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PHYTOCHEMICAL EVALUATION OF CARICA PAPAYA EXTRACTS

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ABSTRACT

Carica papaya Linn. (family- *Caricaceae*) also known as Papaya is a tropical fruit cultivated on a large scale in India and other parts of the world. Not only the fruit, but the whole plant parts possess numerous medicinal properties. It provides many health benefits due to the high content of phyto-constituents present in it. Papaya possesses numerous pharmacological activities like anti-inflammatory, anti-hypersensitivity, hypoglycemic and hypolipidemic, free radical scavenging, wound healing, antitumour, antibacterial, antifungal, diuretic, uterotonic, anti-sickling, anthelmintic, anti-amoebic, antifertility, etc.[1,2,3] The present study focuses on the pharmacognostic evaluation of ripened *Carica papaya* fruit extract. The fruit pulp was collected and dried from the ripe fruits. The dried fruit pulp was extracted successively with petroleum ether, chloroform, ethanol and hydroalcohol by Soxhlation. Dry extract namely CPEAF, CPEE, CPXE, CPHAE, CPCE and CPPEE, thus obtained were analyzed for presence of different phytoconstituents.

KEYWORDS: Carica papaya; Tropical; Free radical; Pharmacognostic; Soxhlation.

INTRODUCTION

Papaya is one such kind of drug possess an excellent medicinal value in all its parts viz. fruits, leaves, seeds and latex.[2]

Papaya consists of a ripened fruit of the plant known as *Carica papaya*, belonging to the family *Caricaceae*. It is a tropical fruit indigenous to America and cultivated on a large scale in countries like Sri Lanka, Tanzania, India, Hawaii, Florida, Philippines, South Africa and Australia. The fruits are big oval in shape and they resemble melon by having central seed cavity thus also known as pepo-like berries sometimes. Fruits weigh up to 20 lbs, and green until ripe, turning yellow or red-orange. Flesh is yellow-orange to salmon (pinkish-orange) at maturity. The edible portion surrounds the large, central seed cavity.[3]

The whole papaya plant contains a wide variety of pharmacologically active constituents. It contains a high nutritional value that helps to prevent the oxidation of cholesterol. It is a rich source of iron and calcium and a good source of vitamins A, B and G. It also contains terpenoids, alkaloids, flavonoids, carbohydrates, glycosides, saponins and steroids. Papaya possess a wide range of pharmacological activities that can be employed in the treatment of various diseases like anti-inflammatory, anti-hypersensitivity, hypoglycemic and hypolipidemic, free radical scavenging, wound healing, antitumour, antibacterial, antifungal, diuretic, uterotonic, anti-sickling, anthelmintic, anti-amoebic, antifertility, etc.[5]

MATERIALS AND METHODS

Collection and Authentication of Plant material

The matured fruits of *Carica papaya* were collected from the local market of Roorkee city in the month of June and were identified and authenticated by the Botanist of S. S. D. P. C. Girls College, Roorkee. A voucher specimen (STIBAS/Corr/2014-15/1313) was deposited in the Department of Botany.

Methods of Preparation of Extracts

The Successive Extraction

Firstly the outer layer and seeds of the fruits were removed and the pulp was collected. The pulp was dried at temperature not exceeding 60ºC using hot air ovens (Universal Hot Air Oven). About 200g of dried fruit pulp was extracted for 8 hours with petroleum ether (40-60ºC) in soxhlet apparatus. The petroleum ether extract was filtered and air dried. The air-dried extract was repacked in the soxhlet apparatus and exhaustively extracted with chloroform for 8 hours. The chloroform extract was filtered and again air-dried. Then extracted plant material was repacked in the soxhlet apparatus and exhaustively extracted with ethanol and water for 8 hours respectively. Extracts filtered and evaporated and their yield, color and consistency were recorded.

Extraction Method for Biflavones

Powdered drug (100 g) of *Carica papaya* was extracted separately in the soxhlet extraction apparatus using ethanol (95%) for 12 Hours. The resultant alcoholic extract was then air-dried and stored in vacuum desiccators. The dried alcoholic extract was suspended in water. The alcoholic extract was mixed with *n*-hexane and the *n*-hexane portion was discarded after separation. To the aqueous portion, dichloromethane was added and the dichloromethane portion was collected and extracted with ethyl acetate. The ethyl acetate portion was collected and solvent was completely removed. The yield of the ethyl acetate fraction was noted. The ethyl acetate fraction was subjected to qualitative chemical test and thin layer chromatography studies for flavonoids.[59]

Extraction Method for Xanthones

Powdered drug (70 g) of *Carica papaya* was cold macerated (at 30-40ºC) using methanol (95%) for 24 hours. The alcoholic extract was then mixed with n-hexane (for the removal of the fatty material may be present in the extract) and the *n*-hexane portion was discarded after separation. Methanolic portion was collected and solvent was completely removed. The yield of the ethanol was noted. The ethanolic extract was subjected to qualitative chemical test and thin layer chromatography studies for Xanthone.[60]

EVALUATION OF EXTRACTS

Pharmacognostic Studies

Macroscopical Evaluation

- **Organoleptic Evaluation** of drugs refers to the evaluation of drugs by colour, odor, size, taste and special features including touch and texture etc.

Phytochemical Screening

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, iridoids, carbohydrates, proteins and amino acids and mucilage. The following tests were carried out to identify the various phytoconstituents present in all the extracts.[62, 73]

Chemical Test for Carbohydrates

- **Charring test**

Carbohydrates on heating in test tube or in presence of Conc. H_2SO_4, produces charring with smell like burning sugar.

- **Molish test**

Aqueous solution of drug/carbohydrate mixed with few drops of Molish's reagent (alpha naphthol) and Conc. H_2SO_4 was added from sidewall of test tube. Formation of purple coloured ring at junction indicates presence of carbohydrates.

- **Iodine test**

It is specific for polysaccharides. Few drops of Iodine solution was added to aqueous solution of drug/polysaccharide. Formation of blue colour, which disappears on heating and reappears on cooling, indicates the presence of starch.

- **Barfoed test**

This test is used to distinguish between monosacchride and disaccharides. Two ml of Barfoed reagent (Cupric acetate, acetic acid and water) was added to 1 ml aqueous solution of drug and boil. Formation of brick red precipitate in 5 min indicates presence of monosaccharide while in 7 min indicates disaccharide.

- **Seliwanoff's test**

This test is used for identification of keto-hexoses or to distinguish between ketoses and aldoses. To 1 ml aqueous solution of drug, 5 ml of Seliwanoff's reagent (resorcinol in 6M HCl) was added and boiled. Formation of cherry red colour in presence of ketose (Fructose) due to formation of hydroxyl methyl furfural, which condensed with resorcinol to produce cherry red colour.

- **Fehling solution test**

It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate.

- Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath.
- Formation of reddish brown coloured precipitate due to formation of cuprous oxide indicates presence of reducing sugar.
- Di, oligo and poly-saccharides having reducing sugars can be tested by first boiling in dilute acid solution followed by neutralization with ammonia. This neutralized aqueous is used for testing.

- **Benedict's test**

It is used for reducing sugars and composed of mainly Copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedicts solution was added and heated almost to boiling. Formation of green, yellow, orange, red or brown colour in order of increasing concentration of simple sugar in the test solution, due to formation of cuprous oxide.

Chemical Test for Starch

- **Jelly test**

To 0.5 gm of starch in a test tube add 5 ml of distilled water and boil on water bath. Formation of translucent jelly indicates presence of starch.

- **Lugol's iodine test**

It is also known as iodine – KI reagent and composed of aqueous Iodine solution in presence of KI. Few drops of iodine – KI reagent was added to the aqueous solution of starch, which produces deep blue to bluish black colour due to presence of amylase. The colour developed disappears on warming and reappears on cooling. Starch amylopectin, disaccharides and cellulose do not produce any colour.

Chemical Tests for Lipids

- **Solubility in polar and nonpolar solvents**

Lipids are insoluble in polar solvents like water and soluble in nonpolar solvents like petroleum ether, benzene and mineral oil.

- **Grease Spot Test**

A simplest test for lipid is based on the ability of lipids to produce a translucent spot on paper

- **Emulsification Test**

0If emulsifiers like bile salts, tween or soap solution is mixed with lipids and water; the lipids broken down into smaller fragments, which remained suspended for long periods of time in water.

Chemical Test for Proteins and Amino Acids

- **Ninhydrin test**

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it gives characteristic deep blue or pale yellow colour due to formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Chemical Test for Alkaloids

- **Dragendroff's Test**

Drug solution mixed with Dragendroff's reagent (Potassium Bismuth Iodide), forms Orangish red colour.

- **Mayer's Test**

Drug solution and few drops of Mayer's reagent (K_2HgI_4), forms creamy-white precipitant.

- **Hager's Test**

Drug solution and few drops of Hager's reagent (Saturated aq. Solution of Picric acid), forms crystalline yellow precipitate.

- **Wagner's Test**

Drug solution and few drops of Wagner's reagent (dilute Iodine solution), forms reddish-brown precipitate.

Chemical Tests of Glycosides

Test for Anthraquinone glycosides

- **Borntragor's Test**

To 1 gm of drug add 5-10 ml of dilute HCl boil on water bath for 10 minutes and filter. Filtrate was extracted with CCl_4/benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red colour in ammoniac l layer due to presence of anthraquinone moiety.

- **Modified Borntragor's Test**

To 1 gm of drug add 5 ml dilute HCl followed by 5 ml ferric Chloride (5% w/v). Boil for 10 minutes on water bath, cool and filter, filtrate was extracted with carbon tetrachloride or benzene and add equal volume of ammonia solution, formation of pink to red colour due to presence of anthraquinone moiety. This is used C-type of anthraquinone glycosides.

Test for Saponins glycosides

- **Foam test**

To 1 gm of drug add 10-20 ml of water; shake for few minutes, formation frothing which persists for 60-120 seconds in presence of saponins.

Test for Steroid and triterpenoids glycosides

- **Liebermann Burchard's Test**

Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add few drops of acetic anhydride followed by conc. H_2SO_4 from side wall of test tube to the $CHCl_3$ extract. Formation of violet to blue coloured ring at the junction of two liquid, indicate the presence of steroid moiety.

- **Salkovaski test**

Alcoholic extract of drug was evaporated to dryness and extracted with $CHCl_3$, add conc. $H_2SO_4$from sidewall of test tube to the $CHCl_3$ extract. Formation of yellow coloured ring at the junction of two liquid, which turns red after 2 minutes, indicate the presence of steroid moiety.

- **Trichloro acetic acid test**

Triterpenes on addition of saturated solution of trichloro acetic acid forms colored precipitate.

Test for Cardiac glycosides

- **Keller Killiani test**

To the alcoholic extract of drug equal volume of water and 0.5 ml of strong lead acetate solution was added, shaked and filtered. Filtrate was extracted with equal volume of chloroform. Chloroform extract was evaporated to dryness and residue was dissolved in 3 ml of glacial acetic acid followed by addition of few drops of $FeCl_3$ solution. The resultant solution was transferred to a test tube containing 2 ml of conc. H_2SO_4. Reddish brown layer is formed, which turns bluish green after standing due to presence of digitoxose.

Test for Coumarin glycosides

- **$FeCl_3$ test**

To the concentrated alcoholic extract of drug few drops of alcoholic $FeCl_3$solution was added. Formation of deep green colour, which turned yellow on addition of conc. HNO_3, indicates presence of Coumarin.

Test for Flavonoid glycosides

- **Ammonia test**

Filter paper dipped in alcoholic solution of drug was exposed to ammonia vapor. Formation of yellow spot on filter paper.

- **Zinc metal test**

To the alcoholic extract of drug Zinc turning and dil. HCl was added, formation of deep red to magenta colour indicates the presence of dihydro flavonoids.

- **Vanillin H_2SO_4 test**

Vanillin H_2SO_4 was added to the alcoholic solution of drug, formation of pink colour due to presence of flavonoids.

Test for Chemical Tests for Tannins

- **Test with Iron salts**

It show color reaction with iron salt like $FeCl_3$ and potassium ferrrocyanide $K_4Fe(CN)_6$ in presence of ammonia. Addition of $FeCl_3$ solution to the solutions of hydrolysable tannins forms bluish black precipitate whereas with condensed tannins it forms greenish brown coloured precipitate.

- **Gelatin test**

To the aqueous solution of gelatin (1% *w/v*) solution of gelatin 0.5-1.0% solution of tannin was added, formation of buff coloured precipitate indicates presence of tannins. Pseudo tannins also show this test positive if tannin is present in sufficient amount.

- **Test for Chlorogenic acid**

Extracts of drug containing chlorogenic acid on treatment with aqueous ammonia converted to green color after exposing with air.

- **Vanillin H_2SO_4 test**

Solution of test drug was mixed with few drops of vanillin HCl. Development of pink colour in presence of tannins due to conversion of phloroglucinol from catechin.

- **Bromine water test**

Condensed tannins are precipitated in presence of bromine water.[62]

RESULT AND DISCUSSION

PHARMACOGNOSTIC EVALUATION

Macroscopic study

The following results were obtained in study of *Carica papaya*. aerial part.

Table 1: Organoleptic Features of *Carica papaya.*

Fruit	
Condition	Fresh
Colour	Yellowish-orange
Odour	Characteristic aromatic
Taste	Sweet
Size	15-45 cm long 10-30 cm in diameter

Characteristics of Various Extracts

Percentage Yield and Physical Characteristics of Various Extracts of *Carica papaya* Aerial Part (Continuous Hot Extraction).

Table 2: Characteristics of Various Extracts.

Extract	**% Dry wt. (g)**	**Colour**	**Odour**	**Consistency**
Petroleum Ether (40-60ºC)	3.48	Yellowish brown	Characteristic	Waxy
Chloroform	3.36	Chocolate brown	Characteristic	Waxy
Ethanol	30.52	Chocolate Brown	Chocolaty	Sticky
Hydroalcohol	43.20	Chocolate Brown	Light chocolaty	Sticky
Ethyl acetate fraction	4.22	Chocolate brown	Characteristic	Sticky
Xanthone	10.76	Chocolate brown	Characteristic	Sticky

Phytochemical Screening of *Carica papaya* Aerial part

Table 3: Qualitative Tests and Their Results Performed on Various Extracts.

Chemical Tests	**Petroleum Ether**	**Chloroform**	**Ethanol**	**Hydroal cohol**	**Ethyl Acetate Fraction**	**Xanthone**
Alkaloids						
Mayer`s reagent	-	-	-	-	-	-
Dragendroff's reagent	-	-	-	-	-	-
Wagner's reagent	-	-	-	-	-	-
Hager's reagent	-	-	-	-	-	-
Saponins						
Froth test	-	-	+	+	-	-
Sterols						
Salkowaski test	+	+	+	-	-	-
Leibermann's reagent	-	-	-	-	-	-
LeibermannBurchard's reaction	-	-	-	-	-	-
Carbohydrates						
Charring test	-	-	+	+	-	-
Molish test	-	-	+	+	-	-
Iodine test	-	-	-	-	-	-
Fehling's test	-	-	+	+	-	-
Benedict test	-	-	+	+	-	-
Barfoed’s test	-	-	-	-	-	-
Starch						
Jelly test	-	-	-	-	-	-
Lugol's iodine test	-	-	-	-	-	-
Lipids						

Solubility test	+	+	-	-	-	-
Grease Spot Test	+	+	-	-	-	-
Emulsification test	+	+	-	-	-	-
Proteins						
Ninhydrin test	-	-	-	-	-	-
Biuret test	-	-	-	-	-	-
Glycosides						
Borntragor's Test	-	-	-	-	-	-
Mod. Borntragor's Test	-	-	-	-	-	-
Foam test	-	-	+	+	+	+
Liebermann Burchard test	-	-	-	-	-	-
Salkovaski test	-	-	+	-	-	-
Trichloro acetic acid test	-	-	-	-	-	-
Keller Killiani test	-	-	-	-	-	-
Legal test	-	-	-	-	-	-
$FeCl_3$test	-	-	-	-	-	-
Flavonoids						
Ammonia test	-	-	+	-	+	+
Shinoda test	-	-	+	-	+	+
Zinc metal test	-	-	+	-	+	+
Vanillin HCl test	-	-	+	-	+	+
Volatile oils						
Sudan III test	-	-	-	-	-	-
Tannins						
Iron salts test	-	-	-	-	-	-
Gelatin test	-	-	-	-	-	-
Ammonia test	-	-	-	-	-	-
Bromine water test	-	-	-	-	-	-

DISCUSSION

The present study was aimed to formulate evaluate the topical anti-ageing cream of *Carica papaya* fruit extract. Different extract namely CPEAF, CPXE, CPEE and CPHAE were prepared and subjected for investigation of antioxidant potential. From the phytochemical investigations, TLC profiling it can be concluded that.

Carica papaya was found to contain, phenolic compounds, flavonoids, fats, triterpenoids, xanthones, glycosides, carbohydrate and alkaloids as their chemical constituents where as other important constituents namely volatile oil, proteins, amino acids and starch were found to be absent.

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