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STANDARDIZATION OF CARICA PAPAYA LEAF EXTRACT

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ABSTRACT

Papaya (Carica papaya linn) is well known for its exceptional nutritional and medicinal properties throughout the world. From the times immemorial, the whole Papaya plant including its leaves, seeds, ripe and unripe fruits and their juice is used as traditional medicine. Nowadays, Papaya is considered as nutraceutical fruit due to its multifaceted medicinal properties. Standardization of drugs means confirmation of its identity and determination of its quality and purity. At present due to advancement in the chemical knowledge of crude drugs various methods like botanical, chemical, spectroscopic and biological methods are used for estimating active constituents present in the crude drugs in addition to its physical constants. Plants have been known to relieve various diseases in Ayurveda. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in Ayurveda. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. In this present review, an attempt has been made to give an overview of certain extractants and extraction processes with their advantages and disadvantages.

KEYWORDS: Standardization, quality, purity, herbal products, phytochemical, extraction, solvent, screening.

INTRODUCTION

Dengue is a rapidly expanding global health problem. One of the most disturbing aspects of the problem of dengue is that there are no effective antiviral agents available to treat dengue complications. Transfusions, growth factor injections and bone marrow transplant have their

limitations. The management of dengue virus infection is essentially supportive and symptomatic and no specific treatment is available for increasing the fallen platelets, which have a significant role in causing the mortality of dengue patient. So there is increased need for research of drugs that could prevent and treat fallen platelets.

The present research work involved in leaf extraction from Carica papaya plant by cold percolation method and Lyophilization followed by fabrication into tablet. The active ingredients of C. papaya tablets regulate the ALOX 12 and PTAFR gene thereby leading to an increased production of megakaryocytes and its conversion into platelets.

Standardization is defined as best technical application consensual wisdom inclusive of processes for selection in making appropriate choices for ratification coupled with consistent decisions for maintaining obtained standards. This view includes the case of "spontaneous standardization processes", to produce de facto standards. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceutical, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in national health care programmes because these drugs are easily available at low cost, safe and people have faith in them. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilise compounds with similar polarity Phytopharmaceuticals and secondary plant product of medicinal importance such as alkaloids, glycosides, terpenoids, Flavonoids and lignans.

Papaya (Carica papaya linn.) is well known for its exceptional nutritional and medicinal properties throughout the world. Papaya, a juicy and tasty fruit, belonging to family Caricaceae is scientifically known as Carica papaya Linn. It is grown in various parts of the world, including India, tropical America and Europe. It is commonly known as Papaya melon tree, Pawpaw or papau, Kapaya, Lapaya, Papyas, Papye, Tapayas, Fan mu gua[1]. Papaya is a powerhouse of nutrients and is available throughout the year. The whole Papaya plant including its leaves, seeds, ripe and unripe fruits and their juice is used as traditional medicine. Papaya plant is normally found in tropical areas around the world. The family name is Caricaceae and the botanical name is *C. papaya* commonly known as the Papaya,

Melon Tree (though technically it is a large herb) or Pawpaw. The Papaya is a short-lived, evergreen plant that can grow up to 25 feet high. Its hollow, fleshy, green or purplish trunk is marked with leaf scars. The Papaya rarely branches. The leaves grow in a spiralled cluster directly from the upper part of the stem on horizontal petioles (leaf stalks) 1 to 31/2 feet long. The leaves are deeply divided and range in width from 1 to 2 feet. Leaves are palmate lobed with 7 lobes. The life of a leaf is 4 to 6 months. Male and female flowers are produced on different plants, though there are hermaphrodite forms in cultivation as well as forms that bear both male and female flowers on the same plant. The flowers are fleshy and waxy and have a light scent. The blossoms are followed by deliciously edible fruits, which, although technically a berry, resemble melons. They have yellowish, thin skin and yellowish, peach, or orange to orange-red flesh with a central cavity filled with small, pea-like, black seeds.

MATERIAL AND METHOD

Fresh Leaves of Carica papaya were collected in the month of November - February. The leaves were collected from local source and authenticated by Dr. Narhare M. V. The leaves were cleaned with tap water followed by distilled water and dried in shade for 10-15 day. The leafy portion was taken and grinded to form fine powder and stored in air tight bottle.

Standardization of Carica Papaya leaf

A. Morphological studies[2]

Papaya leaves were examined to study morphological and organoleptic characters.

B. Determination of moisture

Loss on drying[3]

Weigh about 1.5 g the powered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100^0C or 105^0C, until two consecutive weighing do not differ by more than 0.5 mg. Cool in desiccators and weigh. The loss in weight is usually recorded as moisture. Percentage moisture content was calculated with reference to the shade dried material.

C. Determination of ash value

Ash values used to determine quality & purity of crude drug and to establish identity of it.

Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. these are present in definite amount in a particular crude drug hence, quantitative determination in terms of various ash values helps in their

standardization. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects total ash value. Such variables are removed by treating with acid and acid insoluble ash value is determined.

D. Determination of foaming index[4]

Saponins give persistent foam when shaken with water. Hence, plant material/ extract containing saponins are evaluated by measuring the foaming ability in terms of foaming index.

1. Take one gram of course powder of plant material in a 500 ml conical flask.
2. Add 100ml boiling water and maintain moderate boiling for 30 minutes.
3. Cool and filter.
4. Collect the filtrate /decoction in a 100 ml volumetric flask and adjust the volume to 100 ml by adding sufficient water.
5. Pour the decoction into 10 stoppered test tubes as 1 ml, 2ml, 3ml, etc. Upto 10ml.
6. Adjust the volume of liquid in each test tube to 10 ml by adding sufficient quantity of water and stopper the tubes.
7. Shake the test tubes in a lengthwise motion for 15 seconds (two shakes per second)
8. Allow the test tubes to stand for 15 minutes and measure the height of foam.

E. Determination of swelling index[4]

The term swelling index gives an idea about the mucilage content of the drug; hence it is useful in the evaluation of crude drugs.

Procedure

1. Specified quantity of the plant material (1gm) concerned previously reduced to the required fineness and accurately weighed taken into 25 ml glass stopper measuring cylinder.
2. 25 ml of water added and the mixture was shaken thoroughly every 10 minutes for 1 hour.
3. It was allowed to stand for 24 hours at room temperature.
4. Measure the volume occupied by swollen plant material.

F. Phytochemical screening[4]

The petroleum ether, the chloroform, ethanol and aqueous extracts were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as

alkaloids, steroids, terpenoids, anthraquinone glycosides, flavonoids, tannins and phenolic compounds, steroids, carbohydrates, proteins and amino acids and mucilage. The following tests were carried out to identify the various phytoconstituents present in all the extracts.

Extraction of carica papaya leaf

Defatting of dried powered leaves was done with Soxhlet apparatus using petroleum ether 60^0-80^0c until colourless liquid was derived in siphon tube. Defatted powder was used for extraction. Hot maceration process was extraction process. 99.99% ethanol was used as solvent for extraction. Defatted powderwas added in absolute ethanol. The mixture was kept for two days in room temperature. At the end of first day ethanol containing extract was filtered and collected, then it was resuspended with fresh ethanol. The maceration was continued again for next day. Finally both extract were combined.

Solid extract was obtained by lyophilisation process.

Determination of pH[2]

- **PH 1% solution**

Accurately weighed (1 gm) 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 gm) 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.

TLC[5,6]

TLC finger printing profile was done for alcoholic extracts to find out the nature of compounds present. The solvent system used was *chloroform: acetone: ethanol: conc. Ammonium hydroxide (2: 2: 2 : 1)*, n- butanol: chloroform: acetic acid: ammonia: water (7: 4: 5: 1: 1), benzene: methanol: acetone: acetic acid(70: 20: 5: 5) 10mg/ml of leaf sample of alcoholic extracts was procured from hot extraction method and stock solution of 100 µg/ml of vitamin C using water as a solvent was prepared. Test solution and standard solution was applied on a precoated silica gel 60 F_{254} TLC plate and run in the previously saturated solvent system. The use of 1:1 mixture of ethanolic 2% H_2SO_4 and 0.2% ethanolic p-dimethylaminocinnamaldehyde spray for detection of vitamin C as bright pink orange zone.

RESULTS

A. Macroscopic characters

Papaya leaves were examined to study morphological and organoleptic characters. Sample for microscopy were prepared by embedding in solvent system consists of formalin, glycerine, water (8:1:1) for a week. The sections were taken and then they were seen under microscope (Motic of B1 series) at 10x, 40x, 100x after staining with Phloroglucinol & HCL. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 cm (20–28 in) in diameter, deeply palmately lobed, with seven lobes. Unusually for such large plants, the trees are dioecious. The tree is usually unbranched, unless lopped. Organoleptic property of leaf powder is green to dark green in colour, with smooth surface. The drug powder is irritating with characteristic odour and bitter in taste.

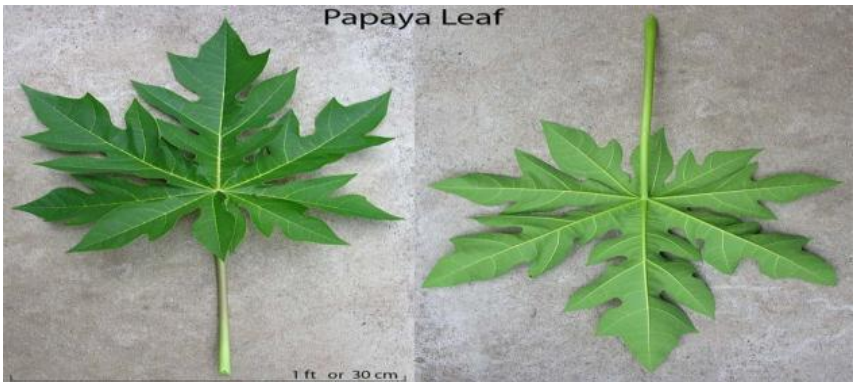

Figure 1.2: Dorsal View. **Figure 1.3: Ventral View.**

T. S. of Carica Papaya leaf shows

[i] Epidermis [ii] vascular bundle [iii] collenchymas [iv] palisade cells [v] stomata [vi] calcium oxalate crystals. [see fig.2 and 3.

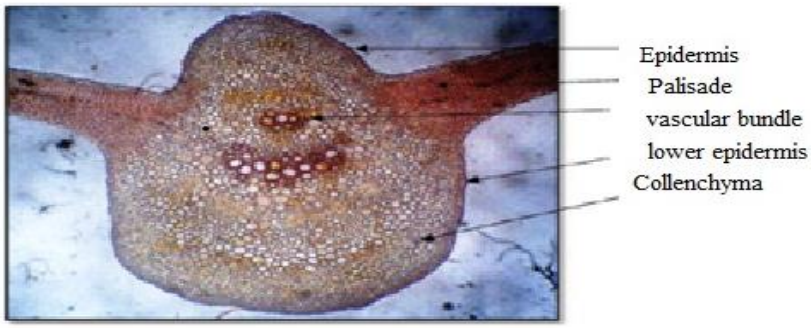

FIG. 2
T .S. of leaf (with Phloroglucinol HCL staining)

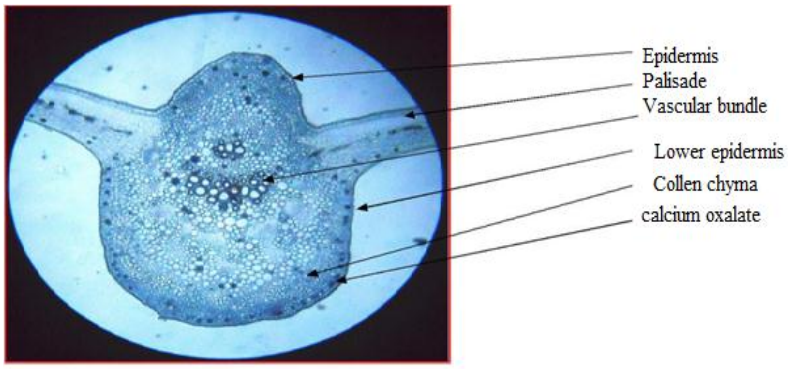

FIG. 3

Powder Analysis: organoleptic properties

Color: Green, Odour: Pleasant, Taste: Bitter.

B. Loss on drying

The mean loss on drying was found to be6.63%.

C. Ash values

The total ash value, acid insoluble ash value and water soluble ash value were found to be 23.33%, 6.66% and 12.25% w/w respectively. Ash value is useful in determining authenticity and purity of drug and these values are important quantitative standards.

D. 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.

E. Swelling index

The swelling index was found to be less than 100.

F. Phytochemical screening for different phytoconstituents

Sr. no.	Test	Petroleum ether extract	Ethanolic extract	Aqueous extract
1	Flavonoids	-	+	+
2	Alkaloids	-	+	+
3	Glycosides	+	+	+
4	Carbohydrates	-	+	+
5	Steroids	+	+	-
6	Proteins	-	+	-

G. pH values

The mean pH value of 1% solution and 10% solution was found to be 6.85 and 4.35, respectively.

I. TLC

R_f value of vitamin c standard and in mixture separated by using three mobile phases enabling the clearest separation of standards on silica gel plates.

Mobile phases for separation of vitamin C.

Sr. No.	Mobile phase	Sample	R_f value of standard and mixture
1.	n- butanol: chloroform: acetic acid: ammonia: water (7: 4: 5: 1: 1)	Standard	nd
		Mixture	nd
2.	Benzene: methanol: acetone: acetic acid (70: 20: 5: 5)	Standard	0.56
		Mixture	0.54
3.	Chloroform: ethanol: acetone: ammonia (2: 2: 2:1)	Standard	0.14

CONCLUSION

The therapeutic value of medicinal plants lies in the various chemical constituents in it. The bioactivity of plant extracts is attributed to phytochemical constituents. For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. *Carica papaya* leaves showed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins and amino acids, phytosterol, phenolic compounds, flavonoids, Terpenoids and tannins in different extracts. The presence of phytosterol in *Carica papaya* leaf was very prominent in all extracts. The saponins, glycosides, proteins and amino acids, flavonoids, terpenoids showed greater intensity of their presence in ethanol. These specifications will be useful as guidance's for quality assessment of the Carica Papaya leaf extract as a good raw material for pharmaceuticals Antithrombocytopenic preparations.

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