2.1 Plant profile of *Carica papaya* Linn.

**Name:** *Carica papaya* Linn.

**Family:** Caricaceae

**Parts used:** Leaf

**Vernacular names**

- **Hindi:** Popaiyah, Papita, Papaya, Papeeta;
- **English:** Papaw Tree, Papaya;
- **Bengali:** Pappaiya, Papeya;
- **Kan:** parangi,
- **Malayalam:** kappalam;
- **Tamil:** paalai, pappali;
- **Telugu:** bappayi, boppayi, madananaba.

**Taxonomical Hierarchy**

<table>
<thead>
<tr>
<th>Domain</th>
<th>Flowering plant</th>
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<tbody>
<tr>
<td>Kingdom</td>
<td>Plantae</td>
</tr>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
</tr>
<tr>
<td>Class</td>
<td>Mangoliopsida</td>
</tr>
<tr>
<td>Subclass</td>
<td>Dilleniidae</td>
</tr>
<tr>
<td>Division</td>
<td>Mangoliophyta</td>
</tr>
<tr>
<td>Super division</td>
<td>Spermatophyte</td>
</tr>
<tr>
<td>Phylum</td>
<td>Steptophyta</td>
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<tr>
<td>Order</td>
<td>Brassicales</td>
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<tr>
<td>Family</td>
<td>Caricaceae</td>
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<tr>
<td>Genus</td>
<td>Carica</td>
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<tr>
<td>Botanical name</td>
<td><em>Carica papaya</em> Linn.</td>
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**Geographical distribution**

It is native to Tropical America and was introduced in India in the 16th century.

![Geographical distribution of papaya in the world](image)
2.2 Introduction

Papaya belongs to small, anomalous family with four genera, of which three is in tropical and subtropical America and one in Africa. The genus Carica contains about 40 species but only three are of horticulture importance. *Carica papaya* Linn, is the common papaya extensively grown in India other variety available is *C. candamarcensis* known as mountain papaya and *C. monoica* grown in the Amazon basin. The fruits, leaves and latex obtained from papaya plant are used medicinally and for various other purposes. Papain, a major chemical compound extracted from fruit and stem latex is used in brewing and wine making and in the textile and tanning industries.

Fresh leaves make a useful dressing for foul wounds and as poultices for sores. The paste of young leaves is used in severe cases of jaundice and infusion has been used for various urinary complaints, gonorrhea, vermifuge and in colic pain. The leaves are smoked in place of tobacco for relief in asthma (Anonymous, 1992; Asolkar et al., 1992). Leaves are used in severe jaundice (Raja Reddy, 1988); to expel guinea worm (Raj and Patel, 1978); as a poultice (Banarjee and Banarjee, 1986); in fracture healing; constipation and indigestion (Megoneitso and Rao, 1983).

2.3 Phytochemistry and analytical reviews

The phytochemical analysis of the *C. papaya* L. leaves has shown the presence of alkaloids, cardiac glycosides and saponins. *C. papaya* L. leaves are an enriched resource of mineral such as Na, K, Ca, Mg, Fe, and Mn (Ranasinghe et al., 2012; Sharma et al., 2013). Benzylisothiocyanate is a glucosinolates compound, which was found in papaya leaf (Tang et al., 1972).

The carpaine and pseudocarpaine were two alkaloids isolated from *C. papaya* L. leaves (Govindachari et al., 1954, 1957, 1965). In another research bioactive compound carpaine was isolated and purified by chromatographic methods and confirmed by spectroscopic methods using LC-MS and 1D/2D-1H/13C NMR and the structure was confirmed by single crystal X-ray diffraction. Quantification of carpaine was carried out by LC–MS/MS using C18 column and ESI-MS detector using 90:10 CH3CN:CH3COONH4 (6 mM) under isocratic conditions and detected with multiple reaction monitoring (MRM) in positive ion mode (Zunjar et al., 2016).
Julianti and his co-workers in 2014 developed and validated carpaine quantitatively after extraction in C. papaya L. leaves with the aid of UPLC-tandem mass spectroscopy, conditions were optimized with respect to solvent, temperature, and number of extraction cycles (Julianti et al., 2014a). Gonga et al., 2015 performed metabolite fingerprinting using 1D and 2D $^1$H NMR and used multivariate statistical analysis to identify parts of plant that contains the maximum strengths of metabolites of phytomedicinal significance. Secondary metabolites such as phenyl propanoids and flavonoids were found in larger concentrations in the leaves as compared to the seeds. UPLC-ESI-MS confirmed the presence of important metabolites in the papaya extracts by NMR analysis (Gonga et al., 2015).

Julianti and his co-workers in 2014 performed a gradient HPLC fractionation of a single injection of the C. papaya L. leaves extract, followed by offline bioassay of micro fractions. Active compounds of C. papaya L. leaves were purified by HPLC-MS and MPLC-ELSD. Structures were established by HR-ESI-MS and NMR spectroscopy. Configuration of compound was confirmed by comparison of experimental and calculated electronic circular dichroism (ECD) spectroscopy data, and by X-ray crystallography (Julianti et al., 2014b). Profiling indicated flavonoids and alkaloids in the active time windows (Figure 2.2). A total of nine compounds were isolated. Four were known flavonols-manghaslin, clitorin, rutin, and nicotiflorin. Five compounds isolated from the alkaloidal fraction were piperidine alkaloids (Julianti et al., 2014b).

The major cyanogenic glycoside found was (2R)-prunasin, along with this (2S)-sambunigrin was also present in small amount. Seigler and his co-workers (2002) were not able to confirm the presence of a cyclopentenoid cyanogenic glycoside, tetraphyllin B, in C. papaya L. leaves and stem. In detailed $^1$H NMR studies of (2R)-beta-D-allopyranosyloxy-2-phenylacetonitrile (1a)/(2S)–beta-D–allopyranosyloxy–2-phenylacetonitrile (1b) and (2R)-prunasin/(2S)-sambunigrin was also done. The diagnostic chemical shift of cyanogenic methine and anomeric protons in 1a/b were sensitive to anisotropic environmental effects and hence, were supported by GLC analysis of the TMS ethers (Seigler et al., 2002). (R)-2-(beta-D-Glucopyranosyloxy)-2-phenylacetonitrile (prunasin) was also isolated from C. papaya L. and C. quercifolia (syn. C. hastata Brign.) (Olafsdottir et al., 2002).

Earlier reports on presence of cyclopentenoid cyanohydrin glycosides in C. papaya L. could not be confirmed, and no cyclopentenoid amino acids could be detected in extracts of C. papaya L. and C. quercifolia. Conversion of [2,3,4,5,6-3H] phenylalanine into triturated
prunasin was demonstrated in both species. On the other hand, when the plants were administered [2-14C]-2-(2'cyclopentenyl) glycine, extracted, and the extracts hydrolyzed with beta-glucosidase (*Helix pomatia*), formation of labelled cyanide was not observed.

The absence of cyclopentenoids, which were typical for the Passifloraceae, and the inability of Carica species to utilize 2-(2’-cyclopentenyl) glycine as a precursor of cyanogenic glycosides were in agreement with the relative phylogenetic position of the Caricaceae and the Passifloraceae. Carica species are thus rare examples of taxa in which glucosinolates and cyanogenic glycosides co-occur, both types of natural products being derived from the same amino acid, phenylalanine (Olafsdottir *et al*., 2002).

### 2.4 Pharmacological activities

#### 2.4.1 Antidengue activity

*C. papaya* L. is an important plant with significant medicinal properties e.g. anti-inflammatory, antimicrobial and wound management (Goyal *et al*., 2009; Ahmad *et al*., 2011). It is also an important source of many phytochemicals shown in the Table 2.1. On administration of papaya in its powder form has significantly increased the PC of DEN infected patients (Sathasivam *et al*., 2009).

In another research it was found that leaf aqueous extracts were responsible for significant increase in PC, white blood cells (WBC) and neutrophils in DEN infected patients (Ahmad *et al*., 2011). The fermented preparations of this fruit have antioxidant activity; it has increased the reduced glutathione concentration in red blood cells (RBC) and decreased the reactive oxygen species (ROS) (Fibach *et al*., 2010).

The papaya extracts also shown the positive effects on other immune responsive cells like macrophages exhibited antiviral properties (Mahbub-E-Sobhani *et al*., 2011; Du *et al*., 2011). The macrophages upon viral infection were responsible to produce antiviral antibodies. So, this can be said that papaya may have many healthy effects on DEN infected patients due to its positive regulation of macrophages and platelets (PLTs) (Figure 2.2).
Figure 2.2: Chemical structures of some phytoconstituents
Table 2.1: Possible mechanism of anti-dengue activity of leaf juice

<table>
<thead>
<tr>
<th>Class</th>
<th>Phytoconstituents</th>
<th>Related activity</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Vitamins</td>
<td>Ascorbic acid, vitamin A, vitamin B&lt;sub&gt;9&lt;/sub&gt; &amp; B&lt;sub&gt;12&lt;/sub&gt; &amp; folic acid</td>
<td>↓CNS, ↑complications in DF, ↑Immune defect by Vitamin A deficiency, ↓Ammonia↑hemoglobin &amp; expression of dihydrofolate reductase, regulate oxidative stress.</td>
<td>Imaga et al., 2010b</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Kaempferol, quercetin, myricetin &amp; rutin</td>
<td>↑<em>In vitro</em> anti-inflammatory activity, ↓acetic acid induced capillary permeability. ↑antimicrobial and anti-inflammatory, ↓NO level, mRNA expression.</td>
<td>Miean and Mohamad, 2001; Rivera pastrana et al., 2010</td>
</tr>
<tr>
<td>Phenolic acids</td>
<td>Ferulic acid, chlorogenic acid, caffeic acid, p-hydroxybenzoic acid &amp; vanillic acid</td>
<td>↑Antioxidant activity, ↑hepatoprotective activity, ↓transaminase activity &amp; protect liver</td>
<td>Canini et al., 2007; Rivera Pastrana et al., 2010; Zhou et al., 2011</td>
</tr>
<tr>
<td>Esters</td>
<td>Benzaldehyde, β damascenone &amp; benzyl isothiocyanate</td>
<td>↑Antihelmintic activity</td>
<td>Lee et al., 2010</td>
</tr>
<tr>
<td>Anthracene</td>
<td>Anthraquinone</td>
<td>↑Proliferation of resting peripheral blood MNC &amp; WBC, ↑Antiviral</td>
<td>Ayoola et al., 2008; Imaga et al., 2010a</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Lycopene, β carotene &amp; β carvone</td>
<td>↓Liver from oxidative stress, ↓HCV related complication</td>
<td>Rivera Pastrana et al., 2010</td>
</tr>
</tbody>
</table>

(Abbr: CNS: central nervous system; NO: nitric oxide; mRNA: messenger ribonucleic acid; MNC: mononuclear cell; WBC: white blood cells; HCV: hepatitis C virus)
Anthraquinone an important photochemical of many plants also found in papaya either in free or in bound form and many studies reported that various anthraquinones have antiviral property against different viruses (Kumar et al., 2007) and also has stimulated the proliferation of resting human peripheral blood MNCs and lymphocyte (Cherng et al., 2008). The derivatives had potential to activate the CD34+ dendritic cells, which is important for immunological responses (Van de Ven et al., 2012).

There are different flavonoids present in papaya one of them is myricetin, a therapeutic compound of papaya possessed in vitro anti-inflammatory activity and can reduce the acetic acid-induced capillary permeability (Miean and Mohamed, 2001; Wang et al., 2010). Another flavonoid named kaempferol showed antimicrobial and strong anti-inflammatory activity by reducing the nitric oxide (NO) levels (Taechowisan et al., 2008; Hamalainen et al., 2007). It was responsible for dose dependent reduction in NO levels, which was governed by reducing inducible nitric oxide synthase (iNOS) proteins and mRNA expression. Ferulic acid, form of phenolic acid is an important subject of antioxidant activity and so can protect the body from many health problems including neural disorders (Srinivasan et al., 2007). Its antioxidant property was basically due to its functional hydroxyl and phenoxy groups. Another phenolic acid named vanillic acid is also present in papaya and some other plants (Shaukat et al., 2003; Jazayeri et al., 2007; Zhou et al., 2011). Vanillic acid possessed strong hepatoprotective activity, as it decreased the activity of transaminase enzyme and disorganized hepatic sinusoids (Itoh et al., 2009). In addition, it also protected the liver from immune-induced liver injuries by decreasing the concentration of inflammatory cytokines, interferon (IFN)-gamma and other liver infecting agents.

Papaya is an important source of some vitamins like vitamin A, B12 and folic acid, which have some contribution in its therapeutic properties (Table 2.1) (Jiao et al., 2010). Vitamin B12 deficiency caused abnormalities in CNS, which have increased the complications in DEN patients with already affected CNS (Scalabrino, 2009). Thus, the presence of vitamin B12 might have healthy impact on DEN infected patients. Deficiency of vitamin A also contributed to immune defects and increased the prevalence of several diseases (Figure 2.3) (Saeed et al., 2005; Uboh et al., 2009; Iribhogbe et al., 2011).

DENV caused aplastic anemia, which was inhibited by the application of folic acid (Albuquerque et al., 2009). Iron-folic acid has decreased the rate of anemia especially in women; by increasing the hemoglobin levels (Casey et al., 2010). It also protected the
endothelial cells from the oxidative stress by increasing the expression of dihydrofolate reductase (Gao et al., 2009). Dihydrofolate reductase regulated the tetrahydrobiopterin and NO superoxide production to suppress the oxidative stress (Crabtree et al., 2011).

Lycopene is an antioxidant compound of papaya. It has shown potential antioxidant property and thus inhibited the liver from oxidative stress (Seren et al., 2008) and has helped in reduction of HCV related complications. Bignotto et al. (2009) studied the anti-inflammatory effects of lycopene in two rat models. He observed that lycopene imposed strong anti-inflammatory activity at 25 and 50 mg/kg concentrations in both paw oedema and ischaemia-reperfusion models. Herzog et al. (2005) noted that lycopene administration caused a decrease in inflammation causing agents like IL-1β, chemokines induce leucocytes (CXC) etc. Thus, lycopene shown an efficient anti-inflammatory agent and it also showed preventive effect on chromosomal aberrations (Aslanturk and Celik, 2005).

These investigations of papaya properties showed that papaya has many therapeutic properties and it is a rich source of highly protective biological compounds, which can treat many health problems.

2.4.2 Antithrombocytopenic activity

Oral administration of standardized C. papaya L. leaf extract (150 mg/kg) in thrombocytopenic rats exhibited significant (p < 0.01) increase in thrombocytes (1014.83 × 10^3 cells/mm^3) and total leukocytes count (TLC), and the reduction in bleeding (BT) and clotting time (CT) as compared to cyclophosphamide (CP)-induced thrombocytopenic group. Histopathological studies showed minimal fibrosis in spleen histology (Anjum et al., 2017).

Chemotherapy-induced TCP is a detrimental side effect of cancer chemotherapy. Hence, their thrombopoietic activity is modest and often associated with unfavorable side effects. C. papaya L. leaf extract has shown to overexpress ALOX-12 and PLT-activating factor receptor gene, which stimulated the megakaryocytes. The mean PC in interventional group was 49.70 ± 12.64/mm^3, which increased to 55.35 ± 15.13/mm^3 (P > 0.05), 147.54 ± 54.35/mm^3 (P < 0.01), and 200.58 ± 51.89/mm^3 (P < 0.01) on post chemotherapy day 7, 10, 13 and 16, respectively. The mean PC in non-interventional group was 47.36 ± 13.11/mm^3, 42.58 ± 12.10/mm^3, 46.36 ± 14.77/mm^3 and 54.23 ± 16.05/mm^3 on post chemotherapy day 7, 10, 13 and 16, respectively, with no statistically significant improvement. Increment in WBC from baseline to day 7 was statistically significant (P < 0.001) as compared to control (Sundarmurthy et al., 2017).
Figure 2.3: Phytoconstituents showing effect on platelets and liver

(Abr: CNS: central nervous system; MNC: mononuclear cell; WBC: white blood cells; HCV: hepatitis C virus; NO: nitric oxide; mRNA: messenger ribonucleic acid; DENV: dengue virus; DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; RNA: ribonucleic acid; T cells: thymus cells; NK cells: natural killer cells)
Antithrombocytopenic activity was assessed on busulfan-induced thrombocytopenic wistar rats. The antithrombocytopenic activity of different bio-guided fractions was evaluated by monitoring blood PC. Two different phytochemical groups were isolated from decoction of *C. papaya* L. leaves: phenolics, and alkaloids. Out of these, only alkaloid fraction showed good biological activity. Carpaine was isolated from the alkaloid fraction and exhibited potent activity in sustaining PC up to $555.50 \pm 85.17 \times 10^9/L$ with no acute toxicity (Zunjar et al., 2016).

Sheikh *et al*., 2014 used local syrup of papaya leaf extract for experimentation to find enhancement of PLTs, mean cell hemoglobin (MCH) and mean corpuscular volume (MCV) in rats at a dose of 0.5 mL for seven consecutive days which were fasted for four hours before dose administration. They showed significant increase in mean PC, MCH and MCV (p<0.05) of the treated animals as compared to control after seven days treatment with *C. papaya* L. leaf formulations.

Oral administration of *C. papaya* L. leaves extract said to have a positive impact on thrombocyte count. A 23-year-old man was administered a calculated dose for five days. Blood samples were tested for complete blood count (CBC) before and after the administration of the juice. Thrombocyte count had increased from 28000/μL to 138000/μL at the end of five days (Siddique *et al*., 2014).

Dharmarathna *et al*., 2013 used total 36 mice for the trial. Fresh *C. papaya* L. leaf extract [0.2 mL (2.0 g)/mouse] was given to the test group. General behaviour, clinical signs and feeding patterns were recorded. Hematological parameters including PLT, RBC, WBC, packed cell volume (PCV), serum biochemistry including serum creatinine, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were determined in blood samples. Tissue samples were examined for possible histopathological changes. Neither group exhibited alteration of behaviour or reduction in food and water intake. Similarly, no significant changes in SGOT, SGPT and serum creatinine levels were detected in the test group. The PC ($11.33 \pm 0.35) \times 10^5/\mu L$ and the RBC count ($07.97 \pm 0.61) \times 10^8/\mu L$ were significantly increased in the test group compared to that of the controls. However, no significant changed were observed in WBC count and PCV (%) in the test group. The PC in the test group started to increase significantly from Day 3 reaching almost a four-fold higher at Day 21. Likewise, the RBC count in the test group increased from 06 ×
10^8/µL to 09 × 10^8/µL at Day 21, while in control group it was found constant (06 × 10^8/µL) (Dharmarathna et al., 2013).

Subenthiran et al. (2013) also conducted study to investigate the PLT increasing property of C. papaya L. leaves juice (CPLJ) in patients with DF. An open labeled randomized controlled trial was carried out on 228 patients with DF and DHF. Approximately half the patients received CPLJ, for 3 consecutive days while the others remained as controls and received the standard management. Their full CBC was monitored 8 hours for 48 hours. Gene expression studies were conducted on the ALOX 12 and PTAFR genes. The mean increase in PC was compared in both groups using repeated measure ANCOVA. There was a significant increase in mean PC observed in the intervention group (P < 0.001) but not in the control group 40 hours since the first dose of CPLJ. The ALOX 12 and PTAFR genes were highly expressed among those on the CPLJ. It was concluded that CPLJ does significantly increase the PC in patients with DF and DHF (Subenthiran et al., 2013; McRedmond et al., 2004). Histopathological organ changes were not observed in either group of mice except in three liver samples of the test groups which has a mild focal necrosis (Dharmarantha et al., 2013).

Ikpeme et al. (2011) was aimed at qualitative evaluation of the ethanol seed, leaf and pulp extracts of C. papaya L. for bioactive compounds and also to investigate their effect on the hematology in male albino rats. A 3 × 4 factorial experimental layout using randomized complete design was adopted. Present result revealed that significant effects (p < 0.05) of the extracts on the hematology of the treated rats. Hence, suggested the use of C. papaya L. extracts in enhancing the production of selected blood parameters, taking issue of dosage into consideration.

Another study was conducted where two milliliters of blood from healthy volunteers and patients with serologically confirmed current DEN infection were freshly collected and used in the assays. Fresh CPLJ at three different maturity stages extracted with 10 mL of cold distilled water. Freshly prepared cold water extracts of papaya leaves (1 mL containing 30 µL of papaya leaf extracts, 20 µL from 40% erythrocytes suspension, and 950 µL of phosphate buffered saline) were used in the heat-induced and hypotonic-induced hemolytic assays. Membrane stabilization properties were investigated with heat-induced and hypotonicity-induced hemolysis assays (Ranasinghe et al., 2012). In dose response experiments, six different concentrations of CPL extract of partly matured leaves showed significant reduction in heat induced hemolysis compared to control. At all three maturity levels showed more than
25% inhibition. Heat-induced hemolysis inhibition activity did not demonstrate a linear dose response relationship (Ranasinghe et al., 2012)

Patil et al (2013) showed evaluation of PLT augmentation activity of CPL aqueous extract in rats. The results revealed the increased PC and reducing clotting time in CP-induced thrombocytopenic rat model. Hettige from Sri Lankan family Physician showed positive results of elevated total WBC and PLTs in DF patients (Hettigue, 2008). Ahmad and his co-workers (2011) also used of CPL extract in DEN treatment, showed enhancement in PLTs within 5 days where patients received 25 mL of CPL extract twice a day for 5 days (Ahmad et al., 2011). A report in the British Medical Journal website described the rapid recovery of PC in two children suffering from DEN. These cases were proved to be positive for DEN by the demonstration of the DEN-antigen in the serum where Kumar (2010) investigated the use of CPL extract for the enhancement of PLTs showed increment within 12 h and 2 days respectively (Kumar, 2010). Kala (2012) administered CPLJ in five patients for the investigation of DF related TCP showed increase in PC by 24 h (Kala, 2012).

A study conducted in Indonesia used C. papaya L. leaves extract capsules (CPC), which contained 70% ethanol extract of CPL. A total of 80 patients were randomized into two groups; one group received CPC in addition to standard treatment, whereas the other group received only standard treatment for DEN. The study found that PLTs in patients with DEN, increased faster who received the CPC (Yunita et al., 2012).

### 2.4.3 Anticancer activity

Intervention to decelerate, arrest, or reverse the process of carcinogenesis by the use of either natural or synthetic agents individually or in combination has emerged as a promising and pragmatic medical approach to reduce cancer risk. Pathak and his co-worker (2014) examined the cancer chemopreventive potential of a flavonoid-rich fraction isolated from C. papaya. The fraction exerted its anticancer properties in vitro through cytoprotection, antioxidative and anti-inflammatory mechanisms and genoprotection in response to isocyanate-induced carcinogenicity. Medium-term anticarcinogenicity and 2-stage skin papillomagenesis studies conducted in benzopyrene-induced lung carcinogenesis and 7,12-dimethyl benz-α-anthracene-mediated skin papillomagenesis mouse models further validated in vitro observations. The study supports the inverse association between dietary flavonoid intake and cancer risk (Pathak et al., 2014). A case control study was conducted at Regional Cancer Center, Medical College, Thiruvananthapuram, on 64 newly diagnosed cases of gall
bladder cancer and 101 cases of gallstones and dietary evaluation was done by dietary recall method. Odd ratios and 95% confidence interval were calculated for various fruits and vegetables and a significant reduction in odd ratio was seen with consumption of papaya (OR, 0.44; 95% CI, 0.2-0.64) (Pandey and Shukla, 2002). Another study has been conducted which has shown positive response against apoptosis or tumor cells by using CPL aqueous extract along with enhanced immunomodulator potential (Nguyen et al., 2013; Otsuki et al., 2010).

2.4.4 Antioxidant activity

Preliminary investigation of the CPL extract possessed significant antioxidant and free radical scavenging abilities using in vitro models in a concentration dependent manner (P<0.05). The extract also reduced hydrogen peroxide induced erythrocyte hemolysis and lipid peroxidation significantly when compared with ascorbic acid (P<0.05). The findings showed that CPL possessed significant bioactive potential which attributed to its synergy (Okoko and Ere, 2012). In another research CPL extract has been used against alcohol-induced gastric damage and blood oxidative stress for protective action in rats showed good results (Indran et al., 2008). Another study showed use of Iranian papaya juice for antioxidant potential in vitro and in vivo, which was found comparable to α-tocopherol (Mehdipour et al., 2006).

2.4.5 Anticoagulant activity

Chandrasekhar et al., 1961 in a study reported to increase the PT and coagulation time in dogs, rabbits, rats and mice. A dose of 2.0 mg/Kg i.v. in dogs increased PT three fold while oral route was ineffective. The duration of anticoagulant action varied with the dose administered. It also showed spasmogenic action on the smooth muscle of guinea pig ileum even in low concentration (1.0 µg/mL).

2.4.6 Central nervous system

The alcoholic extract of leaves (10.0 mg/Kg i.p.) showed dose dependent sedative effect in male rats. The extract (5.0 mg/Kg i.p.) induced central muscle relaxation. The extract (50 mg/Kg i.p.) completely protected the rats against pentylene tetrazol-induced seizures, while 50% protection was observed with dose of 5.0 mg/Kg i.p. The extract at doses of 100 and 200 mg/Kg i.p. showed 100% protection against maximal electroshock-induced convulsions (Gupta et al., 1990).
2.4.7 Hypoglycemic activity

The CPL aqueous extract (0.75 g and 01.50 g/100 mL) significantly decreased blood glucose levels (p<0.05) in diabetic rats. It also decreased cholesterol, triacylglycerol and amino-transferases blood levels. Low plasma insulin levels did not change after treatment in diabetic rats, but they significantly increased in non-diabetic animals. Pancreatic islet cells were normal in non-diabetic treated animals, whereas in diabetic treated rats, CPL could help in islet regeneration manifested as preservation of cell size. In the liver of diabetic treated rats, CPL prevented hepatocyte disruption, as well as accumulation of glycogen and lipids. Finally, an antioxidant effect of CPL extract was also detected in diabetic rats (Juarez et al., 2012). A study compared the effect of fermented papaya preparation in a group of type 2 diabetic patients treated with the hypoglycemic agent glibenclamide and a group of healthy subjects. The result confirmed the empirical experience that the use of fermented papaya can induce a decrease in plasma glucose level both in healthy subjects and type 2 diabetic patients.

2.4.8 Hepatoprotective activity

The ethanol and aqueous extracts (250 mg/Kg for 7 days) showed hepatoprotective against carbon tetrachloride-induced hepatotoxicity in male albino rats as evidenced by biochemical parameters viz., serum aspartate, amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin and glutamate transpeptidase and histological parameters (Balasubramaniam et al., 2002). The extract reduced the elevated levels of enzymes e.g. aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), bilirubin and gamma glutamate transpeptidase (GGTP). The pathological examination showed formation of normal hepatic cords and absence of necrosis and vacuoles (Rajkapoor et al., 2002).

2.4.9 Antifungal activity

Bioactive compounds from vegetal sources are natural antifungic. An ethanol extraction was used to obtain bioactive compounds from C. papaya L. cultivar (cv.) maradol leaves and seeds of discarded ripe and unripe fruit. Both, extraction time and the papaya tissue flour: organic solvent ratio significantly affected the yield. With the longest time and highest flour: solvent ratio produced the highest yield. The effect of time on extraction efficiency was confirmed by qualitative identification of the compounds present in the lowest and highest
yield extracts (Chavez et al., 2011). Gene cloning and characterization was done in papaya leaf for novel recombinant antifungal chitinase (Chen et al., 2007).

Analysis of the CPL extract with phytochemical tests showed the presence of alkaloids, flavonoids and terpenes. Antifungal effectiveness was determined by challenging the different extracts from the best extraction treatment against three phytopathogenic fungi: Rhizopus stolonifer, Fusarium spp. and Colletotrichum gloeosporioides. The leaves extract exhibited the broadest action spectrum. The MIC₅₀ for the extract was 0.62 mg/mL for Fusarium spp. and >10 mg/ml for C. gloeosporioides, both equal to approximately 20% mycelial growth inhibition. Ethanolic extracts from C. papaya L. cv. maradol leaves are a potential source of secondary metabolites with antifungal properties (Chavez et al., 2011).

The leaf extract inhibited the growth of ringworm causing fungi, Epidermophyton floccosum 52.37%, Trichophyton mentagrophytes 24.30% and Microsporum gypseum 20.32%. The cold and hot water extracts of leaves showed antifungal activity (43.8 and 33.3%, respectively) against Pythium aphanidermatum (Mishra et al., 1991; Bhat et al., 1994).

2.4.10 Antibacterial activity

This study conducted by Handayani et al., (2014) revealed that crude alkaloid of CPL could control Staphylococcal enterotoxin A gene-carrying S. aureus by suppressing the expression of sea, in addition to the ability to inhibit the growth of S. aureus. The expression of sea was analyzed using a quantitative reverse transcription real-time PCR. The yield of crude alkaloid extract was 0.48 to 1.82% per dry wt. of CPL. A MIC of crude alkaloid to S. aureus was 0.25 mg/mL. After exposure to the alkaloid at 0.25 and 0.50 mg/mL for 2 h, a significant increase in cycle threshold values of sea was observed. The sea was expressed 29 and 41 times less when S. aureus was exposed to crude alkaloid at one- and two-fold MIC, respectively. The expression of sea was successfully quantified (Handayani et al., 2014).

The alcoholic extract of the plants showed antibacterial activity against S. aureus, S. pyogenes, E. coli, S. pullorum, P. multocida and P. aeruginosa. However, the aqueous extract not showed any activity against any of the test organism (Narang et al., 1962). Carpaine
isolated exhibited slight inhibitory activity against *M. tuberculosis* H$_{37}$Rv (Valsaraj *et al.*, 1997).

### 2.4.11 Larvicidal/pupicidal activity

The CPL extract established the properties of bacterial insecticide, spinosad on larvicidal and pupicidal activity against the chikungunya vector, *Aedes aegypti*. The extract showed larvicidal and pupicidal effects after 24 h of exposure; however, the highest larval and pupal mortality was found in the CPL methanolic extract against the first- to fourth-instar larvae and pupae of values $LC_{50}$ = I instar was 51.76 ppm, II instar was 61.87 ppm, III instar was 74.07 ppm, and IV instar was 82.18 ppm, and pupae was 440.65 ppm, and bacterial insecticide, spinosad against the first to fourth instar larvae and pupae was also found out. Moreover, combined treatment of values of $LC_{50}$ = I instar was 55.77 ppm, II instar was 65.77 ppm, III instar was 76.36 ppm, and IV instar was 92.78 ppm, and pupae was 107.62 ppm. No mortality was observed in the control. The results showed good bacterial insecticidal action and spinosad acted as promising larvicidal and pupicidal against chikungunya vector, *A. aegypti*. This was an ideal eco-friendly approach for the control of chikungunya vector, *A. aegypti* as target species of vector control programs (Kovendan *et al.*, 2012).

### 2.4.12 Antimalarial activity

Mature CPL leaves were widely used to treat malaria in several African countries. An ACT involving a medicinal herb extract and its active constituents provided an indigenous alternative/herbal ACT. The antiplasmodial activity of CPL and/or artemisinic acid were determined by using the Peter's 4-day suppressive test in *Plasmodium berghei* infected mice. The combination of 50 mg/Kg of CPL and 15 mg/Kg of artemisinic acid produced a significant reduction of parasitemia (81.25%), compared to 50 mg/Kg CPL alone (37.70%). The mean survival time of the combinations of CPL and 15 mg/Kg of artemisinic acid, and CPL alone followed a dose-dependent manner (Uhegbu *et al.*, 2008). The ED$_{50}$ of CPL showed good activity. The isobolar equivalent (IE) calculated from the ED$_{90}$ of CPL in combination with artemisinic acid showed antagonistic interaction (Onaku *et al.*, 2011).

### 2.4.13 Antiplasmodial activity

Antiplasmodial activity of CPL extract was confirmed by *in vitro* against four parasites (*Trypanosoma brucei rhodesiense, Trypanosoma cruzi, Leishmania donovani, and*...
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Plasmodium falciparum), and in the Plasmodium berghei mouse model (Julianti et al., 2014a).

2.4.14 Anthelmintic activity

Carpasemine and benzylisothiocyanate isolated from the seeds and CPL aqueous extract after steam distillation of seeds were tested for the activity against Ascaris lumbricoides. It has inhibited energy metabolism as well as altered motor physiology of A. gulli, which ultimately significantly reduced the activity of the parasite in vitro (Kumar et al., 1991). Benzylisothiocynate was mainly responsible for the activity; it did not cause any toxic symptoms at dose of 30 mg/Kg body wt. in rats. Carpasemine was considerably less active. The resin found most potent while leaves extract showed high mortality against Meloidogyne incognita and Rotylenchulus reniformis nematodes (Mahamood et al., 1979; Siddiqui et al., 1987, 1992).

2.4.15 Insecticidal activity

Leaves reported to support the growth and development of the larvae of the pest Diacrisia oblique (Gopalan and Madhusudan, 1981).

2.4.16 Antiulcer activity

The CPL extract reduced the ulcer index; the methanolic extract demonstrated better protection against indomethacin-induced ulcers. The ethanol extract against ethanol-induced gastric ulcers (Kottaimuthu, 2008). The extract protected the gastric mucosa against ethanol effect. The extract significantly reduced the gastric juice volume and gastric acidity (Indran et al., 2008).

2.4.17 Anti-inflammatory activity

Ethanolic extract of CPL has shown anti-inflammatory activity (Owoyele et al., 2008). Oladunmoye (2007) conducted study using CPL ethanolic extract where orogastrically dosed with S. typhi and S. aureus for inflammation in rats (Oladunmoye and Osho, 2007).

2.4.18 Anthelmintic activity

The prevalence was 02.85% and infection was common in males. A paste of CPL with opium and common salt applied for three days was helpful in relief of symptoms and easy extraction of worms from the body (Sanghvi, 1989).