay of papaya leaves using egg albumin method

a) Hexane; b) Chloroform; c) Ethyl acetate; d) Ethanol; e) Diclofenac Na (Standard)

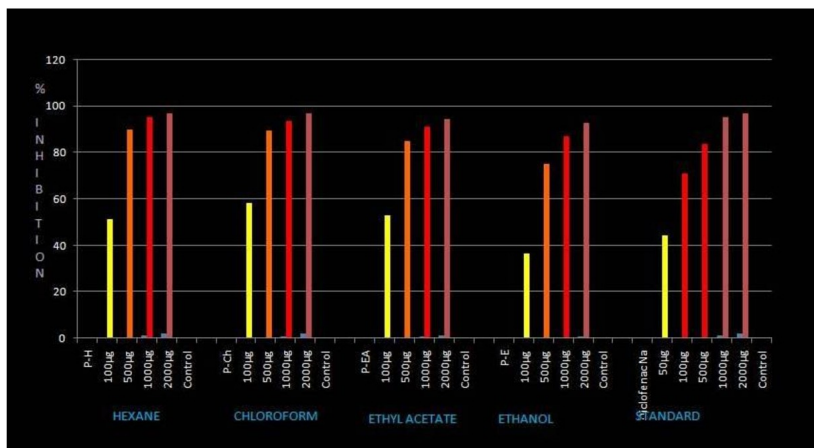


Fig 6 Anti inflammatory activity of papaya leaves using Egg albumin method

CONCLUSION

Papaya is a famous and common fruit known for its medicinal properties world wide. The present study is to investigate the potential of *C. papaya* in the evaluation of antioxidant and anti-inflammatory studies. Papaya extract undoubtedly offers a cheap and possibly valuable treatment for dengue. A herbal medicine being investigated to control the epic problem of dengue is the extract of the leaves of the plant. These evidences are inadequate to comment on the role of papaya leaf extract in dengue. The active compound needs to be identified and the dosage should be standardized to prove its efficacy in dengue beyond doubt. Large-scale randomised studies could establish the efficacy of this natural product in the treatment of dengue. It is also necessary to conduct pharmacokinetic studies to ensure that the active principle is absorbed from the digestive tract. The present study will provide pavement to explore the future application of *Carica papaya* leaves as a raw material in the pharmaceutical industries, especially in the treatment of dengue with clearly defined outcome measures.

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CONFLICTS OF INTEREST

All authors have declared that they do not have any conflict of interest for publishing this research.

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