Summary of thesis entitled

PHYTOCHEMICAL STUDIES ON SOME CULTIVARS OF *Carica papaya* LINN. AND BIOPROSPECTING OF ITS RELATED SPECIES

To

The Maharaja Sayajirao University of Baroda

For the Degree of

DOCTOR OF PHILOSHOPY

IN

CHEMISTRY

By

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December – 2016

Introduction

Platelet is one of the important components of blood whose function (along with coagulation factors) is to stop bleeding by clumping and clotting the injured blood vessel [1]. Platelets, also called thrombocytes (thromb + cyte, "blood clot cell"), are anucleated, and derived from the megakaryocytes of the bone marrow. In every human being, each day about $1x10^{11}$ platelets are produced by cytoplasmic fragmentation of megakaryocytes [2]. They are 2,50,000 to 4,00,000 /µL in human blood [2], [3] with life span of 8 to 14 days [4], [5] and are destroyed in the spleen [3]. The condition of platelet counts less than 1,50,000 /µL is termed as 'thrombocytopenia'. The resulting symptoms are often dramatic and associated with a variety of hemorrhagic sequelae, including epistaxis, petechiae, gastrointestinal bleeding and intracranial hemorrhage.

The line of treatment for thrombocytopenia includes use of corticosteroids, immunoglobulins or splenectomy, however it is not effective for 25 to 30% of patients with chronic ITP [6], [7].

The failure of allopathic armamentarium has channelized efforts into rediscovering the wealth of traditional and complementary medicines (T&CM). While looking for effective traditional remedy for thrombocytopenia in literature, a large number of blogs describing use of *Carica papaya* for the treatment of dengue fever were seen on the social network. Dharmarathna *et al,* have described the enhancement of platelets in rats on administration of decoction of *Carica papaya* leaves [8]. *Carica papaya* leaves are traditionally used for the treatment of dengue and other ailments in Malaysia [9], [10]. Few patents on application of dry *Carica papaya* leaves as platelet booster have also been filed [11], [12].

Inspired by these facts, an attempt was made to conduct a systematic research on an ethanopharmacologically important plant (*Carica papaya*) with a view to rectify the existing lacunae.

To initiate any phytochemical research for development of new drug, authentication of plant material is the most crucial step. The pharmacognostic studies cover this aspect of any crude drug.

The collected plant material was identified and authenticated by Prof. M. Daniel at Department of Botany, The M. S. University of Baroda, Gujarat, India. The voucher specimen of this plant (No. BARO/2010/51) was deposited at the Herbarium, BARO, Department of Botany, The M. S. University of Baroda. Pharmacognostic studies consisting of proximate analysis, morphological studies, anatomical microscopic studies, micromorphological studies, histochemical analysis, powder studies and preliminary phytochemical analysis of the *C. papaya* leaf were performed.

The presence of sphaeraphides, starch grains and rhomboidal calcium oxalate crystals in the leaf powder form **diagnostic characters** of *C. papaya*.

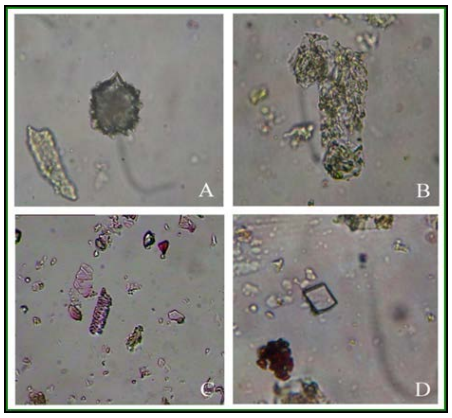


Figure 1: Powder of *C. papaya* showing **A**- Sphaeraphide, **B**- Stomata, **C**- Spiral xylem, starch grains, **D**- Rhomboidal calcium oxalate crystal.

The preliminary screening of various groups of phytoconstituents of decoction of leaves indicated the presence of various phytochemicals like carbohydrates, proteins, amino acids, cardiac glycosides, saponins, flavonoids, alkaloids, tannins, phenolics and iridoids.

Once the authenticity of the plant was established, based on ethnopharmacological data, the crude extract of *Carica papaya* leaves were screened for its pharmacological efficacy and toxicity.

For primary screening of bioactivity, healthy Sprague Dawley rats used as targets, as *in vitro* study of platelet is not possible due to lack of nucleus. The pharmacological activity was studied by monitoring number of platelets. The results indicated increase in platelet count by about 40% irrespective of dose.

The sub-chronic activity carried out by administering 1000 mg of *Carica papaya* leaf extract per body weight of rats for 90 days revealed the *Carica papaya* leaf to be safe for oral administration.

The bioassay guided fractionation study was carried out for the polarity based chromatographically separated fractions as well as fractions separated on the basis of phytochemical groups. The results of bioassay of *Carica papaya* leaf fractions with different polarities suggested the pharmaceutically active compound to be more polar as the fractions extracted with lower polarity solvent systems did not show any significant rise in platelet counts.

The results of screening of different phytochemical groups [Table 1] indicated no conclusive rise in platelet counts with phenolics and saponin groups whereas a substantial rise in platelets is observed in case of alkaloidal extracts.

Table 1: Result of Screening of various phytochemical groups

Extracts	**Platelet Count Mean±SD (10^9/L)**	
	1st day	**7th day**
Control	573.33±48.23	622.33±28.71
Water	663.33±20.26	791.67±13.65
Saponins	515.33±31.26	586.00±37.64
Phenolics	509.67±13.20	543.00±3.61
Alkaloids	611.67±52.70	1004.00±53.51

In order to find the lead compound responsible for the thrombocytopenic activity in *Carica papaya* leaves, thrombocytopenic wistar model was made by optimization of busulfan doses.

The various phytochemical groups were screened for the anti-thrombocytopenic activity on wistar rat model as targets and the results of screening of various phytochemical [Table 2] indicated alkaloidal group to contain the compound/s we were looking for.

Table 2: Result of screening of phytochemical group on antithrombocytopenic model

Extracts	**Platelet Count Mean±SD (10^9/L)**	
	1st day	**20th day**
Control	623.25±105.32	704.75±132.92
Diseased	800.75±142.57	78.75±27.28
Water	782.50±189.19	33.50±15.24
Saponins	883.75±133.30	112.50±24.12
Phenolics	813.50±153.21	72.25±17.69
Alkaloids	973.00±243.96	313.25±50.74

On the basis of these observations, the alkaloidal fraction was further sub-fractionated and the anti-thrombocytopenic activity on the model animal target was studied. The bioassay guided fractionation study revealed that the active pharmacological component present in the *Carica papaya* leaves with anti-thrombocytopenic activity belongs to the alkaloidal group and it could be carpaine, the chief alkaloid component [Table 3].

Table 3: Results of screening of sub-fractions of alkaloidal group on antithrombocytopenic model

Extracts	Platelet Count Mean±SD (10^9/L)	
	1st day	20th day
Control	748.50±203.96	718.75±157.89
Diseased	730.25±135.53	81.50±30.21
Pet. Ether fraction of alkaloids	660.50±99.47	759.75±181.44
Ethyl acetate fraction of alkaloids	578.25±54.79	632.25±116.23
Carpaine (Single compound)	623.00±168.74	555.50±42.99

To understand the apparent better activity of the petroleum ether fraction, quantitation of carpaine in the sub-fractions was thought to be most appropriate approach. To serve the afore said purpose, an LCMS–MS method was developed and validated.

The analysis revealed quantity of carpaine in water, petroleum ether and ethyl acetate extract to be 6.46 ± 0.09 mg/g, 28.56 ± 0.59 mg/g, 25.08 ± 0.53 mg/g respectively.

Once the lead pharmacological group was identified, the next study was about bioprospecting of carpaine in different parts of the plant and **Chapter 4** is about isolation, characterization and quantification of carpaine in different parts of *Carica papaya.*

A modified method by optimizing various parameters i.e. percentage of acetic acid, pH, temperature, bases and time was developed for the extraction of carpaine from different parts of plant. Due to non-chromophoric nature of carpaine it was very difficult to analyze using HPLC with PDA or UV detector, Hence new LCMS method developed for quantification of carpaine was found to be fast, simple and sensitive with good precision and accuracy. The figure of merit [Table 4] for the method were as follows

Table 4: Regression data, LOD, LOQ for carpaine

m/z	component	Regression	r^2	LOD(ng/mL)	LOQ(ng/mL)
479	Carpaine	y=22700x-23.8	0.9991	0.09	0.25

The intraday (n=5) and inter day (n=9) analysis precision RSD% for intraday was 1.35 and precision RSD% for interday was 2.09. The accuracy (recovery) (n=3) was 89.1.

The LCMS-MS analysis confirmed that all the parts of papaya plant contained carpaine. The results as shown in table 5 indicated leaf to contain highest concentration of carpaine followed by fruit pulp, fruit peel, roots, stem, petioles and seeds. Results also shows there was no significant difference between the carpaine content among different age group of leaves.

Table 5: Quantity of carpaine present in different parts of papaya plant

Papaya parts	Content (mg/g)
Leaves (mature)	9.30
Fruit pulp	4.90
Fruit Peel	1.99
Roots	1.86
Stem	1.04
Petioles	0.72
Seeds	0.65
Tendril leaves	0.76
2-3 weeks older leaves	0.57

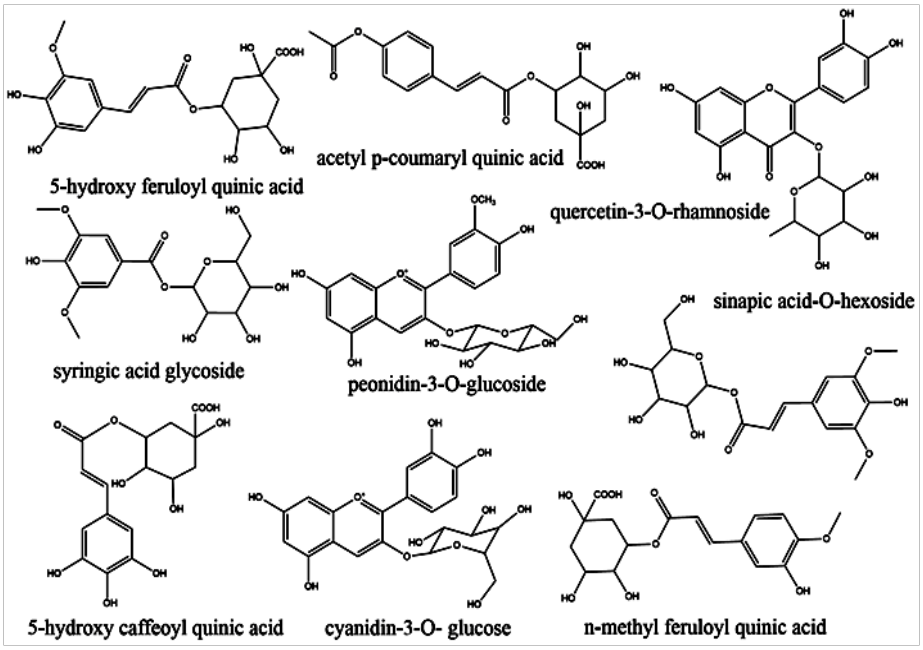
Carica papaya has abundant phenolics in different parts. There are few reports on the study of antioxidant activities of phenolics in different cultivars of *Carica papaya* [13]–[20].

However the study of comparative study of phenolics in different parts of *Carica papaya* has not been reported, hence isolation and identification of phenolics different chromatographic techniques were used including 1D and 2D paper chromatography, HPLC and LCMS-MS method were carried out [**Chapter 5]**.

The highly polar phenolic groups that gave bluish-white fluorescence with Rf ~1 were directly used for qualitative analysis [21]. The 2-D paper chromatography indicated presence of different phenolics (mainly protocatechuic acid) with different colour forming derivatives with p-nitro aniline and sulphanilic acid.

Bioprospecting of phenolics among different parts using HPLC confirmed the presence of protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, syringic acid, p- hydroxy benzoic acid.

The LCMS-MS analysis of isolated phenolic fractions of aqueous extract indicated presence of twenty compounds belonging to a typical hydroxy cinnamic acid and phenolic acid derivatives.



. **Figure 2:** New Phenolics identified from different parts of papaya plant

The quantitative analysis of phenolics and flavonoids along with their antioxidant activities of different parts of papaya plant were performed by total flavonoid content (TFC) and total phenol content (TPC).

Table 6: DPPH scavenging activity, FRAP activity, total flavonoid content (TFC) and total phenol content (TPC) of different parts of *C. papaya* plant

Samples	DPPH IC_{50}(mg/mL)	FRAP IC_{50}(mg/mL)	TPC ± SE (µg GAE/mg)	TFC ± SE (µg Qtn/mg)
Seed	3.11 ± 0.11	4.85 ± 0.27	865.73 ± 0.35	31.41 ± 0.01
Fruit	3.36 ± 0.13	12.43 ± 0.34	981.53 ± 0.53	22.99 ± 0.02
Leaf	13.31 ± 0.48	13.54 ± 0.71	815.29 ± 0.48	9.09 ± 0.01
Root	13.87 ± 0.08	48.15 ± 2.59	315.59 ± 0.19	3.48 ± 0.01
Stem	39.48 ± 0.4	94.24 ± 9.48	298.34 ± 0.11	5.82 ± 0.01

Thus the thesis that *Carica papaya* leaves contains some component that is responsible for the antithrombocytopenic activity has been proved through systematic bioassay guided fractionation and the phytochemical responsible for pharmacological activity is alkaloid (Carpaine), which is present in all parts of the plant and is maximum in the *Carica papaya* leaf.

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