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RESEARCH ARTICLE

Physiochemical Screening of *Carica papaya* Leaves with Specific Reference to Their Pharmacognostical Evaluation

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ABSTRACT

Carica papaya is made to develop pharmacognostical characters of leaf with their morphological, microscopical, and physical characters including histochemical analysis. Morphological evaluation as color, odor, taste, size, shape, surface, and powder microscopy of plant shows the presence of endosperm cell which is polygonal in shape and contains aleurone grains and oil droplet, cell of testa, yellow coloring matter, and starch grains. Quantitative leaf microscopy to determine palisade ratio, stomata index, and vein-islet number is carried out. Peels are removed mechanically through epidermal peeling off and stomatal index (SI) is calculated. The vein-islet number, vein termination number, and palisade ratio of lamina are determined according to the standard method. We prepared the extracts of plant with different solvents for determining the different extractive values by maceration, Soxhlet extraction, successive extraction process, and determination of ash values, pH value, moisture content, and phytochemical screening to show the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, and lipids in the drug extract and fluorescence analysis in different solvent. Analysis of pesticide residues, aflatoxin, and heavy metals are also performed.

Keywords: Aflatoxin, *Carica papaya*, extractive value, fluorescence analysis, heavy metals, pharmacognostical study, phytochemical identification

INTRODUCTION

Carica papaya known as *Papita* belongs to the family Caricaceae. It is distributed throughout the tropics and subtropics where it is extensively cultivated.[1] It is a perennial, herbaceous plant, with copious milky latex reaching to 6–10 m tall.[2] Its erect stem is about 30 cm thick and roughened with leaf scars.[3] Leaves contain alkaloids carpain, pseudocarpain, and dehydrocarpaine I and II, choline, carposide, and Vitamins C, A, and E.[4] Phytochemical screening of the leaves revealed the presence of bioactive compound saponins, cardiac glycoside, alkaloids, vitamins, and mineral constituents.[5] The chief contains is papain. The young leaves are medicinally used in jaundice, urinary complaints, gonorrhea, digestive ailments, bacterial infections, vermifuge, dengue fever, beriberi, abortion, asthma, malaria, and skin problems. It showed the phytochemicals, vitamins, and minerals composition of green, yellow, and brown of leaves.[6] Fresh, green papaya leaf is an antiseptic, while the brown, dried papaya leaf is the best as a tonic and blood purifier.[7]

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MATERIALS AND METHODS

Collection and authentication of plant material

Samples of *Raphanus sativus*, *C. papaya* leaves, were collected from Hamdard University

Campus, New Delhi, India (2018) and samples were identified by Taxonomist Prof. H.B. Singh, Department of Botany, NISCAIR. The specimen was studied in Pharmacognosy and Phytochemistry Laboratory, Vivek College of Technical Education, Bijnor, U.P.

Macroscopical and microscopic study

The fresh leaves were examined to macroscopical and microscopical studies. The dried leaves were examined for powder microscopy using different staining reagents for different types of microscopical characters.

Physicochemical evaluation of drug

Determination of individual extractive values

The amount of soluble components extracted with different solvents from the powder plant material.

Maceration

The air-dried coarse drug powdered was macerated with different solvents such as pet. ether, chloroform, water, and methanol of in a closed flask and place for 24 h, shaking frequently during 6 h and allowing standing for 24 h. After the filtration, evaporated to dryness in a dish and dried at 105°C, to constant weight and get percentage yield.

Soxhlet extraction

The dried coarse powdered drug was packed in a Soxhlet apparatus separately for different solvents such as pet. ether, chloroform, water, and methanol. Each extract was evaporated till to dryness and extractive value was noted.

Successive extraction

The dried coarse powdered drug was subjected for successive extraction in Soxhlet apparatus with different solvents as pet. ether, chloroform, and methanol. The extract was evaporated till to dryness and extractive values were noted.

Determination of ash values

Ash value is an essential parameter of a drug for the extent of adulteration and also establishes the quality and purity of the drug.

Determination of total ash values

After the ignition of medicinal plant yield, total ash constituting in which both physiological and non-physiological ashes were present. The drug was incinerated in a silica crucible at temperature which not more than 450°C, then was cooled and weighed to get the total ash content.

Determination of acid-insoluble ash values

Sand and siliceous earth both forming acid-insoluble ash represent. Ash is boiled with dil. HCl (6 N) for 5 min. After that, the insoluble matter collected on an ashless filter paper, rinsed with hot water, and ignited at a temperature which not more than 450°C to a constant weight.

Determination of water-soluble ash values

The ash was in dissolved distilled water after that the insoluble part of ash collected on an ashless filter paper which ignited at 450°C to a constant weight. The weight of soluble part of ash is noted by subtracting the weight of insoluble part from the ash.

Fluorescence analysis

Fluorescence analysis of the powder drug was exposed to in daylight and ultraviolet (UV) light (254 and 366 nm) and treated with different reagents such as sodium hydroxide, picric acid, iodine, hydrochloric acid, nitric acid, pet. ether, ferric chloride, and chloroform.

Phytochemical screening

The different extracts of the selected drugs such as petroleum ether extract, chloroform extract, methanolic extract, and aqueous extract were reported to preliminary phytochemical investigation for the detection of secondary metabolites. The plant extracts may provide the information regarding

various types of phytoconstituents present such as alkaloids, carbohydrates, flavonoids, protein, saponins, mucilage, resins, fat, and lipids.[8]

Determination of pH

pH 1% solution

Drug was dissolved in distilled water, filtered this, and noted pH of the filtrate with a standardized glass electrode.

pH 10% solution

Drug was dissolved in distilled water, filtered this, and noted pH of the filtrate with a standardized glass electrode.

Determination of moisture content

Excess of water in medicinal plant will encourage the microbial growth and also the presence of fungi and insect resulting in deterioration and hydrolysis. Weighed drug and dried in oven at 105°C temp. for 1 hour, then cool in desiccator and weight.

Loss on drying = Wt. before drying–wt. after drying × 100/wt. sample taken.

Table 1: Macroscopic character of the leaves of *Carica papaya*

Parameters	Observation
Color	Green
Odor	Characteristic
Taste	Bitter
Size	50–70 cm in diameter
Shape	Simple, lobed
Surface	Smooth

Table 2: Quantitative microscopy of the leaves of *Carica papaya*

Parameters	Observation
Stomatal no.	
Upper surface	3±4
Lower surface	6±8
Stomatal index	
Upper surface	24±28
Lower surface	26±30
Vein termination	5±6
Palisade ratio	12±13

Heavy metals residue

Heavy metals were determined such as lead, arsenic, mercury, and cadmium in the leaf extract of the plant using atomic absorption spectrophotometer.[9]

Pesticide residue

According to the American Organization of Analytical Chemist (AOAC) using gas chromatography–mass spectrometry method, pesticides residue was determined such as pyrethroids, organochlorines, and organophosphates in the leaf extract of the plant.[9]

Aflatoxin analysis

According to the AOAC using high-performance liquid chromatography method, aflatoxin were analyzed in leaf extract of the plant.[9]

RESULTS AND DISCUSSION

Macroscopical study

The leaves of *C. papaya* were green in color, simple lobed shape, smooth surface, 50–70 cm in diameter size, bitter in taste, and characteristic odor, as shown in Table 1.

Microscopic study

Transverse section of leaves

Transverse section of leaves through the mid rib showed upper epidermis and lower epidermis

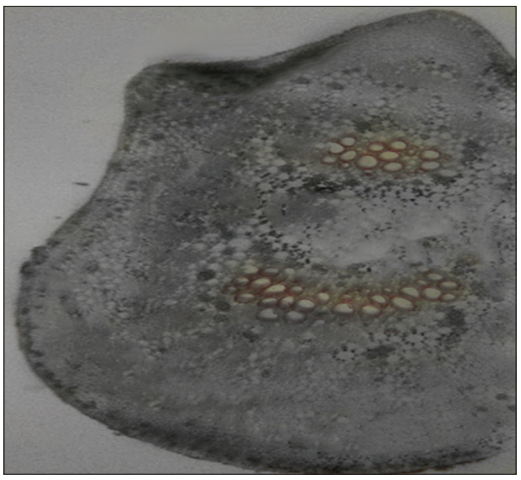

Figure 1: View of vascular bundle

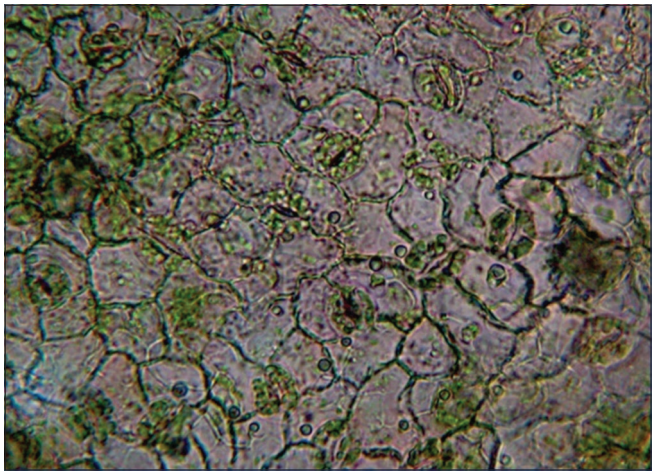

Figure 2: View of stomata

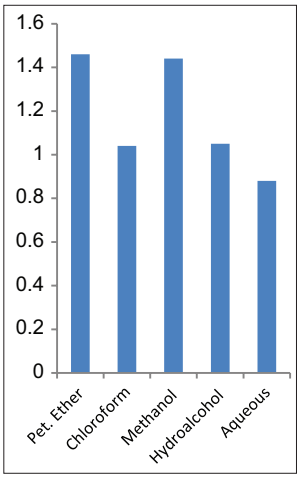

Figure 3: % w/w of maceration extraction

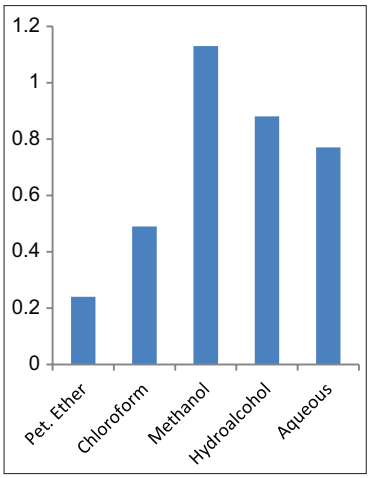

Figure 4: % w/w of Soxhlet extraction

Table 3: Physicochemical evaluation of powder drug of the leaves of *Carica papaya*

Parameters	Result %w/w
Maceration extraction	
Petroleum ether	1.46
Chloroform	1.05
Methanol	1.45
Hydroalcohol	1.06
Soxhlet extraction	
Petroleum ether	0.24
Chloroform	0.49
Methanol	1.13
Hydroalcohol	0.89
Aqueous	0.76
Successive extraction	
Petroleum ether	0.48
Chloroform	0.55
Methanol	0.78
Ash values	
Total ash	1.63
Acid-insoluble ash	1.39
Water-soluble ash	0.16
pH of 1% solution	6.87
pH of 10% solution	5.26
Moisture content	6.40

Table 4: Heavy metals residue analysis of the leaves of *Carica papaya*

Heavy metals	Concentration
Cadmium (Cd)	0.20±0.04
Arsenic (As)	0.37±0.08
Mercury (Hg)	0.45±0.06
Lead (Pb)	0.38±0.09

Table 5: Aflatoxin residue analysis of the leaves of *Carica papaya*

Parameters	Method	Results	MDL (µg/kg)
Aflatoxin B_1	AOAC 990.332	Not detected	1.0
Aflatoxin B_2	AOAC 990.332	Not detected	1.0
Aflatoxin G_1	AOAC 990.332	Not detected	1.0
Aflatoxin G_2	AOAC 990.332	Not detected	1.0

AOAC: American Organization of Analytical Chemist, MDL: Method detection limit

surrounded by well-defined 5–7 layer of collenchyma and sclerenchyma. The endodermis is composed of parenchymatous cells. The pith is found to be absent as the stalk is hollow from inside. A middle portion is covered with xylem and phloem surrounded by parenchymatous cell that, in turn, surrounded by sclerenchyma cells. Numerous fibres are present with cluster crystals. Some xylem vessels (pitted vessels) are also

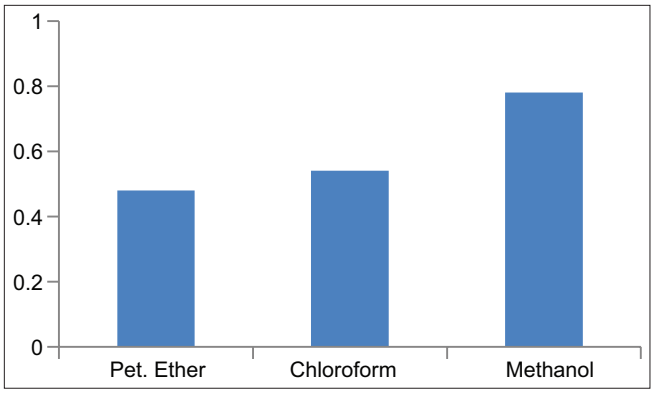

Figure 5: % w/w of successive extraction

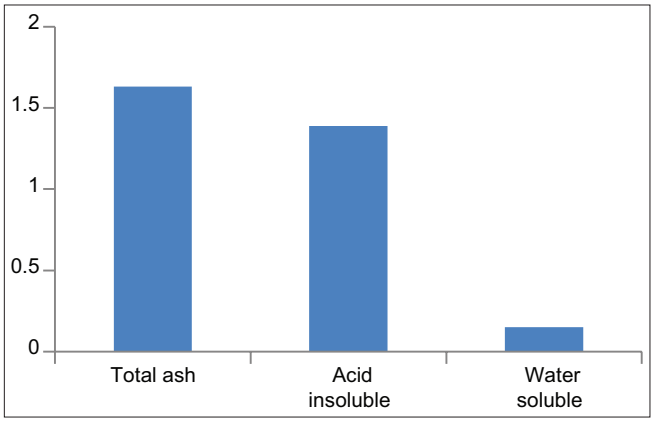

Figure 6: % w/w of ash value

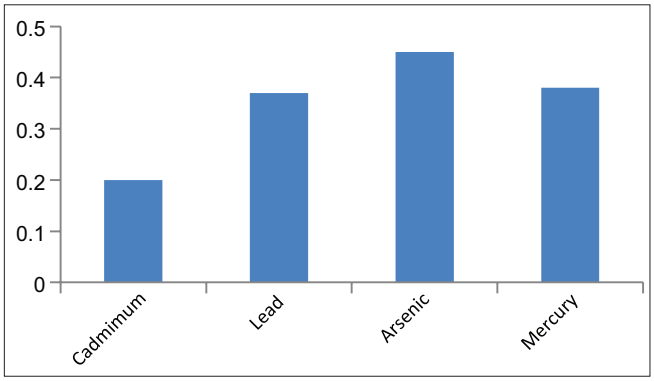

Figure 7: Concentration of heavy metals

visible which are lignified. Cells of palisade and spongy parenchyma are also visible [Figure 1].

Quantitative microscopy

The slides of surface preparation of leaf are prepared and subjected to quantitative microscopic examination, Figure 2. The parameters such as vein termination, vein-islet, and stomatal numbers, stomatal index, and palisade ratio of the leaf are shown in Table 2.

Table 6: Pesticide residue analysis of the leaves of *Carica papaya*

Pesticide	Test method	Results	MDL (mg/kg)
α-BHC	AOAC 790.52/EPA525.5	Not detected	0.01
β-BHC	AOAC 790.52/EPA525.5	Not detected	0.01
γ-BHC	AOAC 790.52/EPA525.5	Not detected	0.01
δ-BHC	AOAC 790.52/EPA525.5	Not detected	0.01
α-Chlordane	AOAC 790.52/EPA525.5	Not detected	0.01
β-Chlordane	AOAC 790.52/EPA525.5	Not detected	0.01
Heptachlor	AOAC 790.52/EPA525.5	Not detected	0.01
Heptachlor_ Epoxide	AOAC 790.52/EPA525.5	Not detected	0.01
α-Endosulfan	AOAC 790.52/EPA525.5	Not detected	0.01
β-Endosulfan	AOAC 790.52/EPA525.5	Not detected	0.01
Endrin	AOAC 790.52/EPA525.5	Not detected	0.01
Endrin_ Aldehyde	AOAC 790.52/EPA525.5	Not detected	0.01
Total DDE	AOAC 790.52/EPA525.5	Not detected	0.01
Total DDD	AOAC 790.52/EPA525.5	Not detected	0.01
Total DDT	AOAC 790.52/EPA525.5	Not detected	0.01
Alachlor	AOAC 790.52/EPA525.5	Not detected	0.01
Butachlor	AOAC 790.52/EPA525.5	Not detected	0.01
Monochlor	AOAC 790.52/EPA525.5	Not detected	0.01
Malathoin	AOAC 790.52/EPA525.5	Not detected	0.01
Methyl–parathion	AOAC 790.52/EPA525.5	Not detected	0.01
Chlorpyrifos	AOAC 790.52/EPA525.5	Not detected	0.01
Ethion	AOAC 790.52/EPA525.5	Not detected	0.01
Diazinone	AOAC 790.52/EPA525.5	Not detected	0.01
Phosphamidon	AOAC 790.52/EPA525.5	Not detected	0.01
Simazine	AOAC 790.52/EPA525.5	Not detected	0.01
Atrazine	AOAC 790.52/EPA525.5	Not detected	0.01
Fenitrothion	AOAC 790.52/EPA525.5	Not detected	0.01
Mevinphos	AOAC 790.52/EPA525.5	Not detected	0.01
Dimethoate	AOAC 790.52/EPA525.5	Not detected	0.01
Phorate	AOAC 790.52/EPA525.5	Not detected	0.01

AOAC: American Organization of Analytical Chemist, MDL: Method detection limit

Physicochemical evaluation

The various physicochemical parameters were determined using air-dried powder plant material, as shown in Table 3. Graphical representation is shown in Figures 3-7.

Table 7: Phytochemical evaluation of the leaves extract of *Carica papaya*

Constituents	Extracts petroleum ether	Chloroform	Alcoholic	Aqueous
Carbohydrate	−	−	+	+
Phenolic compound	+	+	+	+
Alkaloids	−	+	+	+
Flavonoids	−	−	+	−
Lipids	+	−	−	−
Saponins	−	−	+	−
Steroids	+	+	+	−
Amino acids	−	−	+	−
Proteins	−	−	+	−

−: Absent, +: Present

Table 8: Fluorescence analysis of powder of the leaves of *Carica papaya*

Reagent	Daylight	UV light 254 nm	UV light 366 nm
Powder such as	Light green	Dark green	Light green
Powder treated with dist. water	Green	Dark green	Brown
Powder treated with 1 N NaOH	Light green	Dark green	Green
Powder treated with HNO_3	Brown	Black	Dark green
Powder treated with H_2SO_4	Light brown	Dark green	Dark brown
Powder treated with iodine	Brown	Dark brown	Green
Powder treated with conc. HCL	Green	Brown	Black
Powder treated with ammonia	Green	Green	Brown
Powder treated with ferric chloride	Yellowish-green	Black	Light brown
Powder treated with picric acid	Light green	Green	Dark green
Powder treated with pet. ether	Dark green	Black	Dark green
Powder treated with chloroform	Dark green	Brown	Dark green

UV: Ultraviolet

Determination of heavy metal residue

As per the WHO, the determination of heavy metals was carried out in the extract of *C. papaya* leaves using atomic absorption spectrophotometer, as shown in Table 4. Graphical representation is shown in Figure 7.

Determination of aflatoxin residue

The detection of aflatoxin such as B1, B2, G1, and G2 was carried out in the extract of *C. papaya* leaves, as shown in Table 5.

Determination of pesticide residue

According to the AOAC guidelines, pesticide residue was carried out in the extract of *C. papaya* leaves, as shown in Table 6.

Phytochemical analysis

The presence and absence of various phytoconstituents to the preliminary chemical test of extracts are subjected, as shown in Table 7.

Fluorescence analysis

The air-dried powder of the leaves was subjected in lights and UV light with different chemical reagents to be observed, as shown in Table 8.

CONCLUSION

The present study is an attempt to develop the pharmacognostic, physicochemical, and phytochemical standard parameters to be used for identification, purity, quality, and extracts of the leaves of *C. papaya.* Clinical evaluation of these plants in human beings may be carried out for the above promising pharmacological activities.

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