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RESEARCH ARTICLE

ANTIOXIDANT, ERYTHROCYTE MEMBRANE STABILIZATION AND THROMBOLYTIC POTENTIAL OF *CARICA PAPAYA* (LINN.)

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FRAP- Ferric Reducing Antioxidant Potential.

ABSTRACT

The leaf, pulp and seed extracts of *Carica papaya* (Linn.) were tested for the presence of various phytochemicals like tannins, saponins, alkaloids, flavonoids, carbohydrates and proteins. The *invitro* antioxidant potential of *Carica papaya* (Linn.) extracts were evaluated by different methods like DPPH assay. FRAP assay, Nitric Oxide assay and Total antioxidant capacity. The erythrocyte membrane stabilizing property was studied using heat induced hemolytic assay and osmotic fragility test. The thrombolytic potential was assessed by determining the percentage of clot dissolved by the extract. The results show that aqueous, ethanolic and methanolic extracts of *Carica papaya* (Linn.) contains varying amount of phytochemicals and a remarkable antioxidant potential. The results of membrane stabilizing and the thrombolytic properties demonstrate that papaya is one of the obvious choices for various medicinal applications.

INTRODUCTION

Carica papaya (Linn.) is the most widely cultivated tropical plant with a pulpy edible fruit. It is a fast-growing, semi-woody and can grow up to 5-10m tall. (Borokini 2012). It is commonly known as pawpaw, kapaya, lapaya, papyas, Fan mu gua; belonging to the family Caricaceae with only four genera in the world. It is native to North America and has been established in almost all the tropical and subtropical regions over the world (Sheik Fauziya *et al.*, 2013). There are a number of varieties cultivated for various purposes. The Coorg honey dew also known as 'Madhubindu' is commonly cultivated for table as well as processing purposes. The other varieties include Solo, Pusa Dwarf, Pusa Giant, Pusa Majesty, Pusa Delicious, Ranchi (variety from Bihar popular in South India), CO1, CO2, CO3, IIHR39, IIHR54 (developed at IIHR, Bangalore) and Washington cultivated for fruit and papain production purposes. Coorg Honey Dew, Coorg Green, Pusa Delicious and Pusa Nanha are commonly grown in Karnataka and Kerala. The folkloric medicine uses papaya in the treatment of various diseases like dengue fever, antihelmintic, wound healing and various disorders of digestive system. Papaya is also known for its latex which is produced from the bark and unripe fruits. The latex also finds numerous applications in the pharmaceutical industry due to the presence of the protein digesting enzymes (Julia F Morton, 1987). *Carica papaya* (Linn.) extracts have various bioactive components like alkaloids, flavonoids, tannins, saponins, carbohydrates, proteins, fat and steroids. An alkaloid is a naturally occurring nitrogenous organic molecule that has a pharmacological effect on humans and other animals. (Tarek Ismail Kakhia, 2010). Phenolic compounds form a major family of secondary metabolites in plants and represent a diverse group of compounds possessing remarkable antioxidant properties. Tannins are polyphenolic compounds which are known to have anti-nutritional factors. (Rispail *et al.*, 2010). The flavonoids represent a large group of naturally occurring

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compounds with phenylbenzopyran functionality. (Eric Grotewold, 2006). Studies have shown that flavonoids possess numerous pharmacological properties. (Aseel Shakir Mahmood, 2013). Terpenoids and saponins are groups of lipids with steroidal ring known to have anti-microbial and anti-viral properties. Glycosides also possess a steroidal ring beside the aglycone carbohydrate portion. The pharmaceutical action of cardiac glycosides on the heart is well known (Estrada A, 2000). The present study also evaluates the phytochemical constituents of various extracts of *Carica papaya* (Linn.). Antioxidants are of great interest to biologists because of their ability to protect the human body against the damages caused by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). (Winnicka 2006). An antioxidant may be defined as a substance which can delay or inhibit the rate of oxidation of any oxidizable substrate (Resat Apak, 2013). The present study attempts to evaluate the antioxidant, erythrocyte membrane stabilizing and thrombolytic properties of *Carica papaya* (Linn.) extracts.

MATERIALS AND METHODS

The leaves and unripe fruits of *Carica Papaya* (Linn.) were obtained from the local plantation. The leaves were washed, dried in a hot air oven at 55^0C. The unripe fruits were sliced into thin flakes, kept for drying in a hot air oven. The ripe fruits were cut and the seeds were removed and washed thoroughly with distilled water and dried in a hot air oven. After drying the leaves, pulp and seeds were powdered and stored in airtight containers. The chemicals were purchased from Himedia pvt. Ltd., Mumbai and spectrophotometric readings recorded with UV-double beam spectrophotometer (Systronics India Pvt. Ltd.).

Preparation of Extracts

(Vannila *et al*., 2012), (Sabri Fathima Zohra *et al*., 2012). The aqueous extracts of *Carica papaya* (Linn.) were prepared by boiling the powdered leaves, pulp and seed with distilled water for 20 minutes and kept overnight in a refrigerator. The extracts were filtered using Whatmann no.1 filter paper, dried and stored in airtight containers. The ethanolic and methanolic extracts were prepared using Soxlet extraction apparatus and dried using the Rotary Flash Evaporator at 45^0C. The yield was measured in an analytical balance. Table 1 summarizes the yield obtained from the different extracts of *Carica papaya* (Linn.)

Phytochemical Analysis

The qualitative phytochemical analysis was done using suitable methods. **(**Vijalakshmi *et al.,* 2012); (Yadav *et al.,* 2011); (Anusha Bhaskar *et al.,* 2011), (Sulaiman 2011), (Manjamalai *et al.,* 2010); (SMM Shah *et al.,* 2011). The various bioactive components tested include the alkaloids, flavonoids, carbohydrates, glycosides, proteins, fat and steroids.

In vitro Antioxidant study on the *Carica papaya* (Linn.) Extracts

The *invitro* antioxidant potential of the leaf, seed and unripe pulp extracts were evaluated using the suitable methods.

DPPH Assay

The method of Braca *et al.* (2001) was followed. The free radical scavenging activity of the various extracts of *Carica papaya* (Linn.) were measured with formation of stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical using a spectrophotometer at 517nm.

FRAP-TPTZ Assay

The method of Benzie and Strain (1992) was used. The Ferric Reducing Antioxidant Power was determined using 2, 4, 6-tripyridyl–S-triazine (TPTZ) in a spectrophotometer at 595nm.

Nitric Oxide Radical Scavenging Assay

The method of Rajendranath Dixit *et al.* (2013) was followed to evaluate the Nitric Oxide radical scavenging property. The Nitric Oxide Radical Scavenging Activity was measured by the formation of nitrite from sodium nitroprusside using Griess reagent (1% Sulphanilamide in 2.5% ortho-phosphoric acid, 0.1% Naphthyl ethylenediamine dihydrochloride) spectrophotometrically at 540nm.

Superoxide Radical Scavenging Assay

The method of McCord and Fridovich (1969) was followed with slight modification. The superoxide Radical generated by the photo-oxidation of methionine-riboflavin and their scavenging property was determined spectrophotometrically at 560nm using Nitroblue tetrazolium (NBT) as the chromogenic substrate.

Total Antioxidant Assay

The estimation of total antioxidants was done by the method of Palani Samy Hari Prasad and N. Ramakrishnan (2011). The formation of blue- green phosphomolybdenum complex was measured at 695nm.

Estimation of Total Phenolic Content

The method of Singleton *et al.* (1999) was followed to estimate the total phenolic content using Folin- Ciocalteau reagent. The absorbances were measured at 760nm.

Invitro Erythrocyte Membrane Stabilization and Thrombolytic Potential

Heat induced Hemolysis assay

The method of Ranasinghe *et al*. (2012) was followed. The red blood cell suspension was pre-incubated with different extracts of *Carica papaya* (Linn.) for 20minutes at 55^0C in a water bath. Then the absorbance of the suspension was measured at 540nm. Aspirin was used as positive control.

Osmotic Fragility of Erythrocytes

The method of Umapathy *et al.* (2010). The erythrocyte suspension with the extracts was added to serially diluted saline from 0.9% to 0.48% and distilled water. The absorbance of the suspension was measured at 540nm. The absorbance at

0.44% was taken against the absorbance with erythrocyte suspension in distilled water as control.

Thrombolytic Potential

The thrombolytic potential was evaluated by the method of Prasad *et al*. (2007). The extracts were incubated with pre-weighed clot for 90 minutes at 37^0C. The clots were centrifuged, the supernatant discarded and clot weighed again. The percentage of clot dissolved was calculated.

RESULTS

Table 1 and 2 show the respective yield and the phytochemical constituents of the different extracts of *Carica papaya* (Linn.) The results were statistically analyzed and the p value <0.05 were considered to be significant.

Table 1. showing the summary of the yields obtained from different extracts of *Carica papaya* (Linn.)

Extract	Yield Obtained in grams
Ethanolic Leaf Extract	3.882
Ethanolic Pulp Extract	19.243
Ethanolic Seed Fraction 1	5.840
Ethanolic Seed Fraction 2	1.299
Methanolic Leaf Extract	1.875
Methanolic Pulp Extract	12.108
Methanolic Seed Fraction 1	1.028
Methanolic Seed Fraction 2	2.015

In vitro Antioxidant study on the *Carica papaya* (Linn.) Extracts

DPPH assay

Figure 1A, 1B, 1C show the free radical scavenging property of various *Carica papaya* (Linn.) extracts. Among the aqueous extracts the aqueous pulp extract has shown a higher scavenging activity compared to aqueous leaf extract and all the other extracts and ascorbic acid (p value <0.05, <0.001 respectively).

FRAP-TPTZ assay

The Ferric Reducing Power of various *Carica papaya* (Linn.) extracts is shown in Table 3.

Superoxide radical scavenging assay

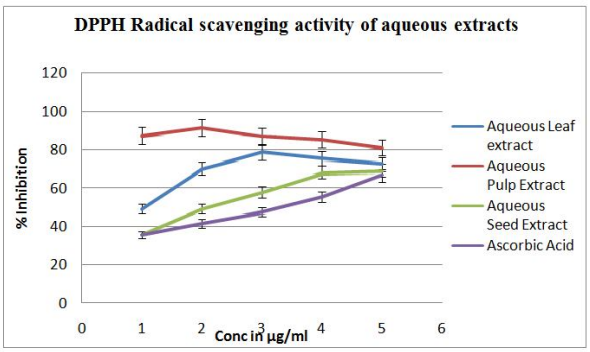
Figure 2A, 2B, 2C and 2D reveal the Superoxide radical scavenging potential of *Carica papaya* (Linn.) The methanolic seed fraction 2 has shown a significantly higher scavenging property. (p value <0.001) compared to ascorbic acid and all the other extracts.

Nitric Oxide radical scavenging assay

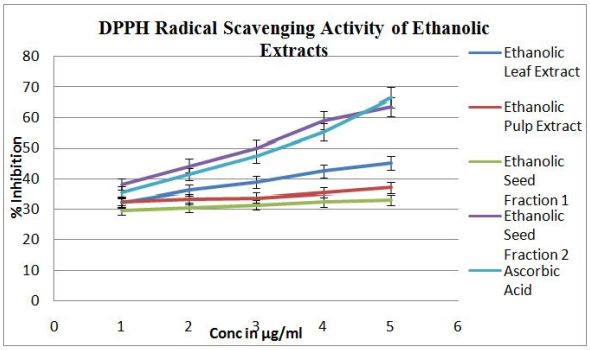
Figure 2 demonstrates the Nitric oxide scavenging potential of *Carica papaya* (Linn.)

Table 2. showing the Phytochemical analyses with different extracts of *Carica papaya* (Linn.)

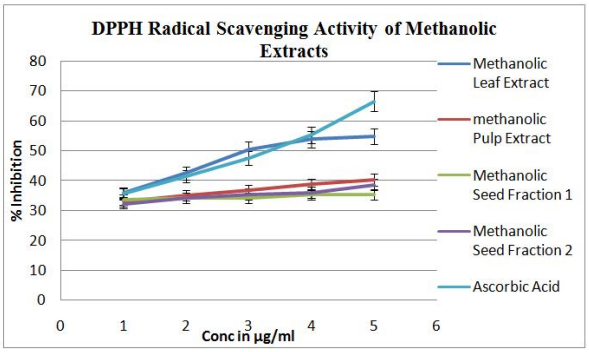
Test	Aq. leaf ext.	Aq. pulp ext.	Aq. seed ext.	Eth. leaf ext.	Eth. pulp ext.	Eth. seed Fr.1	Eth. Seed Fr.2	Met. leaf ext.	Met. pulp ext.	Met. seed Fr.1	Met. Seed Fr.2
Test for Alkaloids:											
Picric Acid Test:	+	-	+	+	+	-	+	+	-	+	+
Test for Flavonoids:											
A. Alkaline Reagent Test:	+	+	-	+	+	-	-	+	+	-	-
B. Ammonia Heat Test:	+	+	-	+	+	-	-	+	+	-	-
Test forTannins:											
Dilute Ferric Chloride Test:	-	-	-	-	-	-	+	-	-	+	-
Test for Saponins:											
Foam Test:	-	+	-	-	+	-	-	-	-	-	-
Test for Carbohydrates:											
A. Fehling's Test:	+	+++	-	-	+++	-	+	-	+	-	-
B. Benedict's Test:	+	+++	-	-	+++	-	+	-	-	-	-
C. Iodine Test:	-	-	-	-	-	-	-	-	+	-	-
Test for Glycosides:											
A. Borntrager's Test:	-	-	-	-	-	-	-	-	-	-	-
B. Salkowski's Test:	+	+	-	-	+	-	-	-	+	-	-
C. Keller-Kilani Test:	-	-	-	+	-	-	-	+	-	-	-
Test for Steroids/Quinones:											
A. Conc. Sulphuric Acid Test:	+	+	-	-	+	-	-	-	-	-	-
B. Acetic Acid Test:											
	+	+	-	+	-	-	-	-	-	-	-
Test for Phenolic Compounds:											
A. Ferric Chloride Test:	-	-	-	-	-	-	+	-	-	+	-
B. Lead Acetate Test:	++	+	-	-	-	++	+	-	-	+	-
Test for Proteins and Amino acids:											
A. Biuret Test:											
B. Millon's Test:	-	-	-	-	-	-	-	-	-	++	+
	-	-	-	-	-	+	+	-	-	++	-
Test for Fat:											
A. Saponification Test:	-	-	+	-	-	-	+	+	+	-	+
B. NaOH- Foam Test:	-	-	+	-	-	-	-	-	-	+	+



1A



1B

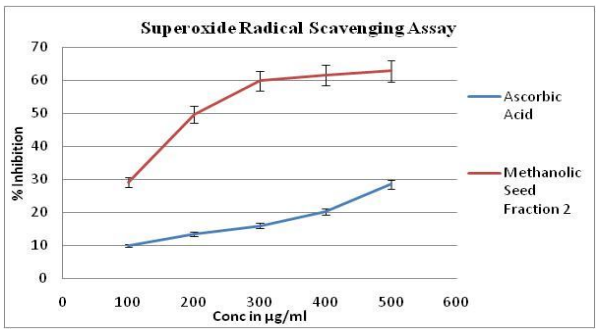


1C

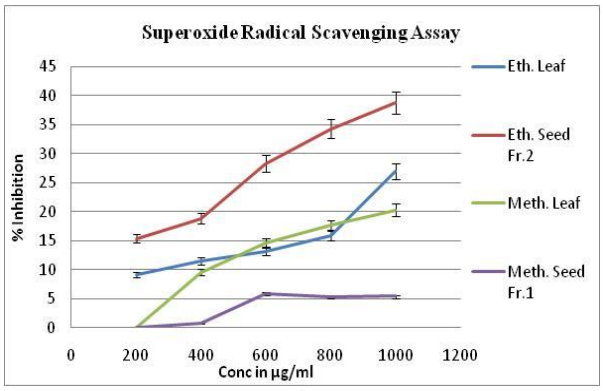
Fig.1 showing the DPPH Assay with aqueous extracts of *Carica papaya* (Linn.) (1A); Ethanolic Extracts of *Carica papaya* (Linn.) and Ascorbic Acid (1B); DPPH Assay with Methanolic Extracts of *Carica papaya* (Linn.) and Ascorbic Acid(1C)

Table 3. showing Ferric Anion Reduction Potential of *Carica papaya* (Linn.) and Ascorbic Acid

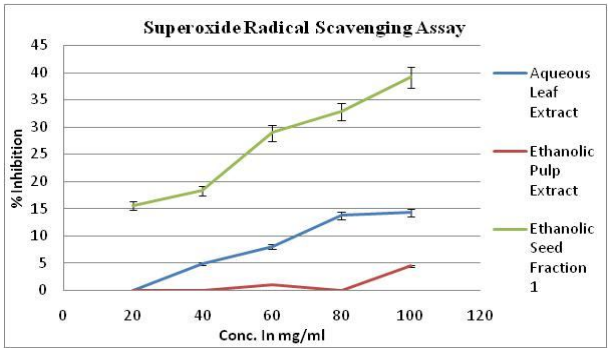
Extract (1mg/ml)	FRAP Value in mg
Ascorbic Acid	25.34±1.55
Aqueous Leaf Extract	2.25±0.50
Aqueous Pulp Extract	2.82±0.58
Aqueous Seed Extract	4.87±1.19
Ethanolic Leaf Extract	6.05±2.09
Ethanolic Pulp Extract	7.66±2.41
Ethanolic Seed Fraction 1	8.88±5.73
Ethanolic Seed Fraction 2	6.38±2.32
Methanolic Leaf Extract	5.27±2.68
Methanolic Pulp Extract	8.90±3.31
Methanolic Seed Fraction 1	8.91±3.54
Methanolic Seed Fraction 2	7.65±4.00



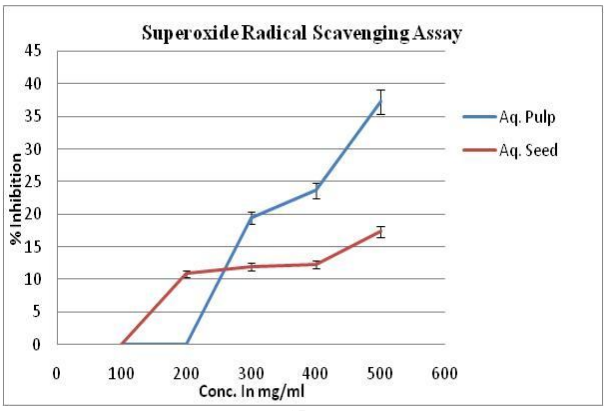
2A



2B



2C



2D

Fig.2. Superoxide Anion Radical Scavenging Assay for Ascorbic Acid and Methanolic Seed Fraction 2 of *Carica papaya* (Linn.) (2A); for Ethanolic and Methanolic Leaf, Seed Extracts of *Carica papaya* (Linn.) (2B); for aqueous leaf, ethanolic pulp and seed fraction 1 of *Carica papaya* (Linn.) (2C); for aqueous pulp and seed extracts (2D)

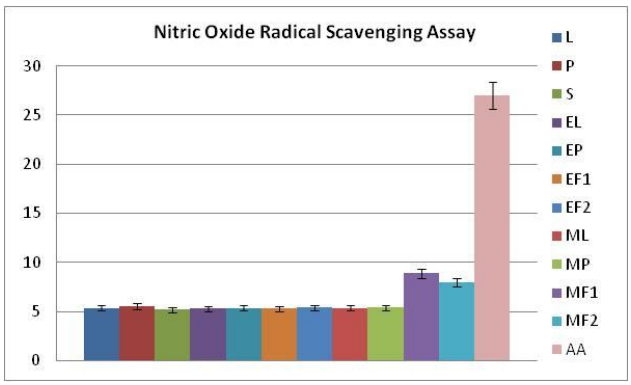


Figure 3. showing Nitric Oxide Radical Scavenging Assay for different extracts of *Carica papaya* (Linn.)

L- Aqueous Leaf Extract
EL- Ethanolic Leaf Extract
EF2- Ethanolic Seed Fraction 2
MF1- Methanolic Seed Fraction 1

P- Aqueous Pulp Extract
EP- Ethanolic Pulp Extract
ML- Methanolic Leaf Extract
MF2- Methanolic Seed Fraction 2

S- Aqueous Seed Extract
EF1- Ethanolic Seed Fraction 1
MP- Methanolic Pulp Extract

Estimation of Total Antioxidants

Table 4 shows the total antioxidants present in different extracts of *Carica papaya* (Linn.).

Table 4. showing Total Antioxidant Capacity of *Carica papaya* (Linn.) Extracts

Extract	Conc. Of extracts	Equivalent Ascorbic Acid in µM
Aqueous Leaf Extract	100mg/ml	62.66±14.93
Aqueous Pulp Extract	100mg/ml	211.67±46.99
Aqueous Seed Extract	100mg/ml	15.67±5.96
Ethanolic Leaf Extract	1mg/ml	1±0.36
Ethanolic Pulp Extract	1mg/ml	33.33±15.27
Ethanolic Seed Fraction 1	100mg/ml	16.67±5.29
Ethanolic Seed Fraction 2	1mg/ml	37±13.5
Methanolic Leaf Extract	1mg/ml	29±13.02
Methanolic Pulp Extract	1mg/ml	35.67±13.05
Methanolic Seed Fraction 1	100mg/ml	66±16.07
Methanolic Seed Fraction 2	10mg/ml	603±113.37

Estimation of total Phenolic content

Table 5 gives the concentrations of phenolic content obtained from different extracts of *Carica papaya* (Linn.)

In vitro Erythrocyte Membrane Stabilization and Thrombolytic Potential

Heat induced Hemolysis assay

The results of heat induced hemolytic assay are shown in Figure 4.

Table 5. showing Total Phenolic Content of *Carica papaya* (Linn.) extracts

Extract	Total Phenolic content in µg
Aqueous Leaf Extract	6.84±1.04
Aqueous Pulp Extract	10.40±1.52
Aqueous Seed Extract	11.83±2.13
Ethanolic Leaf Extract	4.84±1.99
Ethanolic Pulp Extract	6.85±2.71
Ethanolic Seed Fraction 1	10.83±1.78
Ethanolic Seed Fraction 2	45.07±8.05
Methanolic Leaf Extract	25.46±7.52
Methanolic Pulp Extract	9.83±3.12
Methanolic Seed Fraction 1	5.29±2.42
Methanolic Seed Fraction 2	0.71±0.67

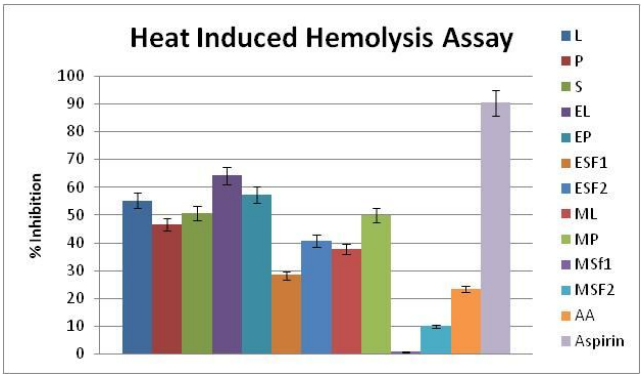


Figure 4. showing Heat induced Hemolysis Assay for different extracts of *Carica papaya* (Linn.)

L- Aqueous Leaf Extract
EL- Ethanolic Leaf Extract
ESF2- Ethanolic Seed Fraction 2
MSF1- Methanolic Seed Fraction 1

P- Aqueous Pulp Extract
EP- Ethanolic Pulp Extract
ML- Methanolic Leaf Extract
MSF2- Methanolic Seed Fraction 2

S- Aqueous Seed Extract
ESF1- Ethanolic Seed Fraction 1
MP- Methanolic Pulp Extract

Osmotic Fragility of Erythrocytes

Figure 5 shows the Osmotic fragility of red blood cells. Ethanolic Seed Fraction 1, Methanolic Pulp Extract and Methanolic Seed Fraction 1 showed significant membrane stabilization (p value <0.001).

Thrombolytic Potential

The thrombolytic potential of various extracts is displayed in Figure 6. Aqueous leaf extract, Methanolic Pulp extract and Methanolic seed Fraction 2 have shown a significant thrombolytic potential (p value <0.001).

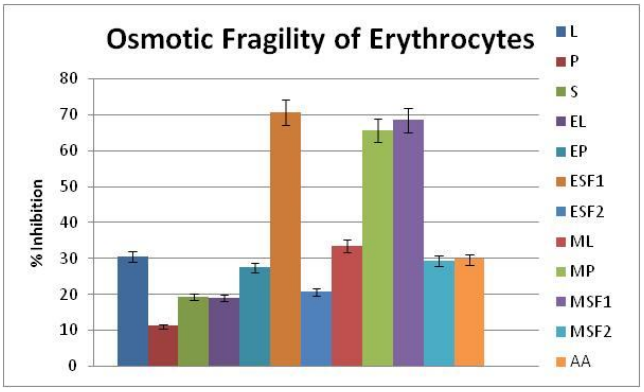


Figure 5. showing Osmotic Fragility of Erythrocytes in different extracts of *Carica papaya* (Linn.)

L- Aqueous Leaf Extract
EL- Ethanolic Leaf Extract
ESF2- Ethanolic Seed Fraction 2
MSF1- Methanolic Seed Fraction 1

P- Aqueous Pulp Extract
EP- Ethanolic Pulp Extract
ML- Methanolic Leaf Extract
MSF2- Methanolic Seed Fraction 2

S- Aqueous Seed Extract
ESF1- Ethanolic Seed Fraction 1
MP- Methanolic Pulp Extract

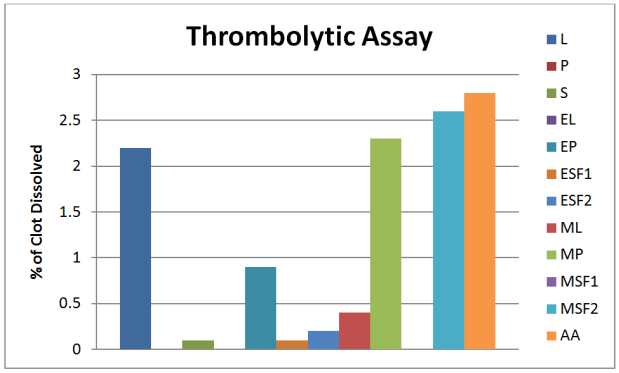


Figure 7 showing Thrombolytic Assay with different extracts of *Carica papaya* (Linn.)

L- Aqueous Leaf Extract
EL- Ethanolic Leaf Extract
ESF2- Ethanolic Seed Fraction 2
MSF1- Methanolic Seed Fraction 1

P- Aqueous Pulp Extract
EP- Ethanolic Pulp Extract
ML- Methanolic Leaf Extract
MSF2- Methanolic Seed Fraction 2

S- Aqueous Seed Extract
ESF1- Ethanolic Seed Fraction 1
MP- Methanolic Pulp Extract

DISCUSSION

From the phytochemical analyses, the aqueous leaf and extract, the ethanolic pulp and seed fraction 2 have a higher amount of bioactive components in them. The aqueous leaf extract showed the highest activity among the extracts with DPPH assay. A higher FRAP value and Nitric Oxide scavenging potential was observed in Methanolic seed Fraction 1. The Methanolic Seed Fraction 2 has shown higher Total Antioxidant capacity, Superoxide anion radical scavenging potential and thrombolytic potential. The Ethanolic Seed fraction 2 possessed a higher total phenolic content. A higher percentage inhibition of heat induced hemolysis was shown by the ethanolic leaf extract and a higher percentage inhibition of hemolysis was shown by ethanolic seed fraction 1 in the osmotic fragility test.

Conclusions

From our results it is evident that the leaf, pulp and seeds of *Carica papaya* (Linn.) have varying amount of bioactive components. The variations in the observed results compared to the previous literatures may be due to environmental variables like the soil composition, climate and geography. The results from the antioxidant studies indicate that the different extracts of *Carica papaya* (Linn.) have remarkable free radical scavenging potential, with erythrocyte membrane stabilization and thrombolytic properties which proves to be a promising agent to counter numerous medical conditions which induce oxidative stress.

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