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ESTIMATION OF BIOACTIVE CONSTITUENTS OF *CARICA PAPAYA* LEAF EXTRACT

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Abstract:
Herbal medicine is referred to as medicinal preparations comprising active ingredients obtained from the herbal plant. The product can be made from the whole plant or any part. Preparations from by-product herbal plants such as oil, gum, and other secretions are also considered herbal medicines. Carica papaya fruit is an excellent source of betacarotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helped in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber. The percentage yield of Carica papaya Hydroalcoholic extract was found to be 5.12 %. The Phytochemical test results showed the presence of Flavonoid, Phenol, Proteins, Reducing sugars and Saponins. Total phenolic content in the Carica papaya Hydroalcoholic extract was found to be 0.435 mg/ 100 mg. The flavonoid content in the plant extract was found out to be 0.765 mg/100mg. The estimation of total protein content was performed and results showed 0.633 mg/100mg of the total protein in the Carica papaya Hydroalcoholic extract. Thus it can be concluded that the Carica papaya contains the bioactive constituents of medicinal value.

Key Words: *Herbal medicine, Carica papaya, Extraction, Phenol, Flavonoid, Protein*

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INTRODUCTION:

Medicinal plant (MP) refers to any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors of the synthesis of valuable drugs. A whole plant or plant parts may be medicinally active. Plants are considered as one of the most important sources of medicines. Among the 2,50,000 higher plant species reported in the world, more than 80,000 species are being used as medicinal. The medicinal plants are extensively utilized throughout the world and are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of world population. The beginnings of the medicinal plants' use were instinctive, as is the case with animals [1].

In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis [2].

Natural products are currently of considerable significance due to their unique attributes as a significant source of therapeutic phytochemicals and their efficacy, safety, and minimal side effects. Bioactive compounds in plants include alkaloids, terpenoids, coumarins, flavonoids, nitrogen-containing compounds, organosulfur compounds, phenolics, etc. A wide spectrum of bioactivities is exhibited by these compounds such as anti-inflammatory, immunostimulatory, anticancer, antioxidant, antimicrobial, etc [3,4].

Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E. The minerals, magnesium and potassium, vitamin B pantothenic acid and folate and fiber. In addition to all this, it contains a digestive enzyme-papain that effectively treats causes of trauma, allergies and sports injuries. All the nutrients of papaya as a whole improve cardiovascular system, protect against heart diseases, heart attacks, strokes and prevent colon cancer. The fruit is an excellent source of betacarotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helped in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber [5].

MATERIAL AND METHODS:

Collection of Plant material:

Leaves of *Carica papaya* was collected from local area of Bhopal (M.P.) in the month of February, 2020.

Extraction of plant material:

Dried powdered of leaves of *Carica papaya* has been extracted with Hydroalcoholic solvent (80:20 ethanol: aqueous) using maceration for 48 hrs, filtered and dried using vaccum evaporator at 40^0C[6].

Phytochemical Screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Estimation of Total Phenolic content:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol**.** 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of Phenol. 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content:

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Estimation of Total protein content:

The amount of protein was estimated by Lowry's method.

Reagents A. 2% Na_2CO_3 in 0.1 N NaOH

B. 1% NaK Tartrate in H_2O

C. 0.5% CuSO4.5 H_2O in H_2O

D. Reagent I: 48 ml of A, 1 ml of B, 1 ml C

E. Reagent II- 1 part Folin-Phenol [2 N]: 1 part water.

1 ml of each BSA (Bovine serum albumin) working standard 50-250 µg/ml or test in test tubes. The test tube with 1 ml distilled water was serving as blank. Added 4.5 ml of reagent I and incubated for 10

minutes. After incubation added 0.5 ml of reagent II and incubated for 30 minutes. Measure the absorbance at 660 nm and plot the standard graph.

RESULTS AND DISCUSSION:

The percentage yield of *Carica papaya* Hydroalcoholic extract was found to be 5.12 %. The Phytochemical test showed the presence of Flavonoid, Phenol, Proteins, Reducing sugars and Saponins. Total phenolic content in the *Carica papaya* Hydroalcoholic extract was found to be 0.435 mg/ 100 mg. The flavonoid content in the plant extract was found out to be 0.765 mg/100mg. The estimation of total protein content was performed and results showed 0.633 mg/100mg of the total protein in the *Carica papaya* Hydroalcoholic extract.

Table 1: % Yield of *Carica papaya*

S. No.	Solvent	% Yield (W/W)
1	Hydroalcoholic	5.12%

Table 2: Phytochemical Screening of *Carica papaya* extracts

S. No.	Constituents	Hydroalcoholic
1.	**Alkaloids** Dragendroff's test Wagner's test Mayer's test Hager's test	 -ve -ve -ve -ve
2.	**Glycosides** Legal's test	 -ve
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
4.	**Phenolics** $fecl_3$	 +ve
5.	**Amino acids** Ninhydrin test	 -ve
6.	**Carbohydrates** Molisch's test	 -ve
7.	**Reducing Sugar** Fehling's test	 +ve
8.	**Proteins** Ethanol test	 +ve
9.	**Saponins** Foam test	 +ve

Table 3: Preparation of calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.135±0.001
2	20	0.247±0.003
3	30	0.364±0.001
4	40	0.474±0.002
5	50	0.581±0.001

***Average of three determination, Mean ± SD**

Table 4: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.232±0.002
2	10	0.415±0.005
3	15	0.617±0.001
4	20	0.815±0.003
5	25	1.021±0.002

***Average of three determination, Mean ± SD**

Table 5: Calibration curve of BSA (Bovine serum albumin)

S. No.	Concentration (µg/ml)	Mean Absorbance
1	50	0.055±0.002
2	100	0.111±0.001
3	150	0.163±0.002
4	200	0.215±0.002
5	250	0.268±0.001

***Average of three determination, Mean ± SD**

Table 6: Total bioactive constituents' content of *Carica papaya*

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)	Total protein (mg/100mg)
1.	Hydroalcoholic extract	0.435	0.765	0.633

CONCLUSION:

Carica papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of three powerful antioxidant vitamin C, vitamin A and vitamin E. The minerals, magnesium and potassium, vitamin B pantothenic acid and folate and fiber. The many benefits of papaya are owed due to high content of vitamin A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties. The percentage yield of *Carica papaya* Hydroalcoholic extract was found to be 5.12 %. The Phytochemical test showed the presence of Flavonoid, Phenol, Proteins, Reducing sugars and Saponins. Total phenolic content in the *Carica papaya* Hydroalcoholic extract was found to be 0.435 mg/ 100 mg. The flavonoid content in the plant extract was found out to be 0.765 mg/100mg. The estimation of total protein content was performed and results showed 0.633 mg/100mg of the total protein in the *Carica papaya* Hydroalcoholic extract. Thus it can be concluded that the *Carica papaya* contains the bioactive constituents of medicinal value.

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