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Nayan Talukdar, Sutila Barchung and
Indrani Barman

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Phytochemical Screening and Study of Total Protein Content, Antimicrobial Activity of the Male Flower of *Carica papaya* L.

Nayan Talukdar¹, Sutila Barchung² and Indrani Barman^{*3}

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ABSTRACT

The male flower carries only stamens and they are also incapable of producing fruits. However, these flowers are reported to have medical significance in various aspects. The present study was aimed to determine the presence of phytochemicals, protein estimation and antibacterial activity of male flower of *Carica papaya* L. The aqueous extract showed presence of carbohydrate, steroid, tannin, flavonoid, saponin, phenolic compounds. The protein estimation was determined by Lowry's method and the value was found to be 300 µg/mL at 660 nm. The antibacterial activity was determined against *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method using standard VA³⁰ antibiotics. From both the test Gram negative bacteria is found to be more effective than Gram-positive bacteria. The finding of the study provided evidences that aqueous extract of *Carica papaya* L. contain medicinally important bioactive compounds which can be an alternative source to replace some commercially available drugs. This study also signifies the folk ware claim of *Carica papaya* L.

Key words: Aqueous extract, Protein estimation, Phytochemicals, Antibacterial activity, Disc diffusion method

India is always having a singular attention for medicinal plants as most of the medicinal plants are available in its various biodiversity. Approximately 80% inhabitants in India depends on plants for therapeutically uses which uses various parts of the plants like leaves, root, bark, flower etc. [1]. In India, different parts of *Carica papaya* L. are used for the treatment of several diseases like dengue, jaundice, stomach disorders, ringworm, roundworm, high blood pressure [2]. *Carica papaya* L. also known as Pawpaw is one of the major fruit crops cultivated in tropical and subtropical zones. Gradually it is becoming an important fruit due to its various medicinal prominence [3]. Male papaya flowers are having some prominent distinguishing features compare to other flowers.

They are large in number, tubular and relatively small. Stamens are having the pollen grains which take part in fertilization and produce fruits. They are also rich in vitamin A, C, E, folate and anti-oxidant molecules. Male papaya flowers are used as a functional ingredient for herbal tea production [4]. Phytochemicals also known as secondary metabolites are bioactive compounds obtained from plants. They are used as a traditional herbal medicine all over the world from different parts of the plant for prevention and treatment of diseases. The present study was conducted on

phytochemical analysis, protein estimation and antibacterial activity of male flower of *Carica papaya* L.

MATERIALS AND METHODS

Collection of the plant material

The flowers were collected from Narengi, Guwahati, Assam, in the month of February and plant materials were identified based on its vernacular name.

Preparation of flowers extract [5-6]

The collected flowers were first washed thoroughly under running tap water until cleanness followed by distilled water and allowed to air dry. After air drying the flowers were chopped into fine pieces and kept for drying under shaded condition. The dried samples were grinded to fine powder with the use of a grinder. Water is used as a solvent for extraction. 10gm of dried flower powder was dissolved in 100mL of distilled water and soaked for 24 hours in conical flask. The mixture was filtered through Whatman No 1 filter paper and later the filtrate was centrifuged at 12,000 rpm for 20mins at 4°C.

Phytochemicals screening [7]

The prepared aqueous extract of the flowers were subjected for presence of phytochemicals namely flavonoids, tannins, phenol, saponins, glycosides, steroid, terpenoids, reducing sugars by following the standard protocol.

* Indrani Barman

✉ rcheindrani@gmail.com

¹⁻³ Programme of Biotechnology, Assam Down Town University, Guwahati - 781 026, Assam, India

Test for flavonoids: In Sodium hydroxide test, the extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color which becomes colorless on addition of dilute acid, indicates the presence of flavonoid.

Test for tannins: Around 2 grams of the extract was heated with 5 mL of ethanol for around 5 minutes followed by cooling and then filtered. To the filtrate few drops of 1% lead acetate was added and observed for the presence of a yellow precipitation which indicates the presence of tannin.

Test for phenol: In Ferric chloride test, to the sample few drops of dilute ferric chloride solution is added. The formation of red, blue, green or purple coloration indicates the presence of phenol.

Test for saponins: In Froth test, the sample was added with distilled and shaken vigorously for the presence of persistent foam atleast for 15 minutes.

Test for glycosides: A quantity of 5 mL diluted sulphuric acid was added to the extract and boiled for 15 minutes in water bath. Few drops of freshly prepared 20% potassium hydroxide solution was added to this solution after cooling. Approximately 10 mL of equal parts of Fehling’s solutions were added and heated for 5 minutes to observe the presence of more dense red precipitate which indicates the presence of glycoside.

Test for steroids: In Salkowski test, 1mL of the extract was dissolved in 10mL of chloroform and equal volume of conc. Sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with fluoresce. This indicated the presence of steroid.

Test for terpenoids: In Liebermann-Burchard test, small amount of the extract was mixed with chloroform and few drops of acetic anhydride solution was added and boiled in a water bath followed by rapidly cooling. Along with the side of the test tube few drops of concentrated sulphuric acid was added. The test tube was observed for the presence of a brown ring at the junction of two layers and upper layer turning to deep red color indicates the presence of terpenoids.

Test for reducing sugar: Benedict’s test: 1mL of extract was added to 2mL of Benedict’s reagent, the solution was then heated to boiling. The change in color confirm the presence of reducing sugar.

Protein estimation [8-9]

Total protein content was estimated by using Lowry’s method. BSA is used for the preparation of standard curve. Around 5 mL of the reagent A (48 mL of 2% sodium carbonate in 0.1N sodium hydroxide, 1ml of 0.5% copper sulphate and 1ml of 1% sodium potassium tartrate) was added to the sample and kept aside for 15 minutes. To this, 0.5mL of freshly prepared reagent B (Folin Ciocalteau: water, 1:1) was added and mixed well. The test tubes were incubated for 30 minutes at dark condition and absorbance was read 660 nm.

Antibacterial activity [10-12]

The antibacterial activity of the extract was determined by disc diffusion method. The flower extract was tested against *Escherechia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria). The bacteria culture was inoculated in nutrient broth for 18 hours allowing the respective bacteria to grow. Later on, Mueller Hinton Agar was prepared and autoclaved along with Petri dish. After that, the media was brought into the laminar air flow and plates were prepared. The cultured broth was inoculated over the solidified media. The disc loaded with extract (10µL) placed over the media by taking standard antibiotic (V³⁰) is placed opposite to the disc and incubated for 24 hours at 37°C.

RESULTS AND DISCUSSION

Plants are composed of various kinds of biological compounds such as carbohydrate, tannin, phenol etc. These compounds are present in plants in various amounts which have made the plant a medicinal plant. *Carica papaya* is also one of those plants that contain many biological compounds which have been proved by using standard phytochemical activity [13]. The preliminary phytochemical screening for the aqueous extract of male papaya flower revealed the presence of several bioactive compounds which could be responsible for the diverse medicinal properties of the plant. Presence of flavonoid, phenol, tannin, carbohydrate etc. were seen in the extraction of the plant (Table 1).

Table 1 Phytochemical analysis of aqueous extract of *Carica papaya* L.

| Phytochemical test | Results |
|--------------------|---------|
| Flavonoids | +ve |
| Tannins | +ve |
| Phenol | +ve |
| Saponin | +ve |
| Glycosides | -ve |
| Terpenoids | -ve |
| Reducing sugar | +ve |

+ indicates present, - indicates absent

These secondary metabolites are also responsible for the defense mechanism showed by plant against various disease-causing microbes [14].

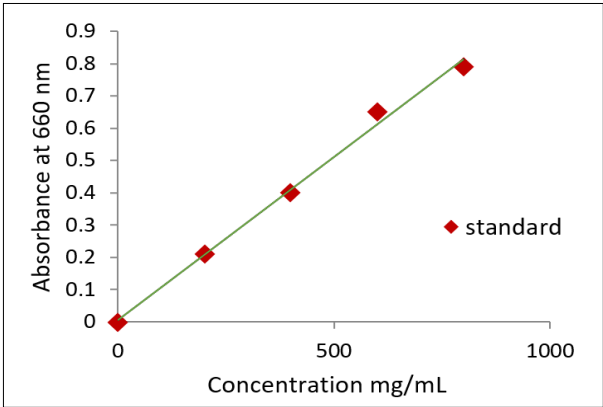


Fig 1 Standard curve of BSA of *Carica papaya* L.

The aqueous extract of male flower of *Carica papaya* L. was further subjected for the estimation of protein by Lowry’s method. The total protein content of the aqueous

extract was found to be 300µg/mL at O.D 660nm using BSA as a standard curved (Fig 1). The extract showed good quantity of protein content, which help the plant to carry its various enzymatic as well as structural and functional role [15].



Fig 2 *Escherichia coli*



Fig 3 *Staphylococcus aureus*

The microbial resistance is raising concern in upcoming time. Hence, sources for antimicrobial activity are always area of interest for the researchers. The Zone of inhibition of aqueous extract of male flower of *Carica papaya* L., against *Escherichia coli* and *Staphylococcus*

aureus is 8 mm and 7 mm respectively (Fig 2-3). The significant antimicrobial activity shown by the aqueous extract of the flower of *Carica papaya* L against *E. coli* and *S. aureus* could be established as a potent antimicrobial agent. The antimicrobial activity shown by the sample could be due to the presence of secondary metabolite profiling [16].

CONCLUSION

The present study confirmed that the aqueous extract of male flower of *Carica papaya* L. are rich in phytochemicals content. It has also the potentiality to possess potent antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Hence, it could be used as a potential source to substitute some of the commercially available synthetic drugs. However, isolation of chemical compounds responsible for its bioactivity along with animal study is necessary.

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