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Research Article

PHYSICO-CHEMICAL AND PHYTOCHEMICAL EVALUATION OF *CARICA PAPAYA* LINN. UNRIPE FRUITS

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ABSTRACT
Present study was performed for the development of quality standards of *Carica papaya* Linn. commonly known as Papita belonging to family Caricaceae is well known for its exceptional nutritional and medicinal properties. The study comprises of physico-chemical and phytochemical evaluation to confirm purity and authenticity of *Carica papaya* L. unripe fruit using WHO guidelines. Microscopy of the fruit showed presence thick cuticle, parenchyma, epicarp, mesocarp endocarp, calcium oxalate crystals, laticifers, etc. Successive extractive value and hot extractive value was found highest in alcoholic extract 48.34 % and 44.90 % respectively (on dry weight basis). Mean ash values (%) are 8.63 (total), 0.79 (acid insoluble ash), and 5.30 (water soluble ash) and moisture content was found to be 9.41 % and the phytochemical analysis revealed the presence of carbohydrates, terpenoids, flavonoids, phenolic compounds in different extracts of *Carica papaya* L. fruit. TLC fingerprinting profile of different extracts were also developed and quantification of β-carotene was also done by using nhexane:acetone (8.5:1.5) as a mobile phase at 415 nm and found to be higher in pet ether *Carica papaya* L. fruit extract 1.55 % w/w.
Keywords: *Carica papaya*, Caricaceae, papaya, WHO guidelines.

INTRODUCTION

Carica papaya Linn belonging to family Caricaceae is commonly known as papaya in English, Papita in Hindi and Erandakarkati in Sanskrit[1-3]. Plant is native to tropical America[4,5] and was introduced in India in 16th century. Besides the fruits being edible, it has a long history and proof of being a very effective medicinal plant. The latex is known for its antiseptic, antiulcer, anticarcenogenic properties. The seeds are considered to be abortifacient and anthelmintic[6]. Traditionally unripe fruit has been known to possess several medicinal properties as laxative, diuretic, hepatoprotective, anti implantation activity and antidote to snake bite, where as ripe fruit has been used as digestive, anti diarrhoeal, expectorant, sedative and anti obesity and psoriasis[7,8]. The clinically fruit has been proved for its uterotonic[9] hypoglycaemic, hypolipidaemic[10], antifertility[11], abortifacient[12], anticoagulant[13], enzyme activity[14] and anti-hypertensive activities[15]. Papain, a major chemical compound extracted from fruit and stem latex is used in brewing and wine making and in textile and tanning industries[7,16]. The bio-efficacy of *Carica papaya* L. is owed mainly to its main phytoconstituent, papain an enzyme, present in fruit and stem latex, vitamins namely vitamin C, B complex and minerals include calcium, phosphorous, iron etc, polysaccharides[17], enzymes (papain, chymopapain), alkaloids (carpaine, pseudocarpaine, carpesamine), glycosides (anthracene derivatives)[18], saponins, flavonoids (kaempferol, quercetin, rutin)[19], phenolic acids e.g. ferulic acid, chlorogenic acid, vanillic acid, along with carotenoids namely β-carotene and cryptoflavin[20]. Fruit has been used in Ayurveda and Unani system of medicine as important part of traditional formulations one of them is Skyzyme (digestive tablets used in gastric troubles, acidity etc). Therefore being an important part of the plant, the fruit has been undertaken for standardization.

MATERIAL AND METHODS

Plant Collection

Fresh unripe fruits of *Carica papaya* Linn were collected in the month of July 2012 from Jamia Hamdard campus, New Delhi, India and authenticated by Dr. H. B. Singh, Scientist F and Head, department of Raw material and Herbal museum, NISCAIR, Pusa Road, New Delhi, India. A voucher specimen (NISCAIR/RHMD/Consult/-2012-13/2158/164) was deposited in the same department, NISCAIR, New Delhi, India. The fruits were washed with water and cleaned simultaneously peeled off, sliced, chopped and were shade dried followed by drying in oven at 35°C for 3 days. The drug was then powdered and kept in air tight container at room temperature away from moisture for further study.

Morphological Studies

Papaya fruits were examined to study morphological and organoleptic characters. Sample for microscopy were prepared by embedding in formalin, glycerine, water (8:1:1) for a week. The sections were cut by razor. The cut sections were seen under microscope (Motic of B1 series) at 10 x, 40 x, 100 x after staining with Phloroglucinol and HCl[21].

Physicochemical Standardization

Extractive values were determined for cold, hot and successive extraction methods where 4 g of coarse powder sieved with 40 mesh size was dissolved in 100 ml of solvent (from non polar to polar). Standard methods were followed to determine the total ash, acid-insoluble ash and water soluble ash values, loss on drying, was determined according to WHO guidelines[21].

Determination of pH

pH 1 % solution

Accurately weighed (1 g) powder drug was dissolved in accurately measured 100 ml of distilled water, filtered and checked the pH of filtrate with a standard glass electrode.

pH 10 % solution

Accurately weighed (10 g) powder drug was dissolved in accurately measured 100 ml of distilled water, filtered and the pH of filtrate was checked with a standard glass electrode.

Loss on Drying

An accurately weighed (2 g) shade dried fruit powder of *Carica papaya* L. was taken in tarred evaporating disc. The crude drug was heated at 105°C in an oven till a constant weight was obtained. Percentage moisture content was calculated with reference to the shade dried material[21].

Determination of Foaming Index

About 1 g of plant material was reduced to a coarse powder, weighed accurately and transferred at moderate boiling for 30 minutes. Cooled and filtered into 100 ml volumetric flask. The detection was poured into 10 ml and adjusted the volume of liquid in each tube with water to 10 ml. Stoppered the tubes and was shaken them in a lengthwise motion for 15 sec; two shakes per second. Allowed to stand for 15 minutes and the height of foam was measured[21].

Determination of Swelling Index

Specified quantity of the plant material (3 g) concerned previously reduced to the required fineness and accurately weighed taken into 25 ml glass stopper measuring cylinder. 25 ml of water added and the mixture was shaken thoroughly every 10 minutes for 1 h. It was allowed to stand for 3 h at room temperature. The mean value of the individual determinations was calculated related to 1 g of the plant material[21].

Determination of Resin Content

The accurately weighed fruit drug (5 g) was rapidly refluxed with acetone (3 × 200 ml) for 6 h and the drug was exhausted for resin content. The excess solvent was removed by distillation on a water bath. The residue so obtained was suspended in water and transferred to separating funnel repeatedly extracted with solvent ether (2 × 200 ml) to extract all resin. The ether extract was cooled over anhydrous sodium sulphate and excess ether removed over water bath. Residue was transferred to a weighed beaker and final weight was noted with reference to air dried drug material.

Table 1: Extractive Values of *Carica papaya* Linn fruit

Extractive values (% w/w)	PE	EA	$CHCl_3$	ACT	ALC	Hyd-ALC	AQS
Cold	00.55	02.44	02.44	06.00	15.96	44.28	11.88
Successive	00.64	04.33	01.83	06.90	48.34	23.31	16.58
Hot	00.77	00.06	07.77	14.77	44.90	40.80	37.16

Table 2: Fluorescence Analysis of Fruit Powder

Treatment with different reagent	Day light	UV 254 nm	UV 366 nm
Distilled water	Buff	Dark buff	Light buff
NaOH (1N)	Buff	Brown	Light buff
Conc H_2SO_4	Buff	Brown	Light buff
Conc HCl	Buff	Brown	Light brown
Conc HNO_3	Buff	Black	Buff
Ferric chloride (5%)	Yellowish brown	Black	Light brown
Petroleum ether	Buff	Dark brown	Light buff
Picric acid	Buff	Yellow	Light yellow
KOH (1%)	Buff	Dark brown	Light yellow

Table 3: Powder Drug Reaction with Different Reagents

Chemical Treatment	Observation	S. No.	Chemical Treatment	Observation
Iodine	Brown	6	Conc HNO_3	Light brown
Ethanol	Buff	7	Conc H_2SO_4	Yellowish brown
Ferric chloride (5 %)	Brown	8	Conc HCl	Brown
KOH (1 %)	Yellowish brown	9	Petroleum ether	Buff
NaOH (1 N)	Light brown	10	Picric acid	Buff

Table 4: Phytochemical Screening of Fruit using Different Extracts

Tests	PE	EA	$CHCl_3$	ACE	MeOH	HA	AQS
Alkaloids	-	-	+	+	+	+	-
Carbohydrates	-	-	-	-	-	+	+
Saponins	-	-	-	-	+	+	+
Glycosides	-	+	-	+	++	++	+++
Proteins	-	+	-	+	+	-	-
Steroids	-	+	+	+	+	+	-
Phenolics	-	+	+	++	-	-	-
Flavonoids	-	+	-	+++	+++	+	-
Terpenoids	+	+	-	+	+	+	-
Tannins	-	-	-	-	-	-	-

Note: +++: highly present; ++: partially present +: weekly present; -: absent

Table 5: TLC Fingerprinting Profile of *Carica papaya* L. Fruit Extracts after Derivatization

Band No	Extract	(No of spots) R_f values in Day light	(No of spots) R_f values at 366 nm	(No of spots) R_f values at 254 nm
1	PF (pet ether fruit)	(5) 0.30, 0.36, 0.43, 0.63, 0.71	(6) 0.16, 0.31, 0.36, 0.43, 0.62, 0.71	(3) 0.31, 0.36, 0.71
2	EF (Ethylacetate fruit)	(5) 0.31, 0.36, 0.43, 0.63, 0.71	(6) 0.17, 0.24, 0.31, 0.36, 0.62, 0.71	(3) 0.31, 0.36, 0.71
3	CF (chloroform fruit)	(4) 0.31, 0.36, 0.63, 0.71	(7) 0.24, 0.31, 0.36, 0.44, 0.53, 0.62, 0.71	(3) 0.31, 0.36, 0.71
4	AF (acetone fruit)	(3) 0.31, 0.36, 0.71	(7) 0.24, 0.31, 0.36, 0.44, 0.53, 0.62, 0.71	(3) 0.31, 0.36, 0.71
5	MF (methanol fruit)	(2) 0.31, 0.36	(4) 0.25, 0.31, 0.36, 0.45	(2) 0.31, 0.36

Table 6: Percent β-carotene content in different extracts

Extract	R_f value	% β-carotene content (% w/w)
Pet ether fruit	0.72	1.55
Ethyl acetate fruit	0.72	0.099
Chloroform fruit	0.72	0.089
Acetone fruit	0.72	0.019
Methanol fruit	0.72	0.003

Figure 1a

Figure 1b

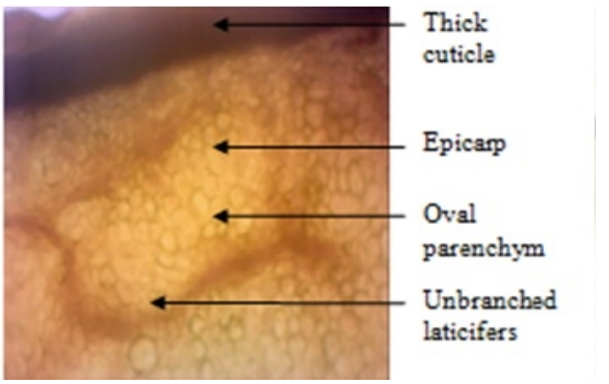


Figure 2a

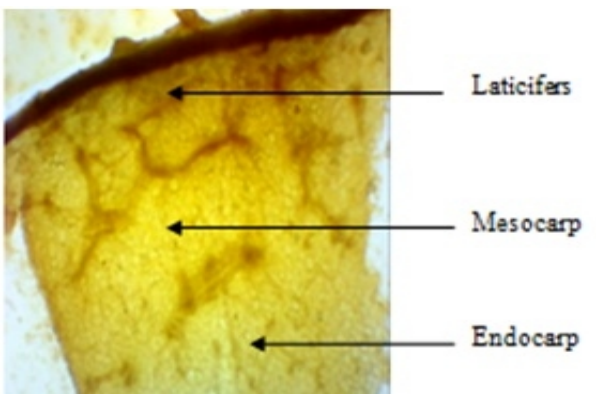


Figure 2b

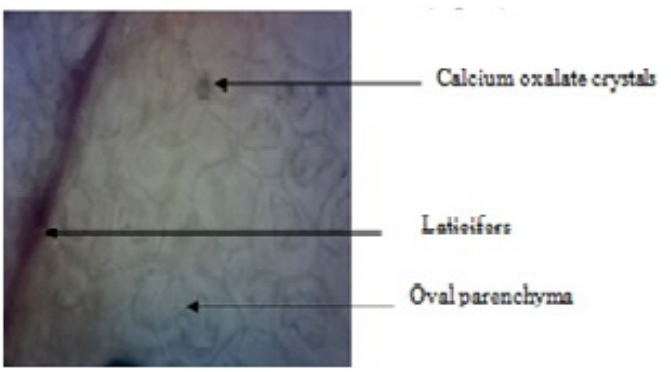


Figure 2c

Figure 2: Transverse Section of Fruit

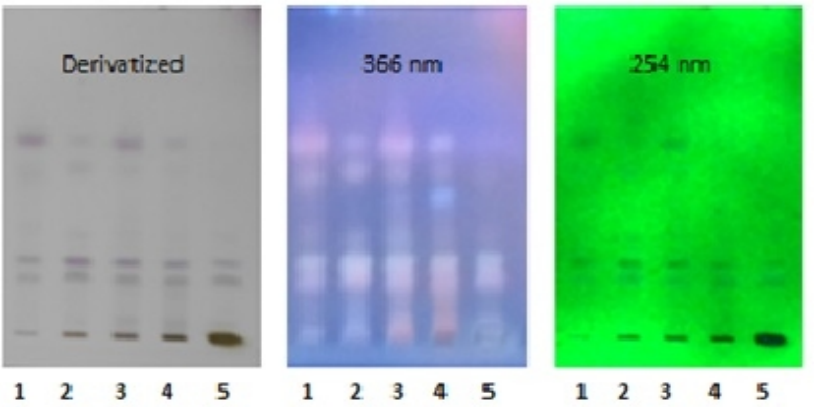


Figure 3: Post Derivatized HPTLC Plates

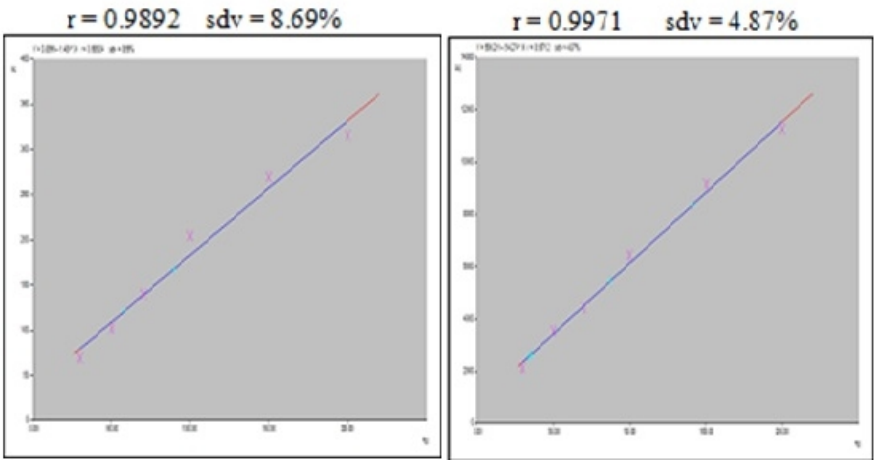
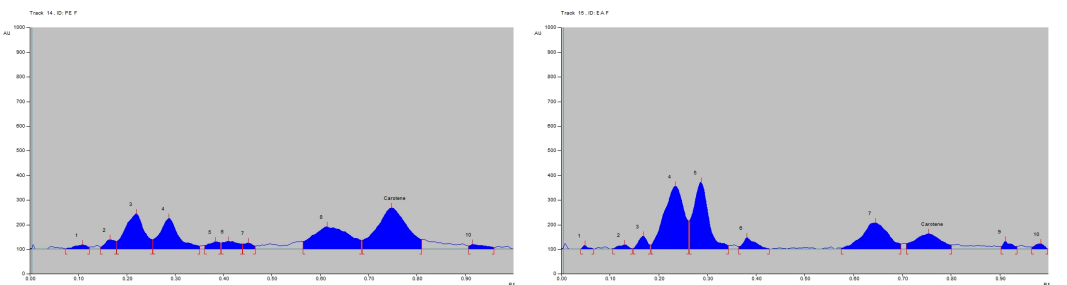
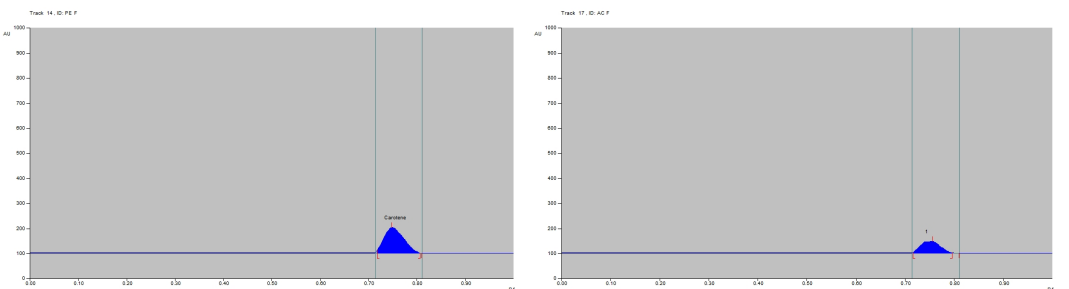


Figure 4(a): Calibration plot for peak height Figure 4(b): Calibration plot for peak area



***Carica papaya L.* pet ether fruit extract** ***Carica papaya* L. ethyl acetate fruit extract**

Figure 5: Multiple Peak Display of *Carica papaya* L. Fruit in Different Extracts



β-carotene in *Carica papaya* L. pet ether fruit extract **β-carotene in ethyl acetate fruit extract**

Figure 6: Single Peak Display of *Carica papaya* L. Fruit in Different Extracts

Fluorescence Analysis
The fruit powder was subjected to fluorescence analysis after being separately treated with water, NaOH, H_2SO_4, HCl, HNO_3, chloroform, ferric chloride, ammonia solution and picric acid. Since many herbs fluorescence when powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powder was studied both in day light and UV light (254 and 366 nm)[22].

Powder Drug Reaction with Different Reagents
The powdered drug was treated separately with different reagents and acids like water, NaOH, H_2SO_4, HCl, HNO_3, chloroform, ferric chloride, ammonia solution and picric acid, the colour shown by that treatment was noted as such and under the microscope[23].

Phytochemical Screening
The phytochemical evaluation of drug was carried out as per the method described. Previously dried powdered fruits (4 g) were extracted in a Soxhlet apparatus with petroleum ether, ethyl acetate, chloroform, acetone, methanol, hydroalcoholic and water (100 ml) successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins and lipids etc[21].

TLC Fingerprinting Evaluation
TLC profiling was done on petroleum ether, ethyl acetate, chloroform, acetone and alcoholic extracts which were subjected to TLC to find out the nature and approximate number of compounds present[24] and quantified at 415 nm.

RESULTS AND DISCUSSION

Macroscopic characters (Figure 1a, 1b)
External colour: green
Shape: big oval or pear shaped
Size: 17-25 cm long, 15-20 cm diameter
Weight: 0.5 to 20 lbs
A**pex**: acuminate to blunt
Surface: smooth
Odour: Nil

Microscopical characters (Figure 2a, 2b, 2c)
Epicarp shows single layer of thin walled cells covered with thick cuticle externally. Mesocarp have wide zone consisting of circular to oval shaped parenchyma cells with scattered and unbranched laticiferous cells. Endocarp composed of 2 to 3 layers of thin walled parenchyma cells. Abundant calcium oxalate crystals are present in mesocarp region of the fruit.

Extractive Value
The extractive values were studied on dried fruit powder as per the procedure described above. All the values were taken in triplicate (Table 1 and Figure 3).

Ash Values
The total ash value, acid insoluble ash value and water soluble ash value were found to be 08.63 %, 00.79 % and 05.30 % w/w respectively. Ash value is useful in determining authenticity and purity of drug and these values are important quantitative standards.

Fluorescence Analysis
The powder of the fruit of *Carica papaya* L. (mesh size 40) was examined under day light and UV light (Table 2).

Foaming Index
The height of the foam in every test tube was found to be less than 1 cm, so the foaming index was less than 100.

Swelling Index
The swelling index was found to be less than 100.

Loss on Drying
The mean loss on drying was found to be 09.41 %.
Resin Content
The mean resinous matter was found to be 03.08 %.

pH Values
The mean pH value of 1 % solution and 10 % solution was found to be 6.51 and 5.60, respectively.

TLC / HPTLC Fingerprinting
The weighed quantity of fruit was extracted in a Soxhlet apparatus for 6 h using twice the amount of solvent (pet ether, ethyl acetate, chloroform, acetone and methanol) at a controlled temperature. The extract was dissolved in the respective solvent (2 mg / ml). The spots were applied with the help of Linomat syringe using Linomat applicator and developed in optimized solvent system (n-hexane: acetone:: 8.5:1.5). Developed plate was derivatized with anisaldehyde-sulphuric acid reagent and dried at 105ºC for 5 minutes and observed in day light and UV light (Figure 3 and Table 5).

Quantitative Analysis of β-carotene in *Carica papaya* L. Fruit by HPTLC
HPTLC was performed on pre coated silica gel aluminium TLC plate $60F_{254}$ (E-Merk, Germany) for qualitative evaluation of β-carotene in *Carica papaya* L. fruit extracts. In brief, concentrated papaya fruit extract (6 µL) and standard markers (2 µL) were loaded on TLC plate with CAMAG system consisting of Linomat V spotting device with nitrogen supply. The mobile phase used for β-carotene was n-hexane: acetone (85:15 v/v). The plates were developed to a distance of 80 mm in a Camag twin-trough chamber previously saturated with mobile phase for 30 minutes. After development, derivatization was carried out with anisaldehyde reagent and heated at 105º C in oven for 5 minutes. Camag TLC visualize-150503 was used for photo documentation of β-carotene at 254 nm, 366 nm and White light. The β-carotene HPTLC chromatogram was obtained using Camag scanner-170422 in conjunction with WinCats-5 software. For the quantitative estimation 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0 µl of standard β-carotene solution (corresponding to 20, 40, 60, 80, 100, 200, 300 ng of standard respectively) was applied on TLC plate. Plate was developed in twin trough chamber to a height of 8 cm. Plate was dried and sprayed with anisaldehyde-sulphuric acid reagent and heated at 105ºC until the colours developed. Densitometric analysis was carried out using Camag TLC scanner in absorbance mode at 415 nm (Figure 4, 5, 6). The chromatogram was integrated using Win Cats software for area calculation (Table 6). Peak area was recorded and calibration curve was plotted using peak area vs. concentration of β-carotene.

CONCLUSION

The quality of a plant product is determined by the climatic conditions of growth and type of hybrid selection, use of fertilizers, harvesting, and drying and storage conditions. The deviation from standard conditions lead to deterioration of products, which are then sold as adulterated products in the market. This work will increase the existing knowledge regarding fruits of *Carica papaya* Linn. may be quite useful for the quality control of various formulations containing *Carica papaya* L. fruits. The main highlight of the work is that it will be helpful to eliminate exhausted and inferior quality fruit available in local markets as this has been the main source of Papain extraction.

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