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A Comparative Study on Phyto-Constitutional Profiling of *Carica papaya* Leaves

Vishvraj V. Devmurari, Cameykumari P. Bhadaniya and Pratik A. Ambasana*

Department of Chemistry, Shree Manibhai Virani and Smt. Navalben Virani Science College, Yogidham Gurukul, Kalavad Road, Rajkot-360005, Gujarat, **INDIA**

Email: pratikambasana@gmail.com

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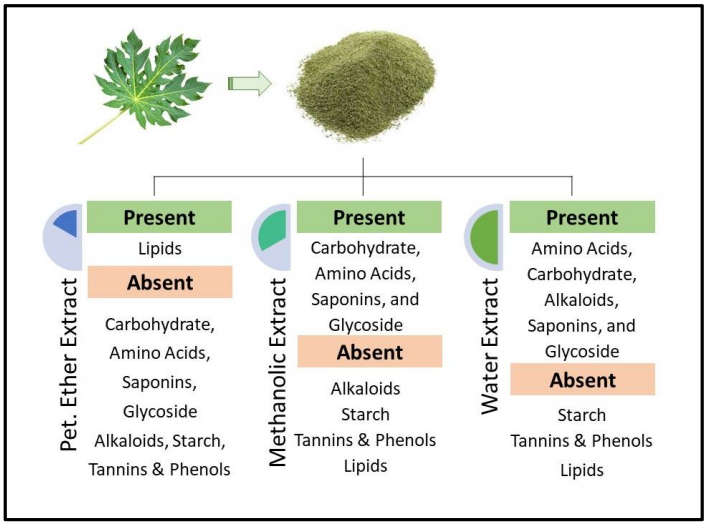
ABSTRACT

Carica papaya (Linn) plant is nature's own basket of various phyto-nutrients, and its leaves are used as the most effective supplement in the treatment of dengue fever. It possesses unparallel activity to increase platelet counts in the body, and hence finds its place as a marketed drug under multiple brand names like Caripill, Caripaya, CPL Tablet, Caripap, etc. for the treatment of dengue fever. This versatile potency of the plant led us to investigate phyto-constitutional profiling of C. papaya leaves. We have evaluated 3 most abundant yet unlike solvents viz. water, methanol, and petroleum ether for extraction to estimate maximum possible phyto-constituents. The report comprises an exhaustive phyto-constitutional profiling of C. papaya leaves in 9 diverse chemical classes with more than 24 chemical tests, supporting the presence of carbohydrate, amino acids, alkaloids, saponins, and glycosides as major chemical constituents.

Graphical Abstract



Keywords: *Carica papaya*, Phytochemical analysis, Phytonutrients, Phyto-constitutes, Extract.

INTRODUCTION

Carica papaya Linn. (family- Caricaceae) is quite inexpensive and abundantly available plant with plethora of nutritive & other pharmacologically active constituents in all of its parts *viz.* fruits, leaves, seeds and latex [1-3]. Various preceding studies of the plant have showed that papaya contains high amount of iron and calcium and quite good number of vitamins. It also contains other phyto-constituents like terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins and steroids in its different parts [4, 5]. The plant possesses high medicinal value that helps in the prevention of the oxidation of cholesterol, along with the treatment of various diseases like anti-inflammatory, anti-hypersensitivity, hypoglycaemic and hypolipidemic, free radical scavenging, wound healing, antitumour, antibacterial, antifungal, diuretic, uterotonicanti-sickling, anthelmintic, anti-amoebic, antifertility, etc [6-9].

Especially leaves of *C. papaya* are known to profusely increase the platelet counts in the body [4] and hence find its place as an approved medication under various names and forms for the treatment of dengue fever. This versatile potency of the plant led us to investigate phyto-constitutional profiling of *C. papaya* leaves.

Solvent Selection: Successful isolation of biologically active compounds from plant material depends on multiple parameters and the type of solvent used in the extraction procedure is the first and foremost influencing factor amongst all other [10-13]. Desirable properties of a good solvent in plant extractions includes, non-hazardous, non-carcinogenic, non-corrosive, low heat of vaporization, high physisorption capacity and non-reactivity towards targeted phyto-constituents, etc. [13, 14]. Keeping all possible parameters in mind like ease of availability, universality, and extractive potency; we have evaluated 3 most abundant yet unlike solvents *viz.* water, methanol, and petroleum ether for extraction to estimate maximum possible phyto-constituents.

Water: Water, being a universal solvent, is used to extract plant products with pharmacological activity. Though it exhibits less consistent activity compared to organic solvents, traditional therapists use predominantly water as a solvent owing to universality in the living beings [15].

Alcohols: Alcohols (Methanol) are efficient solvent for degradation of cell walls and seeds (non-polar character) that releases polyphenols from cells leading to higher pharmacological activity of alcoholic extracts.

Ether: Ether is commonly used selectively for the extraction of coumarins and fatty acids type of scaffolds [16].

MATERIALS AND METHODS

Collection and Authentication of Plant material: The plant material of *C. papaya* was collected from the local market of Rajkot (Gujarat) city in the month of January, 2018 and were identified and authenticated by the Botanist of Shree Manibhai Virani and Smt. Navalben Virani Science College, Rajkot.

Sample preparation: The plant material was washed with distilled water and leaves were cut into small pieces followed by drying at room temperature for 3 days. The dried leaves were grinded to fine powder. The weight of the total dry powder was found to be 105.18 g. 3 different samples of 5 g dried fine powder of *C. papaya* leaves was added to 100 mL methanol, 100 mL distilled water, and 100 mL petroleum ether with constant stirring. All 3 sample mixtures were kept for 24 h in a cabinet and were frequently shaken to allow maximum extraction from the samples. After 24 h, the solutions were filtered and kept for further analysis as sample M, sample W, and sample E respectively.

Physico-chemical Evaluation

Water soluble extractive value: 5 g accurately weighed filtrate was taken in a empty Petri dish and kept for 24 h at room temperature. After the evaporation of solvent, the solid residue remains. From the weight of the residue the percentage of water-soluble extractive value is calculated with reference to air-dried sample.

Alcohol soluble extractive value: 5 g accurately weighed alcoholic filtrate was taken in a empty Petridis and kept for 24 h at room temperature. After the evaporation of solvent, the solid residue remains. From the weight of the residue the percentage of alcohol-soluble extractive value is calculated with reference to air-dried sample.

Petroleum ether soluble extractive value: Petroleum ether soluble extractive value mainly gives fixed oil content of the leaf extract. Suitably weighed quantity (5 g) of the air-dried coarse powder transferred to an extraction thimble, extracted with petroleum ether (40-600) in a continuous extraction apparatus (Soxhlet extractor) for 6 h. Filter the extract quantitatively into a tarred evaporating dish and evaporate the solvent on a water bath. Residue is dried at 110°C to a constant weight and the percentage of petroleum ether soluble extractive was calculated with reference to air-dried sample.

Determination of Ash Value: The total ash, acid insoluble ash and water-soluble ash values were determined from air-dried samples using the reported procedure [17].

Total ash value: About 2 g of powdered material was weighed accurately into a tarred silica crucible. The powder is incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of total ash was calculated with reference to air-dried substance.

Acid insoluble ash: Ash obtained from the total ash was boiled with 25 mL of 2N HCl for a few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible, incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

Water soluble ash: Ash obtained from the total ash was boiled with 25 mL of distilled water for a few minutes and filtered through an ash less filter paper. The filter paper was transferred into a tarred silica crucible, incinerated at 450°C in a muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of water-soluble ash was calculated with reference to air-dried substance.

Phytochemical Evaluation

Qualitative Test for Carbohydrates: Extracts were dissolved individually in 5 mL distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Malesich's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and Conc. H_2SO_4 was added from sidewall of test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Tests for Reducing Sugars and for mono, di and polysaccharides for leaf extract were performed using Standard methods.

Chemical Test for Hexose Sugars

Seliwanoff's Test: This test is used for identification of keto-hexoses or to distinguish between ketoses and aldoses. To 1 mL aqueous solution of leaf extract, 5 mL of Seliwanoff's reagent (resorcinol in 6M HCl) was added and boiled, formation of cherry red colour in presence of ketose

(Fructose) due to formation of hydroxyl methyl furfural, which condensed with resorcinol to produce cherry red colour.

Cobalt chloride Test: To 3 mL of extract 2 mL of cobalt chloride solution was added, boil or 2 min and cooled, to this 2-3 drops of sodium hydroxide solution was added and observed for the change in colour. The appearance of greenish-blue and purplish colour are upper layer greenish blue and lower purplish layer indicates the presence of glucose, fructose or mixture glucose and fructose respectively. Qualitative Test for Starch was performed using Jelly and Lugol's iodine tests. Qualitative Test for Proteins and Amino Acids were performed by standard Ninhydrin Test, Biuret's Test and Xanthoprotein Test

Qualitative Test for Alkaloids: Extracts (3 mL each) were dissolved individually in dilute hydrochloric acid (1 mL, 1%), heated for 2 min in a water bath while stirring continuously and filtered.

Dragendroff's Test: Leaf extract solution mixed with Dragendroff's reagent (potassium bismuth iodide), forms orange-red colour.

Mayer's Test: Leaf extract solution and few drops of Mayer's reagent (K_2HgI_4), forms creamy-white precipitant.

Hager's Test: Leaf extract solution and few drops of Hager's reagent (Saturated aq. solution of picric acid), forms crystalline yellow precipitate.

Wagner's Test: Leaf extract solution and few drops of Wagner's reagent (dilute Iodine solution), forms reddish-brown precipitate.

Qualitative Tests for Tannins and phenols performed using ferric chloride test and lead acetate test

Qualitative Test for Saponin

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

Qualitative Test for Glycoside: 5 mL of distilled water was added to 2 mL of the papaya leaf extract. 2 mL of the H_2SO_4 was also added the mixture and was boiled in water bath for 15 min and allowed to cool. The mixture was neutralized with 20% KOH solution. 1 mL of equal parts of Fehling solution A and B (each) was added to the mixture and boiled for 15 min in a water bath. Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Bontrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranilic glycosides.

$FeCl_3$ Test: To the concentrated alcoholic extract of leaf extract few drops of alcoholic $FeCl_3$ solution was added. Formation of deep green colour, which turned yellow on addition of conc. HNO_3, indicates presence of Coumarin glycosides.

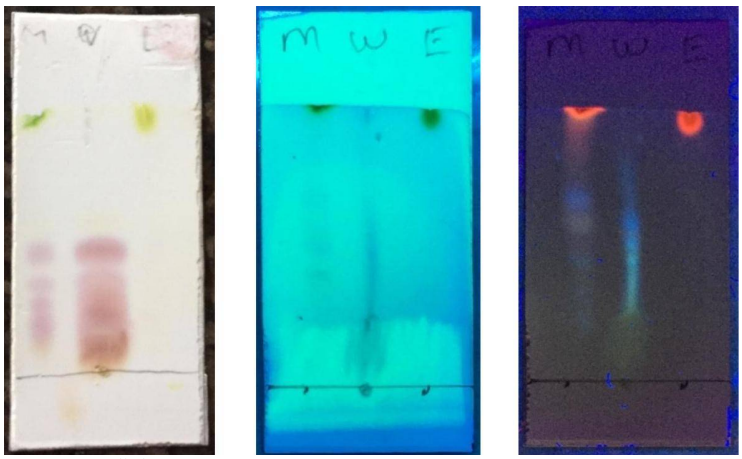
Ammonia Test: Filter paper dipped in alcoholic solution of leaf extract was exposed to ammonia vapour. Formation of yellow spot on filter paper indicates presence of flavonoid glycosides.

Zinc metal Test: To the alcoholic extract of leaf extract Zinc turning and dil. HCl was added, formation of deep red to magenta colour indicates the presence of dihydro flavonoids.

Qualitative Test for Lipids: To 10 mL of extract 0.5N alcoholic potassium hydroxide was added along with a drop of phenolphthalein. The mixture is heated on water bath for 1 h and observed for the formation of soap or partial neutralization of alkali.

Chromatographic Evaluation: For all 3 different extracts of *C. papaya* leaf, chromatographic (TLC - precoated plates) evaluation was performed using following solvent system, especially targeting the separation of amino acids present in the prepared extract samples.

Solvent System - n-Butanol: Acetic acid: Water = 4:1:5
Visualizing agent - 0.2% ninhydrin solution in acetone



Day light (sprayed) UV: 254 nm UV: 366 nm

RESULTS AND DISCUSSION

Results of the aforementioned tests performed over all 3 extracts of leaves of *C. papaya* (Linn.) are mentioned in the table 1.

Table 1. Physico-Chemical Evaluation.

S. No.	Parameter	Result
1	Water soluble extractive value	28 %w/w
2	Alcohol soluble extractive value	22 %w/w
3	Petroleum ether soluble extractive value	14 %w/w
4	Total ash	16.8%w/w
5	Acid insoluble ash	12.9 %w/w
6	Water soluble ash	3.3 %w/w

Extractive value data suggest that leaf material contains high amount of water soluble content, which also supports plant's pharmacological application in water extract form by medical practitioners. Total ash value, acid insoluble ash, and water-soluble ash suggest that the dried plant powder contains very less amount of water thus it resist the microbial growth and very less amount of organic content.

The results of qualitative analysis (Table 2) suggest presence of carbohydrate, amino acids, alkaloids, saponins, and glycoside content in water extract. Methanolic extract also showed presence of carbohydrate, amino acids, saponins, and glycoside content. However, alkaloids were not present in methanolic extract. Petroleum ether extract showed presence of only lipid content in the qualitative analysis.

Table 2. Phyto-chemical Evaluation

S. No.	Parameter	Water Extract	Methanol extract	Pet. Ether extract	Reference Test
1	Carbohydrates	+	+	-	Molisch's Test
		+	+	-	Fehling's Test
		+	+	-	Benedict's Test
		-	-	-	Iodine test
		-	-	-	Barfoed test
		+	+	-	Seliwanoff's Test
		+	+	-	Cobalt chloride Test
2	Starch	-	-	-	Jelly Test
		-	-	-	Lugol's Iodine Test
3	Proteins & Amino Acids	+	+	-	Ninhydrin Test
		-	-	-	Biuret's Test
		-	-	-	Xanthoprotein Test
4	Alkaloids	-	-	-	Dragendroff's Test
		+	-	-	Mayer's Test
		-	-	-	Hager's Test
		+	-	-	Wagner's Test
5	Tannins & Phenols	-	-	-	Ferric Chloride Test
		-	-	-	Lead acetate Test
6	Saponins	+	+	-	Froth Test
7	Glycosides	+	+	-	Modified Borntrager's Test
		-	-	-	FeCl3 Test
		+	+	-	Ammonia Test
		-	-	-	Zinc metal Test
8	Lipids	-	-	+	

APPLICATION

This study reveals the presence of carbohydrate, amino acids, alkaloids, saponins, and glycoside content in water extract, which is mainly used in nutritional and medicinal purpose for the treatment of dengue fever and other such ailments. In addition to this, chromatographic evaluation reveals presence of multiple amino-acids in this medicinally important water extract. These findings can be further investigated by high-end sophisticated analytical techniques, which may provide a lead for development of new medication for dengue fever and specifically with platelet count enhancing activity.

CONCLUSIONS

The present study was aimed towards comparative study on Phyto-constitutional profiling of *C. papaya* (L) Leaves. 3 different extracts from plant leaves were prepared using water, methanol, and petroleum ether as solvents; and subjected for photophysical and phyto-constitutional evaluation. Results of these analyses lead us to conclude that, Leaves of *C. papaya* were found to contain carbohydrate, amino acids, alkaloids, saponins, and glycosides as their chemical constituents; where as other important constituents namely proteins, tannins, phenolic compounds and starch were found to be absent.

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