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Extraction, Phytochemical Analysis and Functional Group Confirmation of *Carica Papaya* Leaf by FTIR Spectroscopic Method

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ABSTRACT

The aim of this study is to extract *Carica papaya* plant leaf, analyse phytochemical analysis, and confirm functional groups using FTIR analysis.To extract Carica papaya and perform preliminary phytochemical analysis to identify phytochemical ingredients contained in the leaf extract and confirm the functional group of the active constituents present in the extract using the FTIR technique.The preliminary phytochemicals of Ethanol, Methanol, and Hydro-Alcohol extracts of Carica papaya were evaluated to determine the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, and tannins to confirm the functional group of the active constituents present in the extract by using the FTIR method analysis. Saponins, steroids, carbohydrates, alkaloids, glycosides, proteins and amino acids, flavonoids, and other related phytochemicals having N-H bonds were found in Carica papaya leaf extract, which was validated by qualitative phytochemical analysis.From the results of the study was concluded that ethanolic extract of *Caricva papaya* has the Alkyl halides, carboxylic acids, amines, amides, Alkenes, Ethers, Alcohol compounds.These amines and carboxylic acids are used for Dengue, Dyspepsia,hyperacidity,dysentery and constipation.

Keywords: FTIR-spectroscopy, Hotperculation extraction, Phytochemical analysis, *Carica papaya*.

INTRODUCTION

Papaya is a nutrient-dense fruit that is readily available throughout the year. Vitamin C, vitamin A, and vitamin E are abundant, as are minerals like magnesium and potassium, B vitamins like pantothenic acid and folate, and fibre. It also contains papaintha, a digestive enzyme that effectively treats trauma, allergies, and sports injuries. All of the nutrients in papaya support cardiovascular health, helping to avoid heart disease, heart attacks, and strokes, as well as colon cancer. Aravind. G, debjit bhowmik duraivel and S, harish.G.(2013)[1]

Table 1 : Botanical Classification

ANGUAGE	REGION	NAMES
Hindi	Haryana, Delhi	Papaya, papita
Bengali	West Bengal	Papaya,Pepe, Papita
Malayalam	Kerala	Omakai
Punjabi	Punjab	Papita
Marathi	Maharashtra	Papai
Tamil	Tamilnadu	Pappali
Kannada	Karnataka	Pharangi
Rajasthani	Rajasthan	Eerankari

Carica Papaya

Carica papaya Linn, also known as papaya in English, Papita in Hindi, and Erandakarkati in Sanskrit, is a member of the Caricaceae family. The plant is tropical in origin and was brought to India in the 16th century. The plant is easily identified by its weak, unbranched soft stem that yields copious amounts of white latex and is crowded by a terminal cluster of huge, long stalked leaves. It grows quickly and can reach a height of 20 metres. Leaves have traditionally been used to cure a variety of maladies, including malaria, dengue fever, jaundice, and immunomodulatory and antiviral activities. Vijay yogiraj, pradeep kumar goyal, chetan singh chauhan, anjugoyal, bhupendra vyas.(2014)[2]

Saponins, alkaloids, tannins, flavonoids, cardiac glycosides, anthraquinones, phlobatinins, anthocyanosides, and phenols were found in the C. papaya phytochemical analysis. The amount and composition of these diverse phytochemicals varies from one plant part to the next, and is influenced by the extraction solvent utilised. According to reports, papaya is a nutritive fruit that contains a tiny quantity of protein and the same number of minerals, primarily iron, calcium, and phosphorus, as well as vitamins A and C and the enzyme papain. Arun kumar srivastava, vinay kumar singh.(2016).[3]

Table 2: Indian Synonyms

Domain	Flowering plant
Kingdom	Plantae
Sub Kingdom	Tracheobionta
Class	Magnoliopsida
Subclass	Dilleniidae
Superdivision	Spermatophyta
Phyllum	Steptophyta
Order	Brassicales
Family	Caricaceae
Genus	Carica
Botanical Name	*Carica papaya* Linn

FTIR Spectroscopy

Fourier transforms infrared spectroscopy, or FTIR, is a universal analytical tool for analysing a wide range of materials, especially for detecting unknown materials. The FTIR method has been used to identify pure compounds, mixtures, impurities, and compositions of many materials. It has also benefited in the understanding of a variety of processes involving a variety of chemicals or materials. According to articles published in the expert literature, infrared spectroscopy has been utilised to analyse structural changes as well as to monitor production (bio production) processes. Mid-infrared spectroscopy is used in pharmaceutical sciences to analyse substance concentration in formulations, investigate substance release from formulations, and investigate other kinetic processes such as substance penetration or distribution. Bremer PJ, Geesey GG.(1991)[4]

FTIR spectroscopy has been used in more advanced studies, including as the characterization of creative materials and disease diagnosis. The use of FTIR for protein structure analysis based on peptide amide linkage is also significant in medical applications. For determining the characteristics of polymeric and biopolymeric materials, FTIR spectroscopy is an important method. [6]Some of the more common applications of FTIR methodology include quality verification of materials, analysis of thin films and coatings, decomposition of polymers and other materials (often combining thermo-gravimetry with FTIR and mass spectrometry), microanalysis of materials to identify contaminants, emissions monitoring, and failure analysis. Vibrational spectroscopy, like FTIR and Raman spectroscopy, can be used to assess the covalent and non-covalent functionalization of biomaterials created by adding various functionalities at the material matrix or surface, such as the immobilisation of active molecules. These complimentary techniques can be used to distinguish between covalent and non-covalent immobilizations. Non-covalent interactions are indicated by the shift of spectral bands characteristic of the material or immobilised molecule in the FTIR spectra of functionalized materials, whereas covalent interactions are indicated by the formation of new functional groups in the FTIR spectra of functionalized materials.] Ashokkumar R, Ramaswamy M.(2014) [5],Salari A, YoungRE.(1998) [6],Garside P, Wyeth P.(2003)[7]

MATERIALS AND METHODS

Plant Collection

The leaves of Carica papaya were collected from plants growing in the omalur village, salem district, Tamilnadu , India during the month of October to November. The leaf was dried at 5 days, then it was blended into coarse powder by electrical grinder. The powdered drug was passed through sieve No.22 to get uniform particle size.

Hot Perculation Method Procedure

All glassware should be soaked in ethanol, drained, and dried in a 102°C hot air oven for 30 minutes. Keeping cool in desiccators is also a good idea. Place a piece of cotton wool at the bottom of a 100ml beaker. Place a cotton wool plug in the bottom of an extraction thimble and set it up in the beaker. Precisely weigh 25 g of sample into the thimble. After adding 1 - 1.5 g of sand, mix the sand and sample with a glass rod. Cotton wool should be used to wipe the glass rod and cotton wool should be placed in the thimble's top. For 2

hours, dry the sample in a 102°C oven. Allow the sample to cool for a few minutes in a desiccator.In the top of the thimble, put the cotton wool shred from the bottom of the beaker. The thimble should be placed in a Soxhlet liquid/solid extractor. Clean and dry a 1000 mL round bottom flask before filling it with 500 mL ethanol. Over a water bath or an electric heating mantle, put together the extraction device. Bring the flask's solvent up to a boil. Füleky G, Czinkota I.(1993) [8]

Adjust the heat source such that the solvent drips at a rate of 6 drops per second from the condenser into the sample chamber. Extraction should be continued for a further 6 hours. It's best to remove sausage meat and other emulsified products in stages: After about 4 hours of extraction, turn off the heat and pour the solvent from the extractor in the flask.After removing the thimble from the extractor, transfer the sample to a 100 mL beaker.

Break up the sample using a glass rod. After returning the sample to the extractor, replace the thimble. After washing the beaker with ethanol, pour the rinsing into the extract. Continue the extraction for another two hours. Remove the extractor and condenser from the extraction unit and turn off the heat. Allow the solvent to evaporate by placing the flask back on the heat source. (The solvent can be distilled and recovered.) Dry the contents of the flask in a 102°C oven until they reach a consistent weight (1-2 hours). Before weighing the flask and its contents, place it in a desiccator to cool. De Castro ML, Priego-Capote F. (2010) [9], Lopez-Bascon MA, De Castro ML.(2020)[10],De Castro ML, Garcıa-Ayuso LE.(1998) [11].

Extraction Procedure for*Carica Papaya*

Dry the crude drug sample at 103◦c for 2hours
↓
Take 5gm of sample + 90ml of 95% ethanol (solvent)(crude drug)
↓
Assemble the extraction in soxhlet apparatus
↓
Extract the crude drug with the solvent at 80-90 c
↓
Continue the process for 6hours
↓
Solvent drips from the condenser into sample chamber at the rate of 6drops/ second
↓
Remove the heat source and drain the solvent from the extract
↓
Repeat the procedure with same sample
↓
Replace the flask on heat source and evaporate off solvent
↓
Cool the flask and weigh the flask and content

Calculation of Extractive Yield

Weight of empty flask (g) = W1
Weight of flask and extracted fat (g) = W2
Weight of sample = S

$$\% \text{ Crude fat} = \frac{(W2 - W1) \times 100}{S}$$

Test For Saponins

preparation of test solution:It was prepared by dissolving extract in water and making it aqueous extract.

Foam Test

The drug extracts were vigorously shaken with water. The formation of Persistent foam shows the presence of saponin.

Libermanburchard's Test

To drug extracts few drops of glacial acetic acid and two drops of Conc. H_2SO_4 were added.

The Colour changes from rose red, violet, blue to green. This changes shows the presence of saponins.

Test for Steroids and Triterpenoid test

Preparation of test solution:The test solution was prepared by dissolving the extract in chloroform and subjected to the following test.

Salkowski test

A few drops of Conc. Sulphuric acid were added to the test solution and allowed to stand for some time. The formation of red colour in the lower layer shows the presence of steroids.

Libermanburchard's test

Some drops of acetic anhydride were added to the test solution; the contents were boiled and cooled. Then concentrated sulfuric acid was added from the side of the tube. The formation of a brown ring at the junction of two layers shows the presence of steroids.

Test for Carbohydrate

Molish test

To 2-3 ml of the test solution, added few drops of Molish reagent solution and was shaken. Conc. Sulphuric acid was

added from sides of test tube.Violet ring was formed at the junction of two liquids shows presence of carbohydrates.

Fehling's test
To 1ml solution of the substance, a mixture of equal parts of Fehling's solution A and B was added and the test tube was heated on a water bath. The formation ofRed colour precipitate shows presence of carbohydrates.

Iodine's test
To 3 ml of the test solution, few drops of iodine solution is added.The formation ofBlue colour appearance shows presence of carbohydrates.

Benedict test
To 5 ml of Benedict's solution, add 1 ml of the test solution and shake each tube. Place the tube in a boiling water bath and heat for 3 mins. Remove the tubes from the heat and allow them to cool. The Formation of green red or yellow precipitate shows presence of carbohydrates.

Barfoed's test
To a small portion of the substance, Barfoed's solution was added and it was boiled. The formation of Red colour precipitate shows presence of carbohydrates.

Test for Alkaloids
Preparation of test solution:The test solution was prepared by dissolving he extracts in Dil.HCL, the solution was filtered. The filtrates was then subjected to the following tests for the detection of the presence of alkaloids.

Dragendorff's test
In 3 ml, of filtrate, few drops of Dragendorff's regent (Potassium bismuth iodide solution) were added. The Formation of Orange coloured precipitate shows presence of alkaloids.

Mayer's test
Few drops of Mayer's reagent (Potassium mercuric solution) were added in 3 ml of test solution. TheFormation of Cream coloured precipitate shows presence of alkaloids.

Small quantity of Hager's reagent (saturated aqueous solution of picric acid) was added in filtrate. The Formation of Yellow colour precipitate shows presence of alkaloids.

Wagners test
Few drops of Wagner's reagent (iodine in potassium iodide) were added in 3 ml filtrate. The Formation of Reddish brown colour precipitate shows the presence of alkaloids.

Muroxide test
To 2-3 ml filterate, add few drops Con.HNO_3 evaporate to dryness. Cool and add 2 drops of NH_4OH. The formation of purple colour shows presence of alkaloids.

Test for Glycosides
Preparation of test solution:It was prepared by dissolving the test sample in alcohol.

Borntrager'stest
(Anthraquinone glycoside)To about 3 ml extract, Dil. Sulphuric acid was added. It was boiled and filtrated. To cold extract equal volume of benzene or chloroform was added. After shaking, organic solvents were well separated. And add ammonium.

Killer Killani test(Cardiac glycoside)
To the test solution few drops of ferric chloride solution and Concentrated Sulphuric acid was added.

Baljet test
Sodium picrate was added to the test solution. Colour of solution changed from yellow to orange colour.

Tests for Proteins and Amino Acids
A. Millon's test
To 3ml extract was added with Millon's reagent and it was boiled which gives white precipitate shows presence of aminoacids.

Ninhydrin test
To 3ml extract was added with 5% Ninhydrin solution and it was boiled. Then it was allowed to cool which gives bluish colour shows presence of aminoacids.

C. Biuret test
To 3ml extract was added with Biuret reagent and violet colour was shows presence of protein.

D. Xanthoprotein test
To 3ml extract, concentrated nitric acid was added and the white precipitate was shows presence of protein.

Test for Flavonoids
A. Shinoda test
a. To dry powder add 5 ml 95% ethanol, Con.Hcl and 0.5gm magnesium turnings which gives pink colour shows presence of flavonoids.

b. To 2 ml of residue add lead acetate solution which gives yellow colour precipitate shows presence of flavonoids.

Tests for Gums and Mucilages
a. To a small amount of extract, 25ml of absolute alcohol was added and then it was filtered. The precipitate was examined for its swelling properties.

b. To the extract, ruthenium red solution was added.

Functional Group Confirmation on FTIR – Analysis
Preparation of Pellets
- Dry IR- grade KBr in a drying oven for at least 1 hour.
- Weigh out 300mg and place in a marble mortar and pestle and grind to fully powder it.
- Carefully weigh 3 mg of the finely ground solid sample and place in the marble mortar and pestle with the KBr.
- Grind for 60 seconds to thoroughly mix.
- Place the KBr compound mixture into an evacuable die on a hydraulic laboratory press. Press in vacuo at 150000 pound of pressure for 6 minutes.

Preparation of Sample
- 2 mg of drug is mixed with 200mg of KBr and grind into a fine powder.
- Take a little amount of the prepared sample and sprinkle it to form a thin layer inside the cavity.
- Keep the sample inside the KBr Pellet press and lock the levers and increase the pressure up to 9 kg/cm^3.
- Carefully remove the pellet and insert inside the sample holder and enter sample name.
- Select sample option above the graph.
- A graph appears due to sample kept, the select file then save as and give the name of the sample.

Extractive Yield

Table 3: Extraction of *Carica papaya* leaft reveals the percentage yield of around 20.7%

S.NO	METABOLITES	TESTS	RESULTS
1.	**SAPONINS**	FOAM TEST	+
2.		LIBERMANBUR-CHARD'S TEST	+
3.	**STEROIDS**	SALKOWSKI TEST	+
4.		LIBERMANBUR-CHARD'S TEST	+
5.	**CARBOHYDRATE**	MOLISH TEST	+
6.		IODINE'S TEST	+
7.		FEHLING TEST	+
8.		BENEDICT TEST	+
9.	**ALKALOIDS**	DRAGENDORFF'S TEST	+
10.		MAYER'S TEST	+
11.		HAGER'S TEST	+
12.		WAGNER'S TEST	+
13.	**GLYCOSIDES**	BORNTRAGER'S TEST	-
14.		KILLER KILLANI TEST	-
15.		BALJET TEST	-
16.	**PROTEINS AND AMINO ACIDS**	MILLON'S TEST	-
17.		NINHYDRIN TEST	-
18.		BIURET TEST	-
19.		XANTHOPROTEIN TEST	-
20.	**FLAVONOIDS**	SHINODA TEST	-

"+" represents – POSITIVE, "-" represents – NEGATIVE

Functional Group Confirmation on FTIR Spectroscopic Method

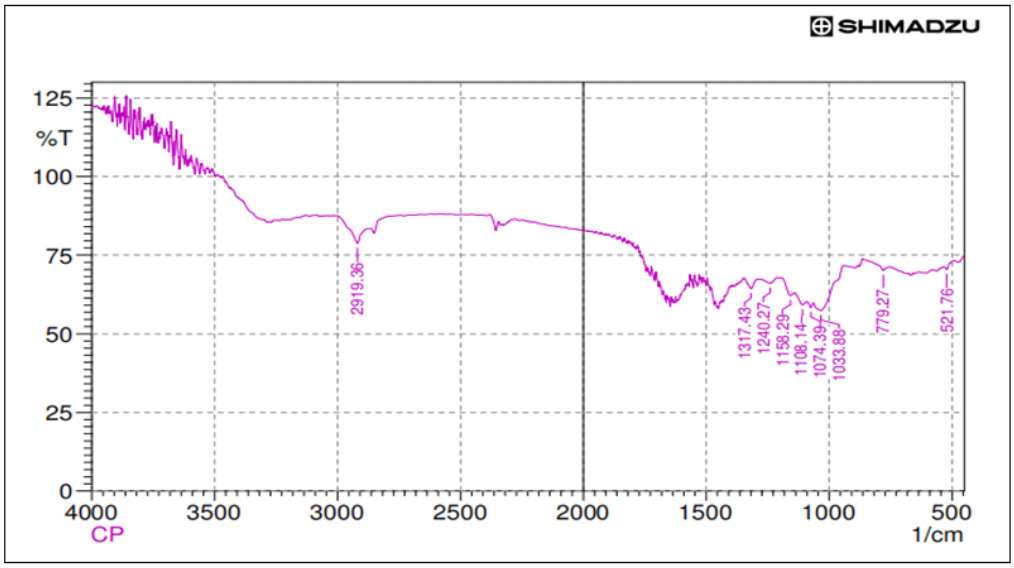

Fig 1: FTIR Graphical Report

Table 4: FTIR Peak

	Peak	Intensity	Corr. Intensity	Base(H)	Base(L)	Area	Corr. Area
1.	521.76	70.43	1.44	533.34	492.83	5.8	0.1
2.	779.27	70.16	1.69	863.17	755.16	15.37	0.32
3.	1033.88	57.33	5.41	1063.78	943.22	24.69	2.55
4.	1074.39	58.36	1.6	1089.82	1064.74	5.7	0.14
5.	1108.14	59.21	1.97	1140.93	1090.78	10.91	0.35
6.	1158.29	62.18	2.37	1193.98	1141.9	10	0.39
7.	1240.27	66.13	1.5	1276.92	1212.3	11.33	0.34
8.	1317.43	64.36	3.21	1344.43	1291.39	9.57	0.54
9.	2919.36	78.75	6.06	3001.34	2878.85	9.82	1.47

Table 5: Results FTIR Functional Confirmation

PEAK	COMPOUND	STRETCHING
2919.36	Carboxylic acid	O-H
1317.43	Alkyl halides	R-F
1240.29	Ethers	Ar-O-R
1158.29	Alcohols	C-O
1108.14	Ethers	Ar-O-R
1074.39	Alcohols	C-O
1033.88	Ethers	Ar-O-R
779.27	Alkyl halides	R-CL
521.76	Alkyl halides	R-Br

DISCUSSION

The role of the plant fruit extract, presence of some functional groups in the *Carica papaya* leaf extract and the synthesized were investigated by FTIR analysis. FTIR analysis was used to identify and get an approximate idea of the possible bio-moleculesthat are responsible for stabilization of fruit extract of *Carica papaya.* The major and strongest vibrational modes in the *Carica papaya* fruit extract are those located at 2919.36, 1317.43, 1240.29, 1158.29, 1108.14, 1074.39, 1033.88, 779.27, 521.76 cm^{-1}. A strong and broad peak at 2919.36 cm^{-1} can be attributed due to presence O-H stretching mode in Carboxiylic Acids. This agrees with the conclusion that fruit extract of *Carica papaya* is composed of saponins, steroids, carbohydrates, alkaloids, glycosides, proteins and amino acids, flavonoids and other similar phytochemicals containing COOH bonds, which was confirmed by using qualitative phytochemicals analysis. The bands at 1240.29 cm^{-1} and 1108.14cm-1 are assigned to Ar-O-R stretching mode in ethers. The fine peak at 1317.43 cm^{-1} was attributed to the R-F stretching mode that shows the presence of alkyl halides and the absorption peak at 1158.29 cm-1 and 1074.39 cm^{-1} corresponds to the alcohol present using *Carica papaya* extract. The narrow bands at 521.76 cm-1 confirm the alkyl halides. This indicates that the biological molecule in the *Carica papaya* leaf extract has a function of stabilization.

CONCLUSION

From the result of the study was concluded that ethanolic extract of carica papaya has the Alkyl halides, carboxylic acids, amines, amides, Alkenes, Ethers, Alcohol compounds. These compounds are effect against dengueprevent heart disease, heart attacks, and strokes, and prevent colon cancer.

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