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Phytochemical Analysis and Physicochemical Study of Carica Papaya Leaves

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ABSTRACT:

The Present paper deals with the Screening of Phytochemical and Physicochemical of Carica Papaya leaves. The results which were obtained showed the presence of various Phytochemicals viz. Alkaloids, Tannins, Resins, Phenols, Carbohydrates, Cardiac Glycosides, Proteins etc. The Physicochemical study revealed the purity and quality of the sample for production of drugs. The detailed discussion is given in the paper.

Keywords: *Carica Papaya, Physicochemical, Phytochemical, Alkaloids, Proteins.*

Introduction

Plants are considered to be the good source for the exploration and discovery of new pharmaceutical compounds as well as medicines, which can be the potential drug for humans as they act as intermediate for synthesis of useful drug [1]. Plants possess various phytochemicals with several bioactivities[2].

Phytochemicals are chemical compounds that occur naturally in plants. They are chemicals produced by plants through primary or secondary metabolism [3-5]. Some are responsible for colour and other organoleptic properties, such as the deep purple of blueberries and the smell of ginger. Plants are composed entirely of chemicals of various kinds Phytochemical has bio-active constitutes such as alkaloids, tannis, flavonoids, saponins and phenolic compounds [6]. The medicinal values of some plants lie in these chemical substances that produce definite physiological actions in the human body. Many of these indigenous medicinal plants are used as spices and food plants [7]. Plant serves as rich resources of natural drugs for research and development. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in plants. The medicinal actions of plants are unique to plant species or groups as the combination of secondary products in a plant are often taxonomically distinct[8].

Most of the phytochemicals posses the biological antioxidant capacity that protects our cells against the oxidative damage and reduces the risk of certain types of cancer. These Phytochemicals tend to prevent the adhesion of pathogens to the human cell wall by physically binding to it[9].

Seeing the defensive properties and other beneficial effects of plants, I have taken, “Phytochemical Analysis and Physicochemical study of Carica Papaya leaves” for the present study.

Classification :

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliophyta

Order - Brassicales

Family - Caricaceae

Genus - Carica

C. Papaya Linn, is an evergreen shrub or small tree, is a member of family Caricaceae, represented with four genera and four species in India. This plant originated in Southern Mexico and Costa Rica and distributed as a plantation Crop in India, Sri Lanka, Hawaii, Australia, and in tropical and subtropical regions[10]. Papaya, the herbaceous perennial plant is also known as Papaya melon tree, Pawpaw or Papua, Kapaya, Lapaya, Papyas, Papye, Tapayas, and Fan mu gua. The entire papaya plant is best with a large variety of phytonutrients and antioxidant, antimicrobial, and anti-dengue properties[11].

In India, boiled leaves of papaya are recommended by Ayurveda practitioners as relief from malarial and dengue fevers as papaya leaf extract is considered effective to elevate platelet count and red and white blood cells in patients after suffering from viral fever[12]. The extract has also been known to protect the patients against the sickling of red blood cells[13]. Tea prepared from the juice extracted from papaya leaves is also used as a synergistic therapeutic dietary supplement for patients suffering from the oxidative stress related diseases because of its strong antioxidant potential [14].

Fig. 1 Carica Papaya leaf

- **Medicinal Use :**
  - Papaya leaves work amazingly to treat dengue fever. It is also great to prevent malarial infection. Papaya leaf contains a compound named – acetogenin that prevents diseases like malaria or dengue.
  - Carica papaya leaf contains active components such as alkaloids, glycosides, tannins, saponins, and flavonoids, which are responsible for its medicinal activity.
  - Papaya leaves extract have strong medicinal properties such as antibacterial, antiviral, antitumor, hypoglycaemic and anti-inflammatory activity.
  - Papaya plant is a source of vitamins A, B and C and also a fair source of calcium and iron [15].

Materials and Methods

Materials which were used in the study are given in table :1

Table- 1 List of Glassware, Instruments and Reagents are:

S.No.	Glass ware	Instrument	Reagent
1.	Conical flask	Water bath	Fehling's solution A &B
2.	Funnel	Weighing machine	Dragendorff's reagent
3.	Glass Rod	Shaker	Wagner 's reagent
4.	Petri Dish	Hot Air Oven	Sodium Hydroxide
5.	Pippetes	Desiccator	Sulphuric Acid
6.	Test tube	Muffle Furnace	Hager's reagent
7.	Measuring cylinder	Grinder machine	Ferric Chloride
8.	Dropper	Sieve	Copper Sulphate
9.	Beaker	Spatula	Distilled water
10.	Crucible	Tray dryer	Hydrochloric acid
11.	Reagent bottle	Filter paper	Ethanol
12.	Capillary tube	Reprostar	Methanol
13.	China Dish	HPTLC	Folin's reagent
14.	Centrifuge Tube	Mixcy	Chloroform
15.	Pippetors	Linomat	Acetone

Sample Collection :

The fresh leaves of Carica Papaya were collected from Majhgawan, Satna.The collected leaves were washed and then dried in sun light for few days. The dried leaves sample were grounded with the electric grinder to get fine powder and then it was sieved by a sieve. Powder was stored in a glass container for further analysis.

The Physicochemical Parameters which were analysed in the present study are:

- Moisture content (LoD at 105ºC)
- Water soluble extractive value
- Alcohol soluble extractive value
- Chloroform soluble extractive value
- Acetone soluble extractive value
- Benzene soluble extractive value
- Total Ash value
- Acid Insoluble Ash

Determination of moisture content (Loss on drying at 105ºC)

The moisture content of the sample is important to determine as it plays role in prevention of microbial growth in the sample powder. In this method, two clean petri plates were taken and weighed them separately and with the help of marker we named Petri plate 1 and Petri plate 2. After this, we took 2 grams of equal sample powder in both the petri plates. These petri plates were kept in hot air oven for 5 hours at 105ºC. After completion of 5 hours, these petri plates were cooled and weighed. Again petri plates were kept on hot air oven for 30 minutes at 105ºC, and these were cooled in a desiccator and weighed. Then the percentage of moisture content was calculated with respect to air dried sample.

Determination of Ash values:

The Ash value is useful to determine the quality and purity of the drug. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc . Different Ash values such as total ash value and acid insoluble ash value were determined.

Determination of Total ash :

Incinerated about 2 gram accurately weighed of drug in a platinum or silica dish crucible at a temperature not exceeding 450°c (6 hours) until free from carbon. Cooled in desiccator and weighed in weighing machine. Finally, percentage of total ash content was calculated with reference to air dried drug.

Determination of Acid Insoluble Ash :

The crucible containing total ash, added 25 ml dilute hydrochloric acid (HCl). The insoluble matter was collected on Gooch crucible, using the ash less filter paper & washed with hot water (250 ml) the filtrate is neutral. Transferred the filter paper containing the insoluble matter to the original crucible dry on a hot plate and ignite to constant weight, cooled for 30 minutes in a suitable desiccator & weighed without delay. The percentage of amount of acid insoluble ash was calculated with reference to air dried drug.

Determination of alcohol soluble extractive : Weighed accurate 2g powder sample and transferred in a beaker. 100 ml alcohol solvent was added. It had been provided continuous shaking for 6 hrs and leave for 18 hrs (Maceration). After this, extract was filtered by using Whatman filter paper no. 1 and weighed in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

Determination of water soluble extractive:

Weighed accurate 2g powder sample and transferred in a beaker. 100 ml water solvent was added. It had been provided continuous shaking for 6 hrs and leave for 18 hrs (Maceration). After this, extract was filtered by using Whatman filter paper no. 1 and weighed in thin orcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined.

Phytochemical Analysis :

Various phytochemical analysis were:

- Alkaloid test
- Flavonoid test
- Saponin test
- Resin test
- Carbohydrate test
- Phenol test

- Cardiac Glycoside test
- Protein test
- Terpenoid test

Stock Preparation : 2 gram of Papaya leaves extract was dissolved in 10 ml of each solvent and 1 ml of each solvent was used as a standard for various phytochemical tests.

1.Test for Alkaloids :-

a)Dragendorff's test: Dissolved a few ml of alcohol or aqueous extract in 5 ml of distilled water then added 1 ml HCL until an acid reaction occurs then added 1 ml of reagent, and observed the change.

b)Wagner's test :-Acidified 1 ml of alcoholic extract with 1.5% w/v of HCI and few drops of Wagner's reagent was added, and change was noted.

c)Hager's test:- 2-3 ml of aqueous or alcohol extract added few drops of Hager's reagent, and the change was noted.

2.**Test for Flavonoid:**

a)Shinoda test : In test tube containing 0.5 ml of alcoholic extracts,10 drops of dilute HCl was added followed by small pieces of magnesium, and observed the change.

3. Test for Saponins:

a)Froth test: In 1 ml of extract, 2 ml of distilled water was added and shaken well and observed the change.

4. **Test for tannin:** To 1-2 ml of extract, added few drops of 5% $\mathbf{FeCl_3}$ (ferric chloride), and the change was noted.

5. **Test for Resin:** For resin test two test were done :

a)With Con.HCl: In 1 ml of extract, few drops of concentrated HCl was added, and the change was observed.

b)With $\mathbf{FeCl_3}$: In 1 ml of extract, few drops of Ferric Chloride was added and observed the change.

6. **Test for Carbohydrate:**

a)Fehling's Test: In 2 ml of aqueous or alcohol extract, added 1 ml of mix of equal parts of Fehling's solution 'A' and 'B' and boiled the contents of the test tube for few minutes, and the change was noted.

b)Benedict's Test: To 1 ml of extract solution, add 2-5 ml of Benedict's reagent and boil for 2 minutes and cooled, and observed the change.

7. **Test for Phenol :**

2 ml of extract was taken and 2 ml of Folin's reagent was added, and change was noted.

8. **Test for Cardiac Glycoside :**

a) Keller Killani Test: To 2ml extract, glacial acid was added, one drop of 5% ferric chloride ($FeCl_3$) and sulphuric acid (H_2SO_4)were also added. Change was observed.

9. **Test for Protein :**

a) Biuret Test: To 1 ml hot aqueous extract, added 5-8 drops of 10% w/v NaOH solution followed by 1-2 drops of 3% w/v $CuSO_4$ solution, and change was noted.

10. **Test for Terpenoid :**

3-5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a layer, and observed the change.

Methodology for High Performance Thin Layer Chromatography:

High Performance Thin Layer Chromatography of the test solutions of sample **Carica Papaya leaves** was carried out on Silica gel 60 F254 percoated plates (0.2 mm thickness; from Merck India Limited Mumbai). A TLC applicator from Camag Linomat-5(Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints.

Procedure:- Applied 10µl each of the test solutions as 8 mm bands and develop the plate in a solvent system toluene : ethyl acetate : formic acid (7:3:0.5) to a distance of 9 cm. Dry the developed plate in air and examined under ultraviolet light, at 254 nm and at 366 nm before derivatisation. Derivatised the plate using 5% methanolic sulphuric acid reagent and heating at 105ºC till the bands are clearly visible and examined the plate under 254nm and 366nm. The Rf values and colours of the bands obtained were recorded.

Derivatization : Possibility of derivatisation is a strong point of HPTLC. Chemical reaction are possible on given plate before or after chromatography, both the possibility have their advantages. However the decision depends on sample matrix level of detection and interference present. Post chromatography derivatization is more popular technique for which several for hundred references in literature are available as compared to a few pre chromatographic derivatization. The results are unique and specific when before Chromatographic development has been recommended. Derivatize the plate using methanol reagent and heating at 105ºC till the band are clearly visible and examined the plate. The Rf value and colour of band resolved were recorded.

Visualization: HPTLC fingerprinting Profile of the test solutions were depicted in given plate indicate the presence of different types of phytochemical. Development of fingerprint profile would serve as a reference standard of authentic sample. The TLC plate was examined under 254 nm, 366 nm, after derivatization 366 nm and visible light.

Rf Value : Measure and record the distance of each spot from the point of its application and calculate the Rf value by dividing the distance travelled by the spot by the distance travelled by the spot by the distance travelled by the front of the Mobile phase.

Preparation of HPTLC :

- **Test solution :** Concentrated solution of sample with methanol.
- **Stationary Phase :** Pre coated plates with Silica Gel.
- **Mobile Phase :** Toluene : Ethyl acetate: Formic acid (7:3:0.5).
- **Spray reagent for derivatization:** 5% Methanolic sulphuric acid.
- **Distance travelled by solvent :** 9 cm
- **Developed Chamber :** Twin through chamber 10×10

Results and Discussion

The Sample was screened for moisture content, Ethanol Soluble extractive, water soluble extractive, chloroform soluble extractive, benzene soluble extractive, acetone soluble extractive, total ash value, acid insoluble ash, Phytochemicals and HPTLC analysis and their results are tabulated below:

1. Physicochemical Analysis

Table No. 2: Loss on Drying (LoD value of Carica Papaya) leaf:

S. No.	Empty Petri dish+2gm Sample weight(A)	After 5 hours post Petri dish weight (B)	After ½ hours of drying post Petri dish weight (C)	Difference (A-C)
1.	27.4365	27.3108	27.3070	0.1295
2.	37.5253	37.4020	37.3981	0.1272
	Average	-	-	**0.1283**

Sample weight= 2 gm

LoD % = Average ×100/Weight of sample

LoD % = 0.1283×100/2 = 6.41%

Extractive value

Table No. 3: Water soluble extractive value of Carica Papaya (leaves):

S. No.	Petri dish pre weight (A)	Amount	Petri dish post or final weight (B)	Difference (B-A)
1.	32.7001	10ml	32.7649	0.0648
2.	36.6176	10ml	36.6820	0.0644
	Average	-	-	**0.0646**

Extractive Value= Average ×500

Extractive Value= 0.0646×500

= 32.3%

Table No. 4: Methanol Soluble extractive value of Carica Papaya (leaves):

S. No	Petri Dish pre weight(A)	Amount	Petri dish post or final weight (B)	Difference (B-A)
1.	23.9424	10ml	23.9917	0.0493
2.	29.6120	10ml	29.6605	0.0485
	Average	-	-	**0.0489**

Average = 0.0489 ,

Extractive Value= 0.0489×500 = 24.45%

Table No. 5: Ethanol Soluble extractive value of Carica Papaya (leaves):

S. No.	Petri dish pre weight (A)	Amount	Petri dish post or final weight(B)	Difference (B-A)
1.	25.4372	10ml	25.4602	0.023
2.	29.8916	10ml	29.9139	0.0223
	Average	-	-	**0.0226**

Average = 0.0226

Extractive value = 0.0226×500

= 11.3%

Ash Value

Table No.6 : Total Ash in 2 gm of sample

S. No.	Weight of Empty crucible (A)	1st weight (B)	2nd weight (C)	3rd weight (D)	Difference (D-A)
1.	16.8104	17.0731	17.0703	17.0701	0.2597
2.	16.4511	16.7101	16.7078	16.7085	0.2574
	Average	-	-	-	**0.2585**

Total Ash % = Average ×100/weight of sample

Total Ash %. =0.2585×100/2

= 12.79%

Table No.7: Acid Insoluble Ash in 2 gm sample

S.No.	Weight of empty crucible (A)	1st weight (B)	2nd weight (C)	3rd Weight (D)	Difference (D-A)
1.	16.8104	16.8260	16.8246	16.8240	0.0136
2.	16.4511	16.4733	16.4713	16.4646	0.0135
	Average	-	-	-	**0.0135**

Acid Insoluble Ash % = Average ×100/2.

Acid Insoluble Ash % = 0.0135×100/2

= 0.67%

Table No. 2, 3, 4, 5, 6, 7, 8, 9, 10 showed the Moisture content (LoD) = 6.41% , WSE = 32.3%, MSE = 24.45% , ESE = 11.3% , BSE = 8.3% , CSE = 9.56% , ASE = 10.8% , Total Ash = 12.79% , Acid Insoluble Ash = 0.67% respectively.

Table No.8: Qualitative Phytochemical Analysis of Carica Papaya leaves:

S.No.	Test	Observation	Water	Metha -nol	Ben -zene	Acetone	Chloroform
1.	**Alkaloids test**						
a.	**Wagner's reagent**	Reddish brown precipitate indicates presence of alkaloids	+	+	-	+	+

b.	**Hager's reagent**	Reddish brown precipitate indicates the presence of alkaloids	+	+	+	+	*
c.	**Dragendorff's reagent**	Orange brown precipitate indicates presence of alkaloids.	+	+	-	-	+
2.	**Flavonoid test**						
a.	**Shinoda test**	Formation of pink colour indicates the presence of Flavonoid	-	-	+	+	+
3.	**Saponins test**						
a.	**Froth test**	Resistance froth of 1-1.5 cm indicate the presence of Saponin	-	-	*	*	*
4.	**Tannin**	White brown colour indicates the presence of tannin	+	+	-	+	-
5.	**Resin test**						
a.	**Con. HCl**	Reddish pink colour indicates the presence of resin	+	+	+	+	+
b.	**$FeCl_3$**	Greenish colour indicates the presence of resin	+	+	+	+	+
6.	**Carbohydrates test**						
a.	**Fehling's test**	Pink or red colour indicates the presence of carbohydrate	-	*	+	+	+
b.	**Benedict test**	Red precipitate indicates the presence of carbohydrate	+	+	+	*	+
7.	**Phenol test**	Violet or brown colour indicates the presence of phenol	+	+	-	+	+
8.	**Cardiac Glycoside test**						
a.	**Keller Killani test**	Reddish brown colour appears at the junction of two liquids layers appear	+	*	+	-	+
9.	**Protein test**						
a.	**Biuret test**	Red or violet colour indicates the presence of protein	+	*	+	+	+
10.	**Terpenoid test**	Reddish brown colour appears	-	-	-	*	-

"+" Present.

"-" Absent.

"*" Not done.

Table- 8, shows the presence of :

- **a.** Alkaloids in all the sample extracts of methanol, water, chloroform, acetone, benzene.
- **b.** Flavonoid were found in chloroform , acetone and benzene, but absent in water and methanol.

c. Saponins were absent in water and methanol.

d. Tannins were present in water, methanol and acetone but absent in benzene and chloroform.

e. Resins were present in all the samples.

f. Carbohydrates were present in all the sample except in water in Fehling's test.

g. Phenols were present in all the samples except benzene.

h. Cardiac Glycosides were present in all samples except acetone.

i. Proteins were present in all four samples including water, acetone, chloroform and benzene.

j. Terpenoids were absent in all samples.

"HPTLC Fingerprint profile of Carica Papaya leaves"

Table No. 9: Rf Value of HPTLC Fingerprint profile on Carica Papaya leaves:

S. No	Rf Values	254nm before derivatization	366nm before derivatization	366nm after derivatization
1.	**Rf1**	0.09(Blue)	0.09(Sky blue)	0.18(Sky blue)
2.	**Rf2**	0.19(Purple)	0.2(Sky blue)	0.33(Sky blue)
3.	**Rf3**	0.95(Green)	0.51(Sky Blue)	0.4 (Green)
			0.55 (Red)	0.42(Sky Blue)
			0.75 (Red)	0.52(Sky blue)
			0.89 (Red)	0.59 (Green)
				0.63(Green)
				0.74(Green)
				0.78(purple)

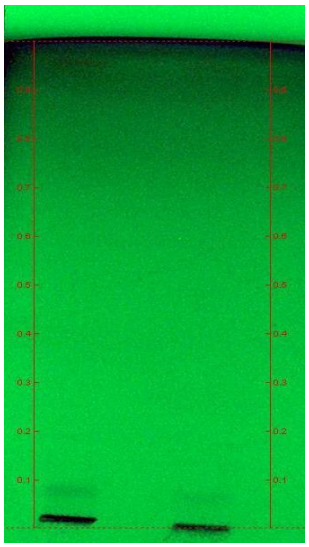
Table 9 shows different spots at:

a) **At 254 nm(before Derivatization) :** Fine spots were observed at 0.09 (Blue), 0.19 (Purple), and 0.95 (Green).

b) **At 366 nm (Before derivatization) :** 6 spots were observed that are 0.09 (Sky blue), 0.2 (Sky blue), 0.51 (Sky blue), 0.55 (Red), 0.75 (Red), and 0.89 (Red).

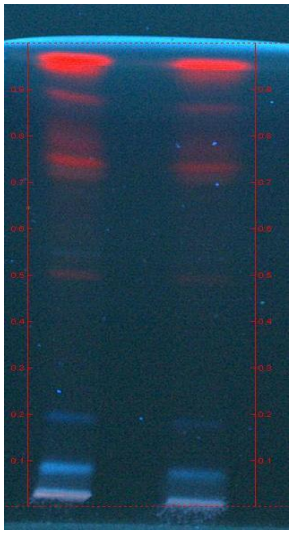
c) **At 366 nm (After derivatization) :** 9 spots were observed that are 0.18 (Sky blue), 0.33 (Sky blue), 0.4 (Green), 0.42 (Sky blue), 0.52 (Sky blue), 0.59 (Green), 0.63 (Green), 0.74 (Green), and 0.78 (Purple).

Fig .1 fig. 2 fig. 3



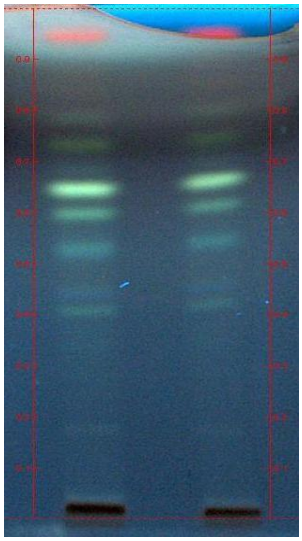
A B

254 nm (BD)



A B

366 nm (BD)



A B

366 nm(AD)

Here ,(BD) refers to Before derivatization, and (AD) refers to after derivatization.

Track A: Test solution of Carica Papaya leaves.

Track B: Test solution of Carica Papaya leaves.

Conclusion

Ayurvedic Herbal Medicines ensure Physical and Mental health without side effects, containing the natural ingredients. The Allopathic drugs have more side effects due to the use of various toxic chemicals. While Ayurvedic medicines help bring Arogya to Human body and Mind. ("Arogya means free from disease").

This study on "Carica Papaya leaves" was undertaken to evaluate the Phytochemical and Physicochemical compositions of the Carica papaya leaves. Phytochemical profiling of young leaves of Carica Papaya revealed the presence of pharmacologically active phytocompounds, alkaloids, phenolics, flavonoids and also, proteins, sugars. These constituents have medicinal value which can be used to treat different ailments. From the present study it can be concluded that Carica Papaya leaves has the potential for the production of drug with proper authentication.

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