

EXTRACTION AND PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF MALE, FEMALE AND HERMAPHRODITE *Carica papaya* PLANTS

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Abstract : The use of plants in medicines has been prevalent since ancient times. WHO has confirmed that about 80% of the world population's health needs are met by herbal medicines. Various parts of the *Carica papaya*, a popular fruit tree grown for their fruit have been traditionally used as ethnomedicine for a number of ailments like diabetes, indigestion, viral infections and menstrual pain. The plants are male, female or hermaphrodite. The present study focusses on preparation of extracts of male, female and hermaphrodite *Carica papaya* leaves using solvents of varying polarities. Chemical characterization of these extracts provides a qualitative analysis of the various secondary metabolites present in each one of them and also gives a comparative study of the chemical composition of these extracts.

IndexTerms –*Carica papaya* leaves, Male, Female, Hermaphrodite, Extraction, Phytochemical Analysis.

I. INTRODUCTION

The usage of *Carica papaya* leaves in folk medicines for treating many maladies has led the researchers to investigate the basis of its therapeutic importance. Various parts of the *Carica papaya* plant including the fruits, shoots, leaves, rind, seeds, roots, and latex have been researched for their phytochemical composition in order to understand and attribute their biological activities to the associated medicinal properties (Runnie I *et al.*, 2004; Otsuki N. *et al.*, 2010; Mahmood T. *et al.*, 2005; Corral-Aguayo RD *et al.*, 2008; Okeniyi JA. *et al.*, 2007). Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. Soxhlet extraction method (Franz Von Soxhlet, 1879) was used to carry out the extraction of plant material using solvents with varying polarity in order to separate the compounds based on their solubility. Many of the secondary metabolites produced by plants have proven medicinal properties. Chemical characterization of these extracts was done using standard assays to gain an insight into the various compounds present in them.

II. MATERIALS AND METHODS

Plant Material: Leaves of Male, Female and Hermaphrodite *Carica papaya* plants were collected from Thrissur District of Kerala. The differentiation of the Male, Female and Hermaphrodite plants was done by analysing the flower anatomy. The leaves were cleaned with water and shade dried. The dried leaves were powdered using electronic blender and stored in air tight containers at room temperature till further use.

Extraction: Three solvents of varying polarities were selected for the extraction. The solvents used were Methanol, Chloroform and Petroleum Ether. Soxhlet Extraction of all the 3 plant materials was done using these solvents for 24 hours. The extracts were then concentrated using Rotary Vacuum Evaporator and further dried in oven at 60 °C to remove the traces of solvent. The nine dried extracts were stored in air tight glass bottles at 4°C till further use.

Qualitative Phytochemical Analysis:

The Leaf Extracts of Male, Female and Hermaphrodite *Carica papaya* plants were screened for the presence of various Phytoconstituents.

Test for Tannins (Sindhu *et al.*, 2012):

10 mg of the Extract was taken. To this, 10 ml of Distilled water was added. The mixture was filtered and 2 ml of FeCl₃ was added to the filtrate.

Positive Result: Greenish Black Precipitate

Test for Terpenoids (Roy A., 2018):

10 mg of the Extract was taken. To this 2 ml of Chloroform and few drops of Concentrated H₂SO₄ was added.

Positive Result: Reddish Brown Colour formation at the Interface

Test for Phenols (Rakesh S., 2012):

10 mg of the Extract was taken. To this, 3-4 drops of FeCl₃ was added.

Positive Result: Bluish Black Precipitate

Test for Cardiac Glycosides (Onwukaeme D *et al.*, 2007):

10 mg of the Extract was mixed with Distilled water. The mixture was filtered and 2 ml of Glacial Acetic Acid, 2 drops of FeCl_3 and 5-6 drops of concentrated HCl was added to the filtrate.

Positive Result: Violet / Brown Ring

Test for Flavanoids (Onwukaeme D *et al.*, 2007):

10 mg of the Extract was taken. To this, few drops of NaOH and few drops of concentrated H_2SO_4 was added.

Positive Result: Intense Yellow Colour

Test for Saponins (Sindhu *et al.*, 2012):

10 mg of the Extract was mixed with 10 ml of Distilled water and shaken vigorously.

Positive Result: Presence of Froth

Test for Alkaloids (Parekh J *et al.*, 2007):

The Extract was mixed with 5 ml of 1% Aqueous HCl on water bath and filtered. To 1 ml of this filtrate, few drops of Dragendorff's reagent were added.

Positive Result: Orange red Precipitate

Quantitative Phytochemical Analysis:

Total Flavonoid Content (Madike LN *et al.*, 2017):

A modified Aluminium chloride method was used for quantifying total flavonoid content of Papaya Leaf Extract. The reaction mixture was prepared by adding 30 μL of 5% NaNO_2 to 500 μL of 100 $\mu\text{g}/\text{ml}$ extract. The tube was then incubated at room temperature for 5 min. After incubation, 30 μL of 10% AlCl_3 was added and, the reaction mixture was further incubated at room temperature for 5 min. Next, 200 μL of 1M NaOH was then added and finally the volume was made up to 1mL using distilled water. The mixture was allowed to stand for 30 min and absorbance was measured spectrophotometrically at 510 nm. Similar experiment was performed for varied concentrations of standard Quercetin (20-100 $\mu\text{g}/\text{mL}$). Quercetin standard curve and its linear equation were used to determine total Flavonoid content in the extract. Flavonoid content was expressed as Quercetin Equivalent (QE) in mg/g dry weight of extract.

Total Phenolic Content (Alhakmani *et al.*, 2013):

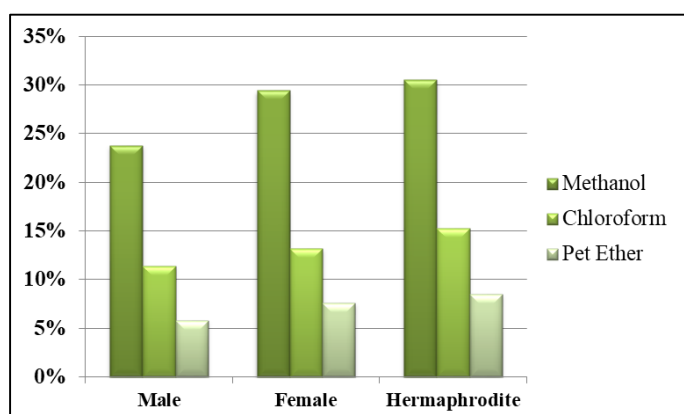
The total Phenolic Content in Papaya Leaf Extract was determined using a modified procedure mentioned by Alhakmani et al. 500 μL of Papaya Leaf Extract (1000 $\mu\text{g}/\text{mL}$) was mixed with 750 μL of Folin-Ciocalteu reagent and incubated for 10 minutes. The solution was then neutralized with 750 μL of 2% Na_2CO_3 solution and incubated in dark for 30 min for colour development. The absorbance was measured at 765nm wavelength on UV-Visible Spectrophotometer. Similar reaction set was prepared using varied concentrations of standard Gallic acid (100-1000 $\mu\text{g}/\text{mL}$). The content of phenolic compounds in Papaya Leaf Extract was determined with the help of standard calibration curve obtained using Gallic acid. The total phenolic content was expressed as Gallic acid equivalent (GAE) in mg/g of dry extract.

III. RESULTS AND DISCUSSION

Extraction Yield:

The yield was found to be the highest with Methanol (30.56% - Hermaphrodite, 29.45% - Female and 25.1% - Male), followed by Chloroform (15.24% - Hermaphrodite, 13.2% - Female and 12.4% - Male) and lowest in Petroleum Ether (8.5% - Hermaphrodite, 7.6% - Female and 7.4% - Male). It was seen that the Percentage Extract Yield of the Leaves of Hermaphrodite Papaya plants was the highest as compared to the Leaves of Female and Male plant (Graph 1).

Graph 1: Percentage Yield of the Extracts in Different Solvents



Qualitative Phytochemical Analysis:

Preliminary Qualitative analysis of the phytochemicals present in the nine extracts showed the presence of Tannins, Terpenoids, Phenols, Cardiac Glycosides, Flavanoids, Saponins and Alkaloids (Table 1)

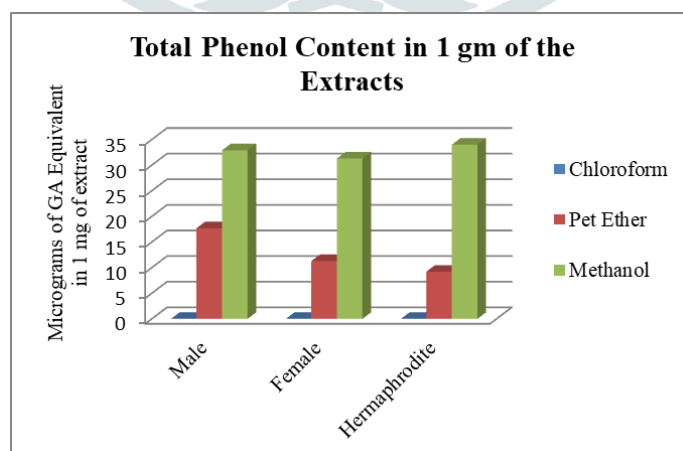
Table 1: Qualitative Phytochemical Analysis of *Carica papaya* Leaf Extracts.

| Phytochemicals | M-M | M-C | M-PE | F-M | F-C | F-PE | H-M | H-C | H-PE |
|----------------|-----|-----|------|-----|-----|------|-----|-----|------|
| Tannins | + | + | + | - | - | - | - | - | - |
| Terpenoids | + | + | + | - | - | - | - | - | - |
| Phenols | ++ | - | + | ++ | - | + | ++ | - | + |
| C.Glycosides | - | - | - | - | - | - | - | - | - |
| Flavoloids | + | + | + | + | + | + | + | + | + |
| Saponins | + | + | + | - | - | - | - | - | - |
| Alkaloids | + | + | + | + | + | + | + | + | + |

M-M : Male Methanolic; **M-C** : Male Chloroform; **M-PE** : Male Pet Ether
F-M : Female Methanolic; **F-C** : Female Chloroform; **F-PE** : Female Pet Ether
H-M : Hermaphrodite Methanolic; **H-C** : Hermaphrodite Chloroform; **H-PE** : Hermaphrodite Pet Ether

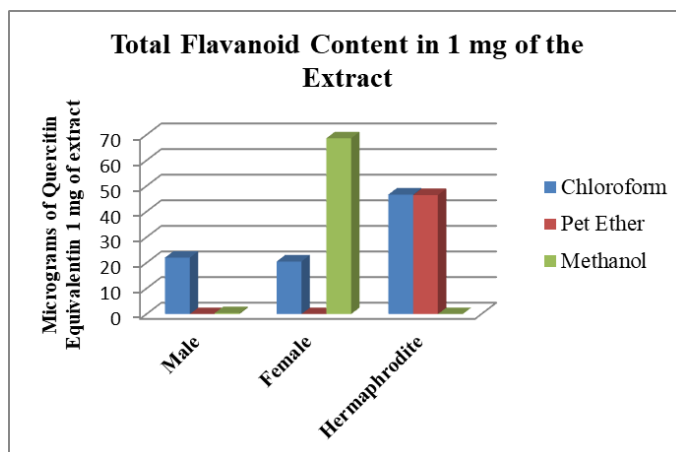
Quantitative Analysis:**Total Phenol Content**

The Total phenol content of the nine extracts showed the highest presence of phenols in the Methanolic Extracts. The Methanolic extracts of the Leaves of Hermaphrodite plants was found to be the highest – 34 micrograms of GAE equivalent in 1 mg of the extract. The chloroform extracts showed no presence of phenols in them (Graph 2).

Graph 2: Total Phenol Content in the Extracts**Total Flavonoid Content**

The Total Flavonoid content of the nine extracts showed the highest presence of Flavonoids in the Methanolic Leaf Extracts of the Female *Carica papaya* plant. The Chloroform and Petroleum Ether extracts of Leaves of Hermaphrodite *Carica papaya* plants showed moderately high amount of Flavonoids in them while the Methanolic Extract of the same showed no presence of Flavonoids (Graph 3).

Graph 3: Total Flavonoid Content in the Extracts



IV. CONCLUSION

Leaves of Male, Female and Hermaphrodite Papaya plants were used for extraction of Phytochemicals using three different solvents of different polarities viz. Methanol, Chloroform and Petroleum Ether. Extraction was done in Soxhlet and the leaves of Hermaphrodite plants showed highest yield in all three solvents. Qualitative Analysis revealed higher number of phytochemicals present in the Methanolic extracts of all the three plant types. Phenols and Flavonoids were present in most of the extracts, hence Quantitative Estimation was conducted. Methanolic extract of Hermaphrodite showed highest micrograms of Gallic Acid Equivalent Phenolic compounds. Methanolic extracts of Female plants showed the presence of highest amount of Flavonoids followed by Chloroform and Petroleum ether extracts of Hermaphrodite plants. Hermaphrodite plants' leaf extracts showed presence of phytochemicals in significant amounts and can be utilized for further analysis. Hermaphrodite plant extracts can have implications in treatments of various ailments and it is evident from the literature. The present study revealed Phytochemical diversity in the leaf extracts of Female, Male and Hermaphrodite Papaya plants.

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