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## Formulation and evaluation of nutraceutical syrup containing *Carica papaya* fruit extract

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### ABSTRACT

The individuals who have felt difficulty in swallowing solid dosage forms, for them use of oral liquid pharmaceuticals has generally been justified on the basis of ease of administration. In the present investigation, Nutraceutical syrup from *Carica papaya* unripe fruit was developed. Formulated dosage forms then subjected to evaluation of production quality by different methods stated as per official compendia. In this work, the papaya syrup was prepared as a Nutraceutical supplement. Papaya syrup is a fruit concentrate used as a common ingredient in the preparation of typical foods, and particularly in cakes. In vitro assays were performed to determine the Nutraceutical ingredients, such as phenolic compounds, cardiac glycosides, vitamins, carbohydrates, proteins, alkaloids and flavanoids qualitatively provided specific information about the composition of the syrup. The final herbal syrup was prepared by mixing one part of extraction with five parts of simple syrup (1:5). Solubility was checked by observing the clarity of solution visually. Then the final herbal syrup was subjected for evaluation and stability studies. Now a day's Accelerated stability studies are used by most of the pharmaceuticals for stability evaluation of all types of formulations. The stability of the syrup was checked on 0 hrs, 24 hrs, 48 hrs & 72 hrs by storing at three different temperatures like 00C, room temperature, and at high temperature i.e 470C. The results of stability study of the final syrup which were stored at 00, at room temperature reveals that some little changes were noticed in 00C stored syrup in specific gravity and viscosity and remaining all the tested physicochemical parameter as well as Phytochemical constituents during 24 hr, 48 hr & 72 hrs are same as 0-day but syrup stored at high temperature becomes tough and black carbon. Finally it was proved qualitatively that the prepared syrup contains maximum number of nutrients as unripe fruit, further we are planning to go for the quantitative estimation of nutrients.

### Introduction

In the long struggle to overcome the powerful forces of nature, the human beings have always turned towards plants for food, shelter, clothing and healing.<sup>1</sup> Worldwide the

interest & use of medicinal plants as a source of pharmacologically active compounds has increased. In developing countries like India, It is familiar that plants are the main medicinal source to treat number of diseases and disorders. The World Health Organization has estimated that 80% of the earth and 6 million inhabitants rely only upon traditional medicines for their primary health care needs and major part of the therapy involves the use of plant extracts or their active principles. Scientists in many parts of the world have proven the effective use of herbal medicine by carrying out wide-ranging research and have proven to humanity<sup>2</sup>.

For effective systemic actions, most significant method of administrating drugs is the oral route of administration. For self administration of medication parenteral route is not regularly used except in few cases like diabetes. The topical administration of drug is inadequately absorbed for showing

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systemic drug actions. To produce perfect systemic effects most of the drugs used are administered by the oral route<sup>3</sup>. Oral route was preferred for the administration of Ayurvedic herbal formulations like oral solutions, syrups, elixirs etc., are prepared and used for the specific effects of the medicinal agents present in that. The medicinal agents present in these preparations, are intended to provide systemic effects. The facts that they are administered in solution form are usually having a greater absorption from the GI tract into the systemic circulation occurs more rapidly than other oral dosage forms of the same medicinal agent. Liquid forms of drugs have certain restriction, but public demand or expectations are very high for such formulations. Likewise, some products which are need to administer regularly as a nutrition supplement are more effective in a liquid form and are used commonly by young children's or the elderly to overcome problem of swallowing the solid oral dosage forms<sup>4</sup>. Most of the orally administered Ayurvedic & Nutraceutical formulations belong to liquid form of drug or nutrition combination<sup>5</sup>. Designing of oral herbal formulations is a challenge in modern pharmaceuticals till date. However the final preparation must satisfy the requirements of pharmaceutical elegance with regard to taste, appearance and viscosity. There are number of medicinal herbs in traditional system of plant derivatives which are time tested, useful to treat number of aliment and also as nutrition supplements.

The term 'Nutraceutical' was coined to represent compounds found in food and herbs that are not technically considered nutrients such as vitamins or minerals, but which may have a profoundly beneficial impact on the health of the body. Common examples of Nutraceutical include the use of glucosamine in arthritic conditions of dogs and cats, and antioxidant compounds that help in the prevention of cancer<sup>6</sup>. This word with "nutra" derived from nutrition and "ceutical" from pharmaceutical— refers to substances that may be considered a food or part of a food and may provide medical and health benefits, with a health care provider and are easily available without a prescription. It was defined as 'a food or part of food that provides medical or health benefits, including the prevention and treatment of disease'<sup>7</sup>.

Nutraceuticals may range from isolated nutrients, herbal products, dietary supplements and diets to genetically engineered "designer" foods and processed products such as cereals, soups and beverages. Doubtlessly, many of these products possess pertinent physiological functions and valuable biological activities. According to the Dietary Supplement Health and Education Act of 1994, the definition of nutraceuticals has been expanded to include vitamins, minerals, herbs and other botanicals, amino acids and any dietary substance for use by humans to supplement the diet by increasing total dietary intake and subsequently increased the use of nutraceuticals dramatically<sup>8</sup>.

## Materials and Methods

### Plant Collection & Authentication

The unripe fruit of *Carica papaya* was collected in Kothuru village and the unripe fruit, leaves and flowers of the plant were sent for the authentication. The plant materials were identified and authenticated by a botanist Dr.

Balasubramaniyan, ABS botanical gardens, Karipatty, Salem, Tamilnadu. The specimen was deposited in the Pharmacology department in the Mother Teresa Pharmacy College for further reference.

### Preparation of Papaya Ethanolic Crude Extract<sup>9</sup>

Fresh matured unripe fruits of *Carica papaya* were collected, washed and peeled. They were sliced into pieces and the seeds discarded. They were then grated and ground to a paste. A quantity of the ground sample (100 g) was weighed and soxhlet-extracted with ethanol (500 mL) at 60°C for 8 to 10 h. Where larger ground samples (500–1000 g) were used, extraction was done under reflux with an appropriate volume of alcohol. The extract was slowly evaporated to dryness under vacuum at 40°C using a rotary evaporator. The residue was then weighed and yield was recorded.

### Phytochemical tests<sup>10</sup>

Phytochemical tests were performed for the identification of plant constituents such as flavonoids, steroids, glycosides, carbohydrates, gums and mucilage, proteins, amino acids alkaloids, vitamins tannins, quinones, saponins and phenols. The tests are as follows.

#### Test for Fixed Oils and Fats

##### Spot test

A drop of extract was placed over a piece of ordinary paper. No translucent spot is visible. This indicates the absence of fat.

##### Saponification test

To a small quantity of the extracts in 20 drops of 40% NaOH and 2 ml of glycerol were added and gently boiled for about 3 minutes until no oil globules were visible and was divided the solution in to 3 parts to carry the following experiments in test tube 1,2,3.

To test tube No.1 saturated solution of NaCl was added, no separation of sapy matter indicates the absence of fats. (salting out process)

To test tube No.2 a few drops of conc. HCl was added and observed no oily layer separated out, indicates the absence of fat.

To test tube No.3 a few drops of CaCl<sub>2</sub> solution was added no precipitated was observed, indicates the absence of fat.

#### Test for tannins & Phenolic compounds

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents

1. 5% FeCl<sub>3</sub> solution gives deep blue black colour
2. Lead acetate solution: White precipitate
3. Gelatin solution: White precipitate
4. Bromine water: Decolourisation of bromine water.
5. Acetic acid solution: red colour solution.
6. Potassium dichromate: Red colour precipitate
7. Dil. iodine solution: Transient red colour

8. Dil. HNO<sub>3</sub>: Reddish to yellow colour.
9. Dil. NH<sub>4</sub>OH & Potassium ferric cyanide solution: Red colour solution
10. Dil. Potassium permanganate solution: Decolourisation.

#### *Test for Proteins & Amino acids*

##### Biuret test

To 2ml of solution, an equal value of 10% NAOH and one drop of 10% CuSO<sub>4</sub> solution were added. A violet colour formation indicated the presence of amino acids

##### Ninhydrin test

To 1ml of ninhydrin solution 1ml test solution was added and heated. No formation of violet colour indicated the absence of amino acids. Ninhydrin is a powerful oxidizing agent, which causes oxidative decarboxylation of α-amino acid yielding CO<sub>2</sub>, NH<sub>3</sub>, aldehyde. The reduced ninhydrin then reacts with the liberated ammonia forming a blue complex. Protein and hydroxyl proteins produce yellow rather than purple colour with ninhydrin.

##### Millon's test

It is a detecting reagent for proteins. No reaction with the test solution indicated the absence of protein and amino acids.

##### Xanthoprotein test

Boiling with conc. HNO<sub>3</sub> - no yellow colour indicates the absence of protein

##### Test for Gum and Mucilages

##### Molisch's test

No reaction with the reagent, indicates the absence of gums and mucilages

##### Test for Alkaloids

1. Dragendorff's test: To 2-3 ml test solution add few drops of Dragendorff's reagent. Orange brown precipitate is formed.

2. Mayer's test: 2-3ml test solution with few drops Mayer's reagent gives precipitate.

3. Hager's test: 2-3ml test solution with few drops Hager's reagent gives yellow precipitate.

4. Wagner's test: 2-3ml test solution with few drops Wagner's reagent gives reddish brown precipitate.

5. Murexide test for purine alkaloids: 2-3ml test solution add 3-4 drops of conc. HNO<sub>3</sub> evaporate to dryness. Cool and add 2 drops of NH<sub>4</sub>OH. Purple colour is observed.

6. Ammonium reineckate test: Add 0.2% hydroxyl amine to a saturated solution of Ammonium reineckate and acidity with dil. HCl, alkaloids give pink precipitate. The precipitate is soluble in 50% of acetone which is used to recrystallize it.

7. Tannic acid test: The test solution is treated with tannic acid solution gives buff coloured precipitate.

8. Picrolonic acid test: The test solution on treatment with picrolonic acid gives yellow precipitate.

#### *Test for Carbohydrates*

##### Molisch's reagent

To the alcoholic solution of extract 10% aqueous solution of α-naphthol was added. Shake and concentrated sulphuric acid was added along the side of the tube. Violet ring at junction of two liquids showed the presence of carbohydrates in the extracts.

##### Fehling's test

2ml of Fehling solution A added 2ml of Fehling's solution B were mixed and added to 2ml the sample solution boiled for 2 minutes and cooled. Yellow precipitate indicated the presence of carbohydrates in the extracts.

##### Barfoed's test

2ml of test solution and 2ml of Barfoed reagent were mixed and boil on water bath. Brick red precipitate formed at the bottom of the test tube, showed the presence of carbohydrates.

##### Benedict's test

5ml of Benedict's reagent and 3ml of test solution were boiled for 2 minutes and cooled. Green yellow precipitate formed show the presence of carbohydrates.

##### Borntrager's test

Anthraquinone derivatives are generally detected by Borntrager's test. In this test the drug powder was boiled with ferric chloride solution and dil. H<sub>2</sub>SO<sub>4</sub>. Filtered while hot, the filtrate extracted with ether and ethereal layer was added with equal volume of ammonia. In the ammonia layer no pink colour developed indicates absence of glycosides in the extract.

#### *Test for Flavonoids*

1. Shinoda test: To extract, add 5ml 95% ethanol or tertiary butyl alcohol few drops conc. HCl and 0.5gm magnesium turnings. Orange, pink, red to purple colour appears.

2. Sulphuric acid test: On addition of sulphuric acid flavones and flavonols dissolve in to it and give a deep yellow solution. Chalcones and aurones give a red or red bluish solution. Flavanes give orange to red colour.

#### *Test for Vitamins*

1. Test for vitamin A: Dissolve a quantity equivalent to 10-15 units in 1ml of chloroform and add 5ml of antimony trichloride solution, a transient blue colour is produced immediately.

2. Test for vitamin C: Dil. 1ml of 2% w/v solution with 5ml of water & add 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2ml of dil. NaOH solution. Add 0.6ml of HCl drop wise and stir. The yellow colour turns to blue.

3. Test for Vitamin D: Dissolve a quantity equivalent to about 1000 units of vitamin D activity in chloroform and add 10ml of antimony trichloride solution, A pinkish red colour appears at once.

**Table 1. Phytochemical constituents in extracts and Papaya syrup**

Phytochemicals	Extract	Nutraceutical Syrup									
		0 day	24 h			48 h			72 h		
			0° C	RT	HT	0° C	RT	HT	0° C	RT	HT
Alkaloids	+	+	+	+	-	+	+	-	+	+	-
Sterols	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	-	+	+	-	+	+	-
Fixed oils and fats	-	-	-	-	-	-	-	-	-	-	-
Protein and aminoacids	+	+	+	+	-	+	+	-	+	+	-
Triterpenoids and saponins	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	-	+	+	-	+	+	-
Flavone and flavonoids	+	+	+	+	-	+	+	-	+	+	-
Vitamins (C)	+	+	+	+	-	+	+	-	+	+	-
Cardiac glycosides	+	+	+	+	-	+	+	-	+	+	-
Andhraquinone glycosides	-	-	-	-	-	-	-	-	-	-	-

**Test for Anthraquinone Glycosides**

1. Borntrager's test: To 3ml extract add dil.H<sub>2</sub>SO<sub>4</sub> boil and filter. To cooled filtrate, add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.

2. Modified Borntrager's test: To 5ml extract, add 5ml of 5% FeCl<sub>3</sub> and 5ml dil. HCl. Heat for 5min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate the organic solvent. Add equal volume of dil. ammonia. Ammoniacal layer turns pink or red.

**Test for Steroids**

1. Salkowski reaction: To 2ml of extract add 2ml of chloroform and 2ml conc. H<sub>2</sub>SO<sub>4</sub>. Shake well. Chloroform layer appear red and acid layers shows greenish yellow fluorescence.

2. Liebermann – Burchard Reaction: Mix 2ml test solution with chloroform. Add 1-2 ml acetic anhydride and 2 drops of Conc. H<sub>2</sub>SO<sub>4</sub> from the sides of test tube. First red then blue and finally green colour appears.

3. Liebermann's reaction: Mix 3ml test solution with 3ml acetic anhydride. Heat and cool. Add few drops of Conc. H<sub>2</sub>SO<sub>4</sub>. Blue colour appears.

**Test for Cardiac Glycosides**

1. Legal's test: To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink to red colour appears

2. Keller killiani test: To 2ml extract, add glacial acetic acid, 1 drop of 5% FeCl<sub>3</sub> and Conc. H<sub>2</sub>SO<sub>4</sub>. Reddish brown colour appears at junction of the 2 liquid layers and upper layer appears bluish green.

3. Liebermann's reaction: Mix 3ml test solution with 3ml acetic anhydride. Heat and cool. Add few drops of Conc. H<sub>2</sub>SO<sub>4</sub>. Blue colour appears.

Blue violet colour (anthocyanins) yellow colour (flavones) yellow to orange (flavonones). With concentrated sulphuric Acid Yellow orange colour (anthocyanins) yellow to orange (flavonones).

**Method of preparation of simple syrup<sup>11</sup>**

666.7 g of Sucrose was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 1000 ml.

**Method of preparation of final herbal syrup<sup>11</sup>**

One part of extraction was mixed with five parts of simple syrup (1:5). Following that, the required quantity of methyl paraben and peppermint oil was added to the above mixture and the final volume was adjusted with simple syrup. Solubility was checked by observing the clarity of solution visually. The final herbal syrup was then subjected for evaluation. The herbal syrup was evaluated for various parameters such as physical appearance (colour, odour, and taste), pH, weight/ml, viscosity and Phytochemical constituent and also subjected to stability studies.

**Results and Discussion****Percentage yield of extraction**

After completion of extraction process the extraction was subjected to evaporation by recovering the ethanol using distillation process. Then pour the concentrated extract in to petridishes for complete evaporation then collect the ethanol free extract in to a separate bottle. The percentage yield was calculated using initial weight of papaya used and final weight of extract. The percentage yield is 2.35%.

**Phytochemical Constituents Present in the Extract**

The following phytochemical constituents were present in the extract. The same were observed in the papaya syrup on day-0, day-1(24 hours) day-2 (48 hours) & day-3 (72 hours) which were kept indifferent temperatures.

**Evaluation test**

**Table 2. Results of Stability Testing of Papaya syrup**

S. No	Sample No.	Time Duration	Temperature	Wt / ml	Specific gravity gm/cc	Viscosity (centi poises)	Colour	Odor	Taste
1	P1	0-day							
2	Q1	24 hours	0°C	1.225±0.005	1.267±0.004	22.982±1.089	Brown	Pine Apple	Sweetish
3	R1	48 hours		1.235±0.005	1.253±0.006	25.212±0.288			
4	S1	72 hours		1.229±0.009	1.270±0.010	25.217±0.279			
5	Q2	24 hours	R.T	1.224±0.006	1.267±0.012	27.392±2.122			
6	R2	48 hours		1.23±0.004	1.252±0.005	26.060±0.370			
7	S2	72 hours		1.230±0.004	1.252±0.005	26.060±0.37			
8	Q3	24 hours	H.T	The syrup becomes tough solid					
9	R3	48 hours							
10	S3	72 hours							

In the past it was the practice in many pharmaceutical manufacturing companies to evaluate the stability of pharmaceutical preparations by observing them for a year or more, corresponding to the normal time that they would remain in stock and in use. Such approach was time consuming. Now a day's Accelerated stability studies are

used by most of the pharmaceuticals for stability evaluation of all types of formulations<sup>12</sup>.

Though the primary aim of this work was to develop Nutraceutical syrup but the stability study will mark an important advancement in the area of phytopharmaceuticals. The prepared Nutraceutical syrup was evaluated

immediately after preparation and all the tested parameter along with specific gravity and viscosity were compared with the changes in accelerated stability testing. The final syrup found to have pH: 4.5, specific gravity 1.23 g/ml and viscosity 23.06. The results of stability study of the final syrup (Table-9) reveal that a little changes were noticed in 00C stored syrup in specific gravity and viscosity and remaining all the tested physicochemical parameter as well as phytochemical constituents during 24 hr, 48 hr & 72 hrs are same as 0-day. But the syrup stored at high temperature that is 470 C becomes tough solid and its colour taste and odor were completely changed. So it is conformed that we should not keep these syrups at high temperatures more than room temperature.

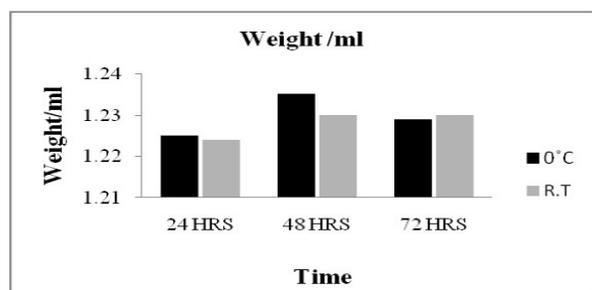


Figure 1. Density of syrup in different time intervals

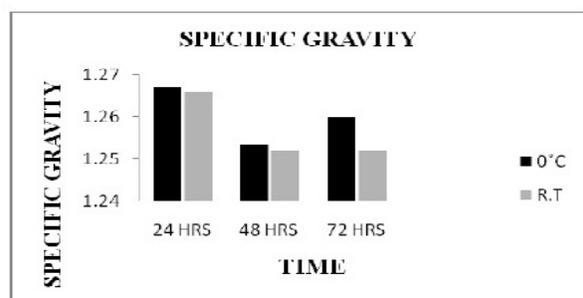


Figure 2. Specific gravity of syrup in different time intervals



Figure 3. Viscosity of syrup in different time intervals

## Conclusion

As the Carica papaya fruit contains more number of nutrients it is difficult to consume more number of fruits. When compared with ripen fruit, unripe fruit contains more nutrition value and less energy. The same was prepared as syrup in this project. This syrup contains maximum of

nutrition values like proteins, vitamins, alkaloids, flavanoids, cardiac glycosides etc... improves the health. As it is an edible fruit more consumption of syrup also does not cause any problem, but it is not recommended to diabetic patients as it contains more amount of sucrose.

Finally it is concluded that the prepared syrup contains maximum of nutrients as unripe fruit it was proved qualitatively further we will go for the quantitative estimation of nutrients and it is also proved that the syrup which is store at 00C, at room temperature contains all the active ingredients as day-0 but syrup stored at high temperature becomes tough and black carbon. So it is conformed that we should not keep the syrup at high temperatures than the room temperature.

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