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Study on Anticancer and Antidiabetic Role of Phyto Components in Different Extracts of *Carica papaya* and Ananas *Cosmosus* Samples

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Abstract: ***In today's world all the medicinal plants found on the earth have their unique medicinal usages and significances. Medicinal plants contain many bioactive compounds that can be used in the process of curing various human diseases. Papaya (Carica papaya) fruit is used as a traditional medicine in India for various ailments, including cancer. Likewise, pineapple (*Ananas comosus*) also used as a therapeutic agent. In this study phytochemicals components were analysed qualitatively and quantitatively using aqueous, ethanol and acetone extracts of Carica papaya and Ananas cosmosus samples. Further antioxidant study was carried out using the above solvent extract by DPPH and FRAP assay methods. Antibacterial study of the plant extracts was performed by disc diffusion method using Staphylococcus aureus and Klebsiella pneumoniae microorganisms. Anticancer activity of ethanolic extracts of Carica papaya and Ananas cosmosus samples was studied with the HCA - 7 colon cancer cell lines and also examined the antidiabetic role of ethanolic extract of Carica papaya and Ananas cosmosus samples with the help of α - amylase and α - glucosidase activity. This present study confirms the presence of various phytocomponents in different proportion in the above plant extracts. These components play a major role in antioxidant and antibacterial activity. The ethanolic extracts of Carica papaya and Ananas cosmosus proved as an efficient therapeutic agent in the treatment of diabetes and cancer.***

Keywords: *Carica papaya, Ananas cosmosus*, Extracts, Anticancer and Antidiabetic activity

1. Introduction

The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. The "backbone" of traditional medicine are the medicinal plants. Fruits are an excellent source of essential vitamins and minerals, and they are high in fibre (Aravind, 2013). Fruits contain many bioactive compounds. Many wild fruits and tropical fruits are safe to consume and some have been developed as medicines. Due to different genotypes and environmental concerns, fruits contain rich phytochemicals, therefore, medicinal valuable fruits are often considered to be healthy foods. That fruits could have the potential to prevent and treat some diseases (Avaloo, 2010). *Carica papaya* is a succulent fruit with a yellowish orange colour of a large plant of the family Caricaceae. Papaya is a soft tropical fruit having commercial importance because of its high nutritive and medicinal value. (Attendorf, 2019). *Ananas comosus* is a tropical plant with an edible fruit; it is the most economically significant plant in the family Bromeliaceae. Pineapples grow as a small shrub; the individual flowers of the unpollinated plant fuse to form a multiple fruit. (Gautam. S, 2010). Pineapple is a digestive aid and a natural Anti - Inflammatory fruit. A group of sulfur - containing proteolytic (protein digesting) enzymes (bromelain) in pineapple aid digestion.

In the present study the two fruit samples, *Carica papaya* and *Ananas cosmosus* are selected. These fruit samples are used to study the various phytochemical components, Analysis of minerals, Antioxidant activity of DPPH assay and FRAP assay, Antibacterial activity against the gram positive and negative bacteria, Antidiabetic activity in Alpha amylase and Alpha glucosidase and their Anticancer activity.

2. Materials and Methods

2.1 Collection of fruit samples

The healthy and ripen *Carica papaya (*papaya) and *Ananas cosmosus (*pineapple) was selected and purchased from the local markets in and around Erode, Tamil Nadu. The fruits were rinsed and peeled with well - sterilized knife. The fruit was cut into thin strips.

2.2 Sample preparation and extraction

The fruits were taken. It weighs (1: 5 ratio) about 10g grounded of pulp was dissolved in 50ml of each of the solvents Aqueous, Ethanol and Acetone to form crude extracts. The conical flasks with extracts were covered by cotton plugs to avoid evaporation. The extracts were placed in shaking incubator at 250 rpm for 48 hrs. After shaking they were filtered with muslin cloth and again filtered with filter paper twice. Prepared crude extracts were evaporated to dryness, extract amount was measured and a final concentration was prepared and used as primary sample for phytochemical constituents, antioxidant activity, antibacterial activity, anticancer and antidiabetic activity.

2.3 Preliminary phytochemical screening

- **Test for Carbohydrates Molish's test:** To a few drops of extract, 2 ml of Molish reagent is added. The mixture is shaken well and 2.0 ml of Concentrated Sulphuric Acid is added slowly along the sides of the test tube and allowed to stand. A reddish or violet ring formed at the junction of two solutions indicates the

presence of Carbohydrates.

- **Test for Reducing Sugar Fehling's test:** To a few drops of extract, 2ml of Fehling's reagent is added. The mixture was shaken well and kept in a boiling water bath for five minutes. A formation of brick red precipitate indicates the presence of Reducing sugar.
- **Test for Alkaloids Mayer's test:** To a few drops of extract, two drops of Mayer's reagent is added by the side of the test tube. A green coloured precipitate confirms the test as positive.

Wagner's test:
To a few drops of extract, two drops of Wagner's reagent is added by the side of the test tube. A reddish - brown coloured precipitate confirms the test as positive.

- **Test for Saponins Foam test:** To a few ml of extract, 20ml of distilled water was added in the test tube and the test tube is continuously shaken for 10 minutes. The formation of foam confirmed the presence of Saponins.
- **Test for Tannins**

Lead Acetate Test:
To a few ml of extract, add few drops of 1% lead acetate. The mixture is shaken well. A yellowish precipitate indicates the presence Tannins.

- **Test for Flavonoids Acid test:** To a few ml of extract, few drops of diluted sulphuric acid is added. Orange colour develops which indicates the presence of Flavonoids.
- **Test for Terpenoids Acetic anhydride test:** To 2 ml of extract, 2 ml of acetic anhydride and concentrated sulphuric acid is added. Formation of blue, green rings indicate the presence of Terpenoids.
- **Test for Amino Acids Ninhydrin test:** To a few drops of extract, few drop of Ninhydrin solution is added in a test tube. A characteristic blue colour indicates the presence of Amino acids.
- **Test for Proteins Biuret Test:** Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink colour, which indicates the presence of Proteins.

Millon's test:
To a few ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of Proteins.

- **Test for Glycosides Libermann's test:** To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish - brown ring indicates the presence of Glycosides.
- **Test for Cardiac Glycosides:** In a test tube, added 5 ml of extract and 2 ml of glacial acetic acid and 1 drop of ferric chloride and then add 1 ml of concentrated sulphuric acid slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of Cardiac Glycosides.
- **Test for Total Phenols Ferric Chloride Test:** To 2 ml of extract, 3% of Ferric chloride is added. Formation of deep blue colour indicates the presence of total Phenol.
- **Test for Quinone:** To few drops of extract 5 ml of Hydrochloric acid is added. Formation of yellow precipitate indicates the presence of Quinone.
- **Test for Anthraquinones:** To 2 ml of extract, 2 ml of 10% Ammonium hydroxide is added. Formation of bright pink colour indicates the presence of Anthraquinones.
- **Test for Steroids:** To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic anhydride is added reddish brown colour is formed. To this added 1 ml of concentrated sulphuric acid. Formation of violet to blue green colour indicates the presence of Steroids.
- **Test for Fatty acids:** To a few ml of extract are pressed in filter paper and dried. The transparency appeared in the filter paper indicates the presence of Fatty acid.
- **Test for Cholesterol:** To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic anhydride is added. To this added 1 ml of Concentrated Sulphuric Acid. Formation of violet to blue green colour indicates the presence of Cholesterol.

2.4 Estimation of secondary metabolites

- Estimation of alkaloid (Sigh and Archana Sahu method)
- Estimation of flavonoid (Aluminium Chloride Method)
- Estimation of phenol (Mallick and Singh., 1980)
- Estimation of tannin (Folin's Ciocalteau Method)

2.5 Biochemical analysis

- Estimation of glucose (Anthrone Method)
- Estimation of protein (Biuret Method)
- Estimation of cholesterol (Zak's Method)

2.6 Estimation of vitamins and minerals

- Estimation of vitamin c (Omaya et al., 1971)
- Estimation of iron (2, 2' - Dipyridyl Method)
- Estimation of magnesium (Titan Yellow Method)

Statistical analysis
The *Carica papaya* and *Ananas cosmosus* were analysed to determine their components. All experiments were done and mean values are presented. Results were expressed as Mean±SD (Standard Deviation).

2.7 Evaluation of scavenging activity

- DPPH radical scavenging assay (Brand Williams et al., 1995)
- Ferric reducing antioxidant power (Oyaizu, 1986)

2.8 Antibacterial activity - disc diffusion method (kirby - Bauer method)

The crude extracts of *Carica papaya L* and *Ananas cosmosus*, tested for antibacterial activity using disc diffusion method (Kirby - Bauer method). The selective microorganisms (*Klebsiella pneumonia*, *Staphylococcus aureus and E. coli)* are collected in a separate nutrient agar plate from department of Biotechnology, KASC, Erode.

Principle
Micro - organism are naturally susceptible to the action of a specific antibiotic and are inhibited by it. The susceptibility is based on genetically determined characteristic of individual species. Disc method is the simplest method to

perform the susceptibility test. Sterile discs are impregnated with antibiotic are placed on a agar plate (i. e.) heavily and uniformly inoculated with an actively growing culture of the organism. The test organisms grow in a smooth lawn of confluent growth on the plate except in a clear zone around the antibiotic disc, which inhibits the growth of the organism and indicates the susceptibility of the organism.

Preparation of culture suspension

Nutrient broth media is the best growth media for the growth of bacteria. The nutrient broth was sterilized and cooled. Then the collected microorganisms were suspended in nutrient broth and it was allowed to grow at 37ºC for 24 hours in bacterial incubator. After incubation, note turbidity in broth and used for anti - bacterial assay. The strains were maintained and stored at 4ºC.

Preparation of disc

The papaya and pineapple were prepared as per the mentioned procedure, the fruits were macerated with different solvents as Ethanol, Acetone and Distilled water. The mixture was condensed to obtain three kinds of different extracts. Every extract that was obtained from every solvent was tested in different concentrations. Disc was prepared from those extracts by which it was cut into disc shape with the measurement of 9 mm diameter. Here these extracts itself was used as a disc instead of impregnating sterile disc with the sample solutions.

Determination of zone of inhibition

The 20ml of sterilized nutrient agar medium containing Agar - Agar was poured on to each of the respective Petri dishes and allowed to solidify for 10 - 15 minutes. Then 100 µl of bacteria inoculums were spread using cotton swab on to the respective solidified Petri dishes. Discs taken from sample were placed on the surface of agar plates and were incubated for 24 hrs at 37ºC in a bacterial incubator. After incubation, the antibacterial activities were then determined by measuring the clear zone of inhibition in millimetre (mm).

2.9 In - vitro Anticancer activity Cell line

The human Cancer cell was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin - ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96 - well plates at plating density of 10, 000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO2, 95% air and 100% relative humidity. After 24 hrs, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hrs at 37^0C, 5% CO2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

3 - [4, 5 - dimethylthiazol - 2 - yl]2, 5 - diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate - dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 hrs of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 ˚C for 4 hrs. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

Calculation

The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control x 100

The % cell inhibition was determined using the following formula.

% Cell Inhibition =100 - sample/Abs (control) x100 Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software.

2.10 Antidiabetic activity

1) Alpha amylase enzyme inhibition assay Materials required

The Alpha amylase Enzyme: (Type VI B: From porcine pancreas, 5, 00, 000 U) [15.8 U/mg solid at pH 6.9 and stored at 2 - 8˚C and their Substrate level is starch 1%. The Positive Control is Acarbose - Stored at RT - Glucobay (Bayer pharma, India) and Sodium dihydrogen orthophosphate (NaH2PO4.2 H2O) - Himedia (RM - 1255) is stored and Disodium hydrogen phosphate (Na2HPO4.2H2O) - Himedia (RM - 257) - stored at RT. The indicator is iodine solution 1% and to absorbed at UV - Visible Spectrometer.

Procedure

Added 390 ml of 0.02M Phosphate buffer pH 7/ Positive control/ Different concentration of test samples + 10 µ L of α - amylase and pre - incubated at 37 ˚ C for 10 mins and added 10ml starch and reincubated at 37˚ C for 1hr and Added 0.1 ml 1% iodine solution + 5ml of distilled water and measured OD at 565 nm

2) Alpha – glycosidase enzyme inhibition assay

Material required

Alpha - glucosidase enzyme - isolated from rat intestine (stored at - 20° C). Substrate: Sucrose - Himedia, India (RM 3063) - Stored at RT and their positive control is Acarbose - stored at RT - Glucobay (Bayer Pharma, India) and total protein estimation kit (Biuret method) - Span diagnostics (B - 0211) - Stored at 2 - 8° C and added 390 ml of 0.02M Phosphate buffer pH 7/ Positive Control/ Different concentration of test samples +10 µ L of - amylase and pre - incubated at 37° C for 10 mins and re - Incubated at 37° C for 1hr and measured OD at 565 nm and added 10 ml of Starch and added 0.1 ml 1% Iodine solution+5ml of distilled water. Sodium dihydrogen orthophosphate (NaH2PO4.2H2O) - Himedia, India (RM - 1255) and stored at RT. Disodium hydrogen phosphate (Na2HPO4.2H2O) - Himedia (RM - 257) - stored at RT. Glucose reagent - ErbaAGAPPE diagnostics, India (AFP 11208100) - Stored at 2 - 8° C and absorbed at UV - Visible Spectrometer.

Procedure

Added 225 ml of 80mM Phosphate buffer pH 7.0/ Positive control/ Different concentration of test samples +75 ml of Alpha - glucosidase and pre - incubated at 37° C for 30 mins and kept in a boiling water bath for 2 mins, cooled and then added 250 ml of glucose reagent and incubated at RT for 10 mins and measured OD at 510nm.

3. Results and Discussion

3.1 Preliminary phytochemical screening

The preliminary phytochemical screening of *Carica papaya* and *Ananas cosmosus* in different extracts were shown in Table - 1 and Table - 2.

Table 1: Preliminary phytochemical screening of *Carica papaya*

S. No	Experiments	Extracts		
		Aq	Eth	Ace
1	Carbohydrates	++	++	+
2	Reducing sugar	-	+	-
3	Alkaloids	+	++	+
4	Saponin	-	-	+
5	Tannins	+	+	+
6	Flavonoids	++	++	++
7	Terpenoids	+	+	-
8	Amino acids	-	-	-
9	Proteins	++	+	+
10	Glycosides	-	+	-
11	Cardiac Glycosides	-	-	-
12	Phenol	+	++	++
13	Quinone	-	-	-
14	Anthroquinone	-	-	-
15	Cholesterol	+	+	+
16	Fatty acids	-	-	-
17	Steroids	+	++	-

(++) Highly present, (+) Present, (-) Absent

Ethanol and Acetone extracts of *Carica papaya* answered the maximum phytochemical tests when compared with the aqueous extracts.

Table 2: Preliminary phytochemical screening of *Ananas cosmosus*

S. no	Experiments	Extracts		
		Aq	Eth	Ace
1	Carbohydrates	+	++	++
2	Reducing sugar	+	-	-
3	Alkaloids	+	++	++
4	Saponin	-	-	+
5	Tannins	+	+	+
6	Flavonoids	+	++	++
7	Terpenoids	+	+	-
8	Amino acids	-	-	-
9	Proteins	+	++	+
10	Glycosides	-	+	-
11	Cardiac Glycosides	-	-	-
12	Phenol	+	++	++
13	Quinone	-	-	-
14	Anthroquinone	-	-	-
15	Cholesterol	+	++	+
16	Fatty acids	-	-	-
17	Steroids	+	+	-

(++) Highly present, (+) Present, (-) Absent. Ethanol and Acetone extracts of *Ananas cosmosus* sample answered the maximum phytochemical tests when compared with the aqueous extracts.

3.2 Estimation of Secondary metabolites

3.2.1 Estimation of Alkaloid, Flavonoid, Phenol and Tannin invarious extracts of *Carica papaya* sample

Aqueous**,** Ethanol and acetone extracts of *Carica papaya* sample were examined for the occurrence of alkaloid, flavonoid, phenol and tannin.

Table 3: Alkaloid, Flavonoid, Phenol and Tannin invarious extracts of *Carica papaya sample*

Components	Extracts of Phytocomponents (mg/g)		
	Aqueous	Ethanol	Acetone
Alkaloid	5.5±.61	7.0±1.21	8.0±1.01
Flavonoid	5.4±1.98	7.4±0.22	5.6±1.06
Phenol	3.9±0.01	7.5±1.61	4.5±1.12
Tannin	3.6±0.56	4.8±0.90	4.3±1.01

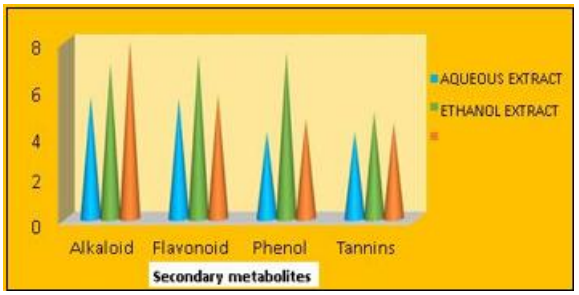

Figure 1: Alkaloid, Flavonoid, Phenol and Tannin in various extracts of *Carica papaya sample*

Ethanol extract of *Carica papaya* showed the high proportion of flavonoid (7.4±0.22 mg/g) and phenol (7.5±1.61 mg/g) and tannins (4.8±0.90 mg/g).

3.2.2 Estimation of Alkaloid, Flavonoid, Phenol and Tannin in various extracts of Ananas cosmosus sample

Aqueous**,** Ethanol and acetone extracts of *Ananas cosmosus* sample were examined for the occurrence of alkaloid, flavonoid, phenol and tannin.

Table 4: Alkaloid, Flavonoid, Phenol and Tannin invarious extracts of *Ananas cosmosus sample*

Components	Extracts of Phytocomponents (mg/g)		
	Aqueous	Ethanol	Acetone
Alkaloid	4.0±0.99	6.0±0.26	8.5±2.12
Flavonoid	6.7±1.16	8.0±2.2	5.5±1.01
Phenol	4.7±1.01	6.0±0.73	5.0±1.06
Tannin	2.8±1.01	3.5±0.01	4.4±0.99

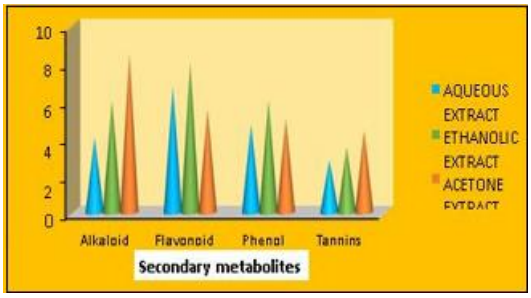

Figure 2: Alkaloid, Flavonoid, Phenol and Tannin invarious extracts of *Ananas cosmosus sample*

Ethanol extract of *Ananas cosmosus* showed the high proportion of flavonoid (8 mg/g) and phenol (6 mg/g). The present study confirms, when compared with both the fruit samples ethanol extract of the *Carica papaya* contains high concentration of some secondary metabolites (flavonoids, phenol and tannins) Occurrences of these phytocomponents in different proportion play a significant role in the medicinal properties of those samples.

3.3 Estimation of Biochemical analysis

3.3.1 Estimation of Glucose, Proteins and Cholesterol in various extracts of *Carica papaya* sample

Aqueous, Ethanol and Acetone extracts of Carica papaya fruit samples were examined for the occurrence of glucose, proteins and cholesterol.

Table 5: Glucose, Protein and Cholesterol content in various extracts of *Carica papaya* sample

	Extracts of phytocomponents (mg/g)		
	Aqueous	Ethanol	Acetone
Glucose	15.61±2.22	19.01±2.01	16.32±2.01
Protein	28.91±3.91	21.30±0.91	25.64±2.90
Cholesterol	20.41±2.21	17.67±1.01	18.56±2.01

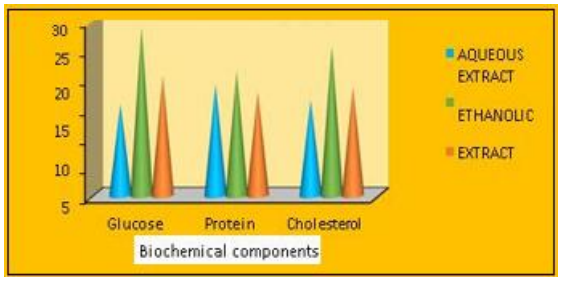

Figure 3: Glucose, Protein and Cholesterol content in various extracts of carica papaya sample

The biochemical study reveals that the Carica papaya has high glucose concentration as (19.01±2.01 mg/g) in ethanolic extract. High protein concentration (28.91±3.91) was shown in aqueous extract. Meanwhile, cholesterol concentration (20.41±2.21) was also high in aqueous extract.

3.3.2 Estimation of Glucose, Proteins and Cholesterol in various extracts of Ananas cosmosus sample

The various extracts of *Ananas cosmosus* fruit samples were examined for the occurrence of glucose, proteins and cholesterol.

Table 6: Glucose, Protein and Cholesterol content in various extracts of *Ananas cosmosus* sample

	Extracts of phytocomponents (mg/g)		
	Aqueous	Ethanol	Acetone
Glucose	17.91±1.89	17.50±1.21	18.01±2.01
Protein	20.61±2.22	27.83±1.32	26.54±1.00
Cholesterol	20.44±2.22	16.90±0.98	19.01±2.35

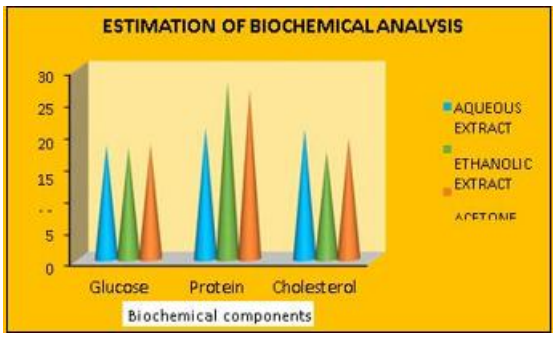

Figure 4: Glucose, Protein and Cholesterol content in various extracts of *Ananas cosmosus* sample

The biochemical study reveals that the Ananas cosmosus has high glucose concentration as (18.01±2.01 mg/g) in acetone extract. High protein concentration (27.83±1.32) was shown in ethanol extract. But, cholesterol concentration (20.44±2.22) was high in aqueous extract.

3.4 Estimation of Minerals and Vitamins

3.4.1 Estimation of Minerals and Vitamins in various extracts of *Ananas cosmosus* sample

The amount of Iron, Magnesium and Vitamin C in various extract of *Carica papaya* were estimated by colorimetric method.

Table 7: Estimation of Minerals and Vitamin in various extracts of *Carica papaya* sample

Minerals and Vitamins	Extracts (mg/g)		
	Aqueous	Ethanol	Acetone
Iron	19.21±0.84	20.01±1.56	23.21±1.67
Magnesium	1.39±0.24	1.43±0.61	1.32±0.09
Vitamin C	17.52±0.77	18.21±0.5 4	14.59±0.91

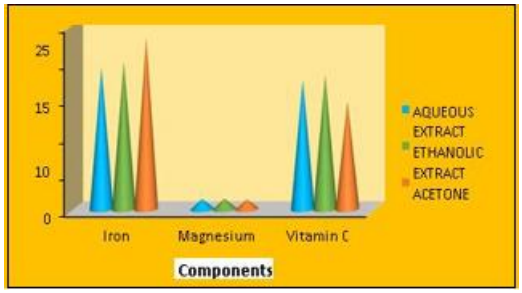

Figure 5: Minerals and Vitamin in various extracts of *Carica papaya* sample

The mineral estimation in acetone extract of Carica papaya showed high concentration of Iron as (23.21±1.67 mg/g). Magnesium concentration was high in ethanol extract as (1.43±0.61 mg/g). In Vitamin C has high concentration in ethanol extract as (18.21±0.54 mg/g).

3.4.2 Estimation of Minerals and Vitamins in various extracts of *Ananas cosmosus*

The amount of Iron, Magnesium and Vitamin C in various extract of *Ananas cosmosus* were estimated by colorimetric method.

Table 8: Estimation of Minerals and Vitamin in various extracts of *Ananas cosmosus* sample

Minerals and Vitamins	Extracts (mg/g)		
	Aqueous	Ethanol	Acetone
Iron	20.04±0.94	24.50±1.08	21.24±1.02
Magnesium	1.40±0.45	1.46±1.89	1.46±1.89
Vitamin C	16.71±0.96	19.21±1.01	16.71±0.96

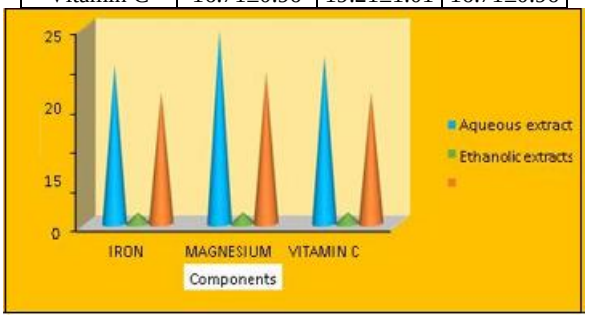

Figure 6: Minerals and Vitamin in various extracts of *Ananas cosmosus* sample

The mineral estimation in ethanolic extract of Ananas cosmosus showed high concentration of Iron as (24.50±1.08 mg/g). Magnesium concentration was high in acetone extract as (1.54±1.26 mg/g). In Vitamin C has high concentration in ethanol extract as (19.21±1.01 mg/g).

3.5 Evaluation of Scavenging activity of various extracts of *Carica papaya* and *Ananas cosmosus*

3.5.1 DPPH' Radical Scavenging Assay

The free radical scavenging activity of various extract of *Carica papaya* and *Ananas cosmosus* was determined by DPPH method and result were presented in table 9 and figure7 were seen scavenge the stable DPPH radical directly to different extents over at different concentrations.

Table 9: DPPH Radical Scavenging Assay in Ethanol, Acetone and Aqueous Extract of *Carica Papaya* and *Ananas Cosmosus*

Sample	%inhibition of samples		
	Aqueous	Ethanol	Acetone
Carica papaya	62	88	74
Ananas cosmosus	64	76	70

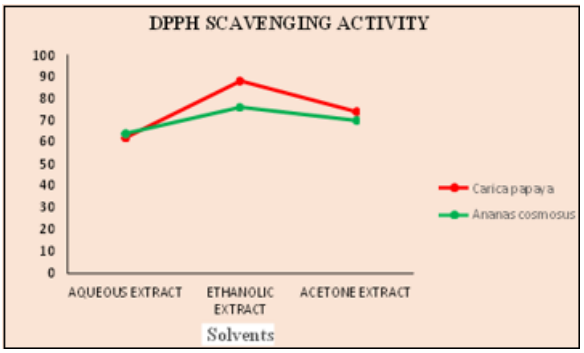

Figure 7: DPPH Radical Scavenging Assay in Ethanol, Acetone and Aqueous Extract of *Carica Papaya* and *Ananas Cosmosus*

3.5.2 FRAP Radical Scavenging Assay

The free radical scavenging activity of various extracts of *Carica papaya* and *Ananascosmosus* was determined by Frap reducing antioxidant power method and result were presented in table 10 and figure 8 were seen scavenge the stable FRAP radical directly to different extents over at different concentrations.

Table 10: FRAP Radical Scavenging Assay in Ethanol, Acetone and Aqueous Extract of *Carica Papaya* and *Ananas Cosmosus*

Sample	%inhibition of samples		
	Aqueous	Ethanol	Acetone
Carica papaya	66	91	81
Ananas cosmosus	56	82	83

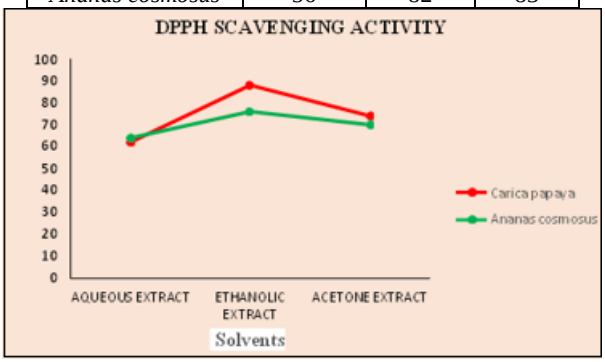

Figure 7: DPPH Radical Scavenging Assay in Ethanol, Acetone and Aqueous Extract of *Carica Papaya* and *Ananas Cosmosus*

Ethanol extract showed the maximum level than other extracts. This study confirms that the antioxidant activity of *Carica papaya* and *Ananas cosmosus.*

3.6 Antibacterial assay of *Carica papaya* and *Ananas cosmosus*

The extracts of Carica papaya and Ananas cosmosus had been tested for their antibacterial activities and an interesting antibacterial profile have been observed against

Staphylococcus aureus and Klebsiella pneumoniae species. The activities of extracts are mentioned in the terms of zone of inhibition (mm).

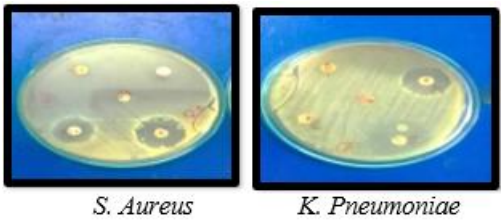

Figure 8 Antibacterial Assay of C*arica papaya*

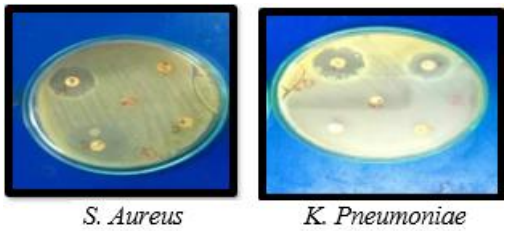

Figure 9: Antibacterial Assay of *Ananas cosmosus*

The antibacterial activity of various extracts of *Carica papaya* and *Ananas cosmosus* was compared with commercially available antibiotic discs in gram positive bacteria S. aureus sample kanamycin (1.9mm), (2.4mm), (2.7mm), tetracyclin, (2.2mm), (2.5mm), (2.8mm), sample (0.6mm), (1.4mm), (1.9mm) and Ananas cosmosus has kanamycin (1.7mm), (2.2mm), (2.9mm), tetracyclin, (2.3mm), (2.5mm), (2.6mm), sample (0.2mm), (1.5mm), (1.7mm).

The antibacterial activity of various extracts of *Carica papaya* and *Ananas cosmosus* was compared with commercially available antibiotic discs in gram negative bacteria K. Pneumonia sample kanamycin (1.6mm), (2.2mm), (2.4mm), tetracyclin, (2.0mm), (2.4mm), (2.5mm), sample (0.7mm), (2.2mm), (2.6mm) and Ananas cosmosus has kanamycin (1.4mm), (2.1mm), (2.5mm), tetracyclin, (2.1mm), (2.3mm), (2.7mm), sample (0.4mm), (2.3mm), (2.5mm). It shows the antibacterial activity in various extracts of samples.

Table 11: Antibacterial Assay of *Carica papaya*

Name	*Caricapapaya*	Zone of Inhibition in (mm)		
		Disc	Kenamycin	Tetracyclin
S. aureus	Aqueous	0.6	1.9	2.2
	Ethanol	1.4	2.4	2.5
	Acetone	1.9	2.7	2.8
K. pneumoniae	Aqueous	0.7	1.6	2.0
	Ethanol	2.2	2.2	2.4
	Acetone	2.6	2.4	2.5

Table 12: Antibacterial Assay of *Ananas cosmosus*

Name	*Ananas cosmosus*	Zone of Inhibition in (mm)		
		Disc	Kenamycin	Tetracyclin
S. aureus	Aqueous	0.2	1.7	2.3
	Ethanol	1.5	2.2	2.5
	Acetone	1.7	2.9	2.6
K. pneu moniae	Aqueous	0.4	1.4	2.1
	Ethanol	2.3	2.1	2.3
	Acetone	2.5	2.5	2.7

3.7 Anticancer Activty by MTT Assay

The anticancer activity of Carica *papaya* were conducted for the ethanol extract on colon cancer cell lines with different concentrations.

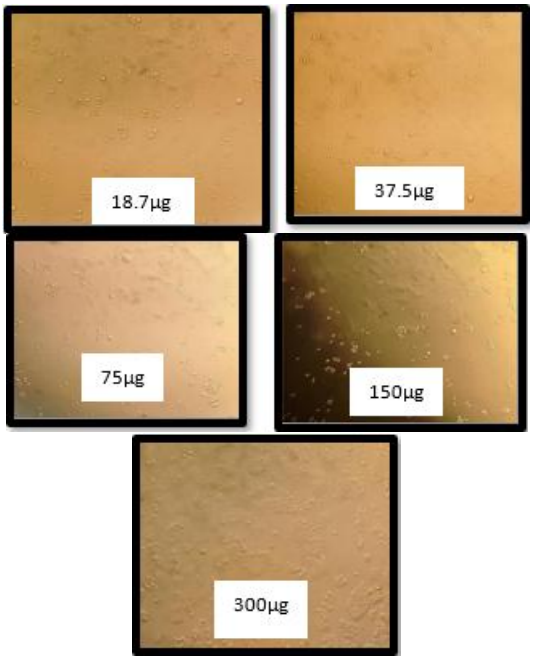

Figure 10: Anticancer activity of Ethanol extract of C*arica papaya* on colon cancer cell lines

Table 13: Anticancer activity of Ethanol extract of *Carica papaya* on Colon cancer cell lines (HCA 7 cell line)

Conc (µg/ml)	% Cell inhibition
18.7	3.14389
37.5	13.6638
75	24.7279
150	37.3639
300	54.5344
IC 50	170.17 µg/ml
R^2	0.989

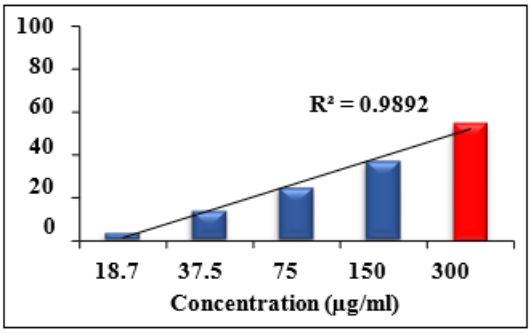

Figure 11: Anticancer activity of Ethanol extract of *Carica papaya* on Colon cancer cell lines (HCA 7 cell line)

3.8 Anticancer activity *of Ananas cosmosus*

The anticancer activity of *Ananas cosmosus* were conducted for the ethanol extract on colon cancer cell lines with different concentrations.

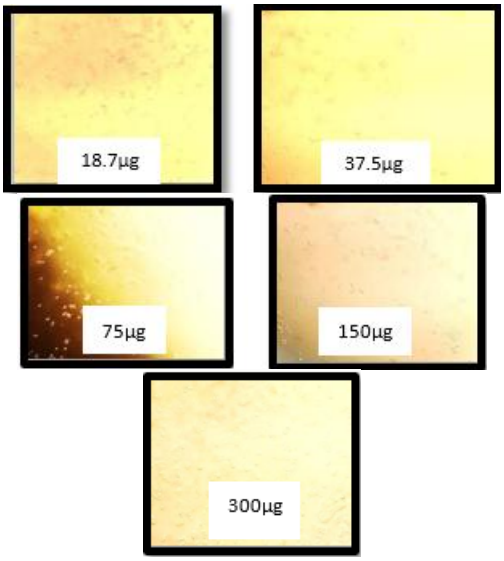

Figure 12: Anticancer activity of Ethanol extract of *Ananascosmosus* on colon cancer cell lines (HCA 7 cell line)

Table 14: Anticancer activity of Ethanol extract of *Ananascosmosus* on colon cancer cell lines (HCA 7 cell line)

Conc (µg/ml)	% Cell inhibition
18.7	3.14389
37.5	13.6638
75	24.7279
150	37.3639
300	54.5344
IC 50	170.17 µg/ml
R^2	0.989

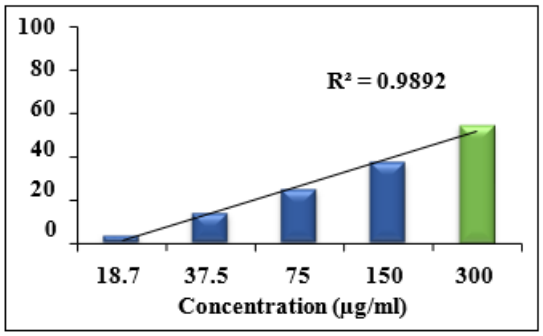

Figure 13: Anticancer activity of Ethanol extract of *Carica papaya* on Colon cancer cell lines (HCA 7 cell line)

The Carica papaya shows the greater efficiency of anticancer activity than Ananas cosmosus.

3.9 Antidiabetic activity in *Carica papaya*

3.9.1 Alpha amylase enzyme inhibition assay

The antidiabetic activity of alpha amylase enzyme inhibition of *Carica papaya* were conducted for the ethanol extract and results are showed in Table 15 and Figure 14.

Table 15: Alpha amylase enzyme inhibition assay of *Carica papaya*

% of the inhibition Conc of the		% of the inhibition
Sample	Papaya	Standard Acarbose (Standard)
10 µg	28%	15.60%
20 µg	36%	42.25%
30 µg	52%	62.39%
40 µg	67%	75.65%
50 µg	83%	90.26%

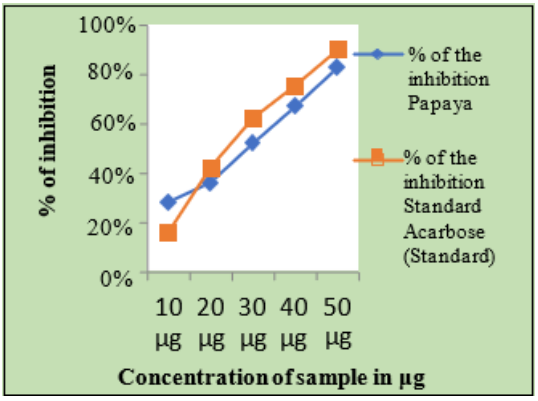

Figure 14: Alpha amylase enzyme inhibition assay of *Carica papaya*

Alpha glucosidase enzyme inhibition assay

The antidiabetic activity of alpha glucosidase enzyme inhibition of *Carica papaya* wereconducted for the ethanol extract and results are showed in Table 16 and Figure 15.

Table 16L: Alpha glucosidase enzyme inhibition assay of *Carica papaya*

Conc of the sample	*% of the inhibition Papaya*	*% of the inhibition Standard Acarbose (Standard)*
10 µg	31%	30%
20µg	42%	47.73%
30 µg	65%	59.34%
40 µg	72%	73.48%
50 µg	87%	89.56%

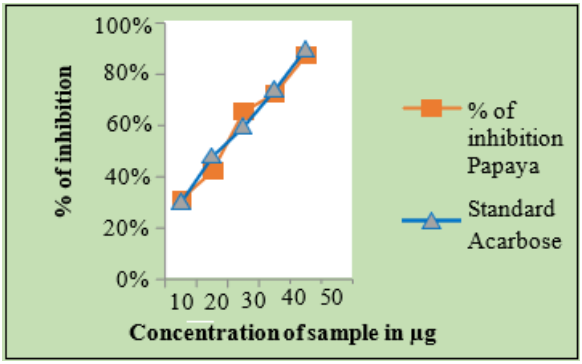

Figure 15: Alpha amylase enzyme inhibition assay of *Carica papaya*

3.10 Antidiabetic activity in *Ananas cosmosus*

The antidiabetic activity of alpha amylase enzyme inhibition of *Ananas cosmosus* were conducted for the ethanol extract and results are showed in Table 17 and Figure 16.

3.10.1 Alpha amylase enzyme inhibition assay

The antidiabetic activity of alpha amylase enzyme inhibition of *Ananas cosmosus* were conducted for the ethanol extract and results are showed in Table 17 and Figure 16.

Table 17: Alpha amylase enzyme inhibition assay of *Ananas cosmosus*

Conc of the sample	*% of the inhibition Pineapple*	*% of the inhibition Standard Acarbose (Standard)*
10 µg	23 %	14.89%
20 µg	30%	44.78%
30 µg	51%	68.89%
40 µg	62%	77.56%
50 µg	79 %	92.52%

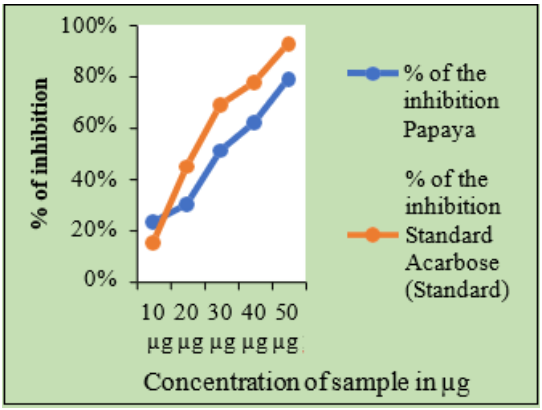

Figure 16: Alpha amylase enzyme inhibition assay of *Ananas cosmosus*

3.10.2 Alpha glucosidase enzyme inhibition assay: The antidiabetic activity of alpha glucosidase enzyme inhibition of *Ananas cosmosus* were conducted for the ethanol extract and results are showed in Table 18 and Figure 17.

Table 18: Alpha glucosidase enzyme inhibition assay of *Ananas cosmosus*

Conc of the sample	*% of the inhibition Pineapple*	*% of the inhibition Standard Acarbose (Standard)*
10 µg	23%	14.89%
20 µg	30%	44.78%
30 µg	51%	68.89%
40 µg	62%	77.56%
50 µg	79%	92.52%

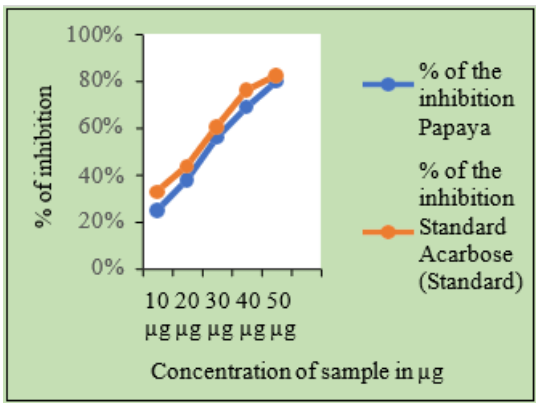

Figure 17: Alpha glucosidase enzyme inhibition assay of *Ananas cosmosus*

The Carica papaya shows the greater efficacy of anticancer activity than Ananas cosmosus.

4. Summary and Conclusion

According to findings, *Carica papaya* and *Ananas cosmosus* has many phytochemical components, has been studied in vitro, and has strong action with favourable safety profile for treating patients with various diseases or illnesses. the biological activity of fruits is concerned with the phytochemicals present in fruit samples. Phytochemicals screening plays an important role in the study of cytotoxic and antidiabetic activity of the fruit sample. The fruit extract of both samples taken to understanding its processes may help to avoid diabetes and cancer. The statistical method to show the correlation among the biological activities of the of both the samples. These in vitro studies of anti - cancer and anti - diabetes may become a good source of phytochemicals, food products, and nutraceuticals to be utilized and cure of various ailments.

The extracts are prepared using ethanol, acetone and aqueous as a solvents and Phytochemical studies revealed that the fruit sample contain the important components like Carbohydrates, Alkaloids, Flavonoids, Proteins, Phenol, Tannins. The present study concluded that the preliminary qualitative test can be help in detection of such bioactive principles leading to discovery of new drugs and the bioactive components such as flavonoids content were highly observed in ethanol extract of *Carica papaya* and *Ananas cosmosus*.

After the phytochemical screening the equal proportion of ethanol, acetone and aqueous extract of papaya and pineapple was secondary metabolites such as alkaloids, flavonoids, phenol and tannins were biochemical estimated to highly presence of flavonoids, cholesterol and proteins. The ethanol extract of *Carica papaya* and *Ananas cosmosus* is a good source of biochemical compounds, vitamins and minerals such as Iron, Magnesium making a suitable functional source for improving nutraceutical and nutritional effects. It found that ethanol extract of *Carica papaya* has high amount of alkaloids, flavonoids, phenol and tannins than *Ananas cosmosus* sample.

The present study further confirms the antioxidant and antibacterial activity of both the fruit samples. The ethanol extract of *Carica papaya* showed the maximum activities than the *Ananas cosmosus* sample. This highlights the medicinal proportion of anticancer and antidiabetic activities of the papaya fruit sample.

An MTT assay was performed to determine the anti - cancer activity of ethanolic extract of *Carica papaya* and *Ananas cosmosus* showed HCA 7 cells from human colon cancer was investigated in vitro. These findings provide important information to improve health and prevent cancer by improving the consumption of papaya and pineapple and its products and its application in food product development. The anticancer activity starting from low to high concentrations when compared with the control using MTT assay. The results showed % cell inhibition and cell

viability at different concentrations.

The investigation confirms that ethanolic extract of *Carica papaya* and *Ananas cosmosus* exhibits antidiabetic activity. The elucidation of bioactive compounds from ethanol extract & also confirm its antidiabetic property by *alpha amylase and alpha glucosidase enzyme inhibition assay*. This study provides that fruit of *Carica papaya and Ananas cosmosus* have anti - diabetic efficacy. Thus, considering its relative hypoglycemic potency, they may serve as useful therapeutic agents for treating diabetes.

5. Future Prospects

Cancer is an irregular proliferation of cells that starts with a gene mutation that alters cellular function, is triggered by several factors, and can be inherited or acquired. The anticancer activity of papaya and its components' strength, focusing on its implication in cancer prevention and treatment.

In view of the growing incidence of cancer, increasing the pace of the creation, development and testing of new antitumor agents, the improvement and expansion of new high - tech systems for preclinical *in vitro* screening is becoming very important.

Prevalence of diabetes mellitus, a chronic metabolic disease characterized by hyperglycemia, is growing worldwide. The majority of the cases belong to type 2 diabetes mellitus. Inadequate regulation of the blood sugar imposes serious consequences for health. Conventional antidiabetic drugs are effective, however, also with unavoidable side effects. On the other hand, medicinal plants may act as an alternative source of antidiabetic agents. The medicinal valuable of fruits having efficacy to prevent diabetes mellitus and invitro studies by enzyme inhibition assay provides prevention and treatment.

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