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Estimation of Tannins present in Fruit Pulp and Fruit Shell of *Couroupita Guianensis*

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ABSTRACT

Tannins have a complex molecular structure which is produced by most of plants acts as protective material for plants having many pharmacological properties like astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities. The complexity of structure made very difficult to isolate tannin so estimation of total tannins became a necessary part in studies. In this work the successful attempt was made to estimate total tannins from fruit shell and fruit pulp of plant Couroupita guianensis by the simple spectrophotometry method. Gallic acid was used to obtain standard curve and 30min refluxed extracts of dried fruit shell and pulp with suitable dilutions (beer limit 10-22µg) were analyzed at 700nm. Calibration curve shows coefficient of variance (r^2) = 0.9996. Hydrolyzed tannins found in the shell was 21.42±0.22 which was very high that pulp 3.77±0.13 as that of free tannins of shell was 14.26±0.25 which was also more than the pulp 5.16±0.42. Total tannins found in shell (35.68±0.39%) was very high amount than tannins from shell (8.93±0.44%) are calculated from the data. This study exposes the higher tannin containing source in the form of fruit shell of Couroupita guianensis. The seasonal variation in occurrence of tannins is in process.

Key words: *Tannins, Folin-Denis method, Couroupita guianensis.*

INTRODUCTION

Tannins are high molecular weight polyphenolic compounds present in plants. They are soluble in water and polar organic solvents. These tannins are classified as hydrolysable and condensed tannins based on their chemical structure and biological activity [1,2]. Tannins are found very useful as they increase the effect of other active principles. Tannins have astringent property which is having more significant value in medicinal use. They promote rapid healing of wound and tissue formation. They are used in many diseases and disorders like ulcers, hemorrhoids, burns, inflammation of gums. Tannins are taken orally in diarrhea conditions, heavy metal poisoning. Recent studies report that tannins can be used in the treatment of AIDS and in other viral infections. They also show antioxidant activity [3], free radical scavenging [4], antimicrobial activities. Along with this they are used for the manufacture of Gallic acid and pyrogallol, and sometimes as a reagent in analytical chemistry. They are the basic need of the leather industry for leather production.

Couroupita guianensis (Cannon Ball) is a large deciduous tropical tree 90' tall and indigenous to the Amazon rainforest. The leaves, up to 6" long, are simple with a serrate margin; it flowers in racemes; the yellow, reddish and pink with stunning fragrant. Flowers are large 3" to 5 " waxy aromatic smelling growing directly on the bark of the trunk (cauliflory). Fruits are large globose woody look like big rusty cannonballs hanging in clusters, like balls on a string. The fruit contains small seeds in a white, unpleasant smelling edible jelly, fruits edible and occasionally eaten, but the smell of white flesh discourages most people. Plant is used primarily as ornamental cause of hard shells of fruit. They are also used to make containers and utensils. Cannon ball flowers are considered of special significance in Buddhist culture in Sri Lanka. In Tamil Nadu, it is called Nag lingam flower. The Sivalingamshape is visible at the center of the flower and snake shaped pollen is the specialty of this flower and it has very good fragrance. This rare flower can be used for Shiva Pooja. Plant is indigenous to rainforest of the Guianas in Northeastern South America; a popular ornamental in Caribbean and SE Asian botanic gardens and listed as a rare tree and flower in India [5,6].

The photochemical screening shows presence of carbohydrates, proteins, glycosides, alkaloids and tannins. The cannon ball tree also studied for various activates by different parts of plant extracts like antioxidant activity, leaves shows antimicrobial activity, antifungal, analgesic activity. The literature evidences are found for use of this plant for the treatment of malaria, toothache. The fruit pulp, bark and flowers are used for medicinal applications and have antimicrobial and fungal activity [7, 8, 9]. it is one of the ingredients in the many preparations which cure gastritis, scabies, bleeding piles, dysentery, and scorpion poison [10].

The photochemical investigation shows presence of tannins in different parts of plants and the group of activities including antioxidant, antibacterial, antifungal, analgesic strengthens the possibilities for presence of large amount of tannins in the various parts of plant. The tannin estimation from different parts of plant like leaves, stem are reported but along with this the study of tannins present in fruit shell and pulp is important. So in this study we performed the tannin estimation of Cannonball fruit pulp and fruit shell.

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MATERIALS AND METHODS

Materials:

Plant was identified and authenticated by Dr. Madhukar Bachulkar, Taxonomist and Principal, Vijaysinha Yadav College of Arts and Science, Pethvadagaon, Dist- Kolhapur, Maharashtra. Sample was collected in month of May. Fresh fruits after removing outer shell were used in experiment. The fruit pulp and shell was dried under shed for 12-15 days. Then dried samples were powdered using electric blender (Bajaj). Powder samples were stored in air tight containers in cool and dry place. Standard curve was obtained using Gallic acid (J. P. Pharma) with the help of double beam UV/Visible spectro-photometer (Jasco-V-630) and tannin content was determined by Folin-Denis method [11]. All the chemicals and reagents used were analytical grade (Merck and Loba).

Methods:

Preparation of reagent and solutions: [12]

Folin-Denis reagent:

Sodium tungstate 10 gm, Phosphomolybdic acid 2 gm andPhsosphoric acid 5 ml were taken in the 250 ml round bottom flask with 75 ml of double distilled water. Mixture was refluxed for 2h. After cooling the volume was adjusted to 100 ml with double distilled water.

Sodium carbonate solution:

Accurately weighed 35g of sodium carbonate was dissolved in sufficient quantity of distilled water by heating at 60-80°C. Finally volume was made to 100 ml with double distilled water.

Standard Gallic acid solution:

Gallic acid 100µg ml^{-1}was prepared by dissolving accurately weighed 10 mg Gallic acid in sufficient quantity of double distilled water and volume was made to 100 ml with same.

Test solution for free tannins:

2g each of air dried powder sample was refluxed with 75 ml of double distilled water for 30 min for complete extraction of tannins. Whole the mixture after cooling was filtered through Whatmann filter paper no. 41. Filtrate was centrifuged at 2000 rpm for 20 min. Supernatant was collected in 100 ml volumetric flask and volume was made to 100 ml by double glass distilled water.

Test solution for hydrolysable tannins:

2g each of dried powder samples was refluxed with 0.1 ml hydrochloric acid and 75 ml of double glass distilled water for 30 min. Whole the mixture after cooling was filtered through Whatmann filter. Filtrate was centrifuged at 2000 rpm for 20 min. Supernatant was collected in 100 ml volumetric flask and volume was made to 100 ml by double glass distilled water.

Preparation of working standards:

From standard Gallic acid solution aliquots were pipette out as 0.1, 0.12, 0.14, 0.16, 0.18, 0.20 and 0.22 ml. in 10ml volumetric flasks. To each flask was added Folin-Denis reagent (0.5 ml), sodium carbon-ate solution (1 ml) and volume was made with double distilled water up to 10 ml. This gives working standard solutions of 10, 12, 14, 16, 18, 20 and 22µg ml-1.

Calibration curve:

The absorbance of so formed blue color solution was measured at 700 nm within 30min of the reaction. Calibration curve was plotted by recording absorbances against concentration of Gallic acid in seven working standards. Gallic acid obeyed Beer's Law in the concentration range of 10-22µg ml^{-1}. By using quantitative modes of instrument the slope, intercept and correlation coefficient values were obtained. The concentration in sample solution was calculated by using formula Abs=A+B*C, where A=0.1131, B=0.0616, C=concentration of Gallic acid and correlation coefficient (r^2) was 0.9996. Calibration curve as shown in **Fig. 1**, absorbances are shown in **Table. 1 & 2** shows data of calibration curve.

Tannin estimation:

Tannin estimation done by folin-denis method and total tannin content of fresh fruit shell and fruit pulp was carried out. The data of experiment of tannin estimation of fruit shell and fruit pulp is shown in **Table. 3 & 4** respectively. **Table. 5** represents the % total tannin contents in fruit shell and fruit pulp.

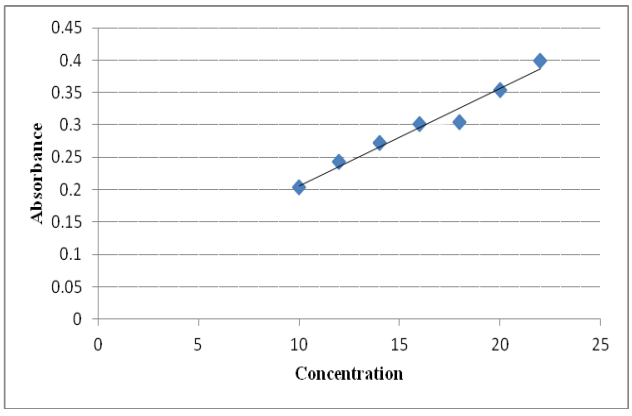

Fig. 1: Calibration curve of Gallic acid

Table No. 1: Absorbances of calibration curve

Concentration (µg mL^{-1})	Absorbance
Blank	0.008
10	0.2033
12	0.2427
14	0.2718
16	0.3013
18	0.3048
20	0.3541
22	0.3987

Table No. 2: Data of calibration curve

λmax	700nm
Beer's law limit (µg ml $^{-1}$)	10 to 12
Regression equation data:	
Slope	1.723
Intercept	0.0214
Correlation coefficient (r^2)	0.9996

Table No. 3: Tannin content of fruit shell

Sample	Absorbance*	Concentration* (µg mL^{-1})
Fruit shell sample for hydrolysable tannins	0.2675±0.21	45.32±0.36
Fruit shell sample for free tannins	0.2671±0.51	30.17±0.11

*Average of three determination ±SEM

Table No. 4: Tannin content of fruit pulp

Sample	Absorbance*	Concentration* (µg mL^{-1})
Fruit pulp sample for hydrolysable tannins	0.1516±0.34	8.562±0.71
Fruit pulp sample for free tannins	0.1995±0.37	11.26±0.12

Table No. 5: % tannin contents in fruit shell and fruit pulp

	% Tannin* (Mean± SEM) w/w		
Analyte	**Hydrolysable tannins**	**Free tannins**	**Total tannins**
Fruit shell	21.42±0.22	14.26±0.25	35.68±0.39
Fruit pulp	3.77±0.13	5.16±0.42	8.93±0.44

RESULTS AND DISCUSSION

Tannins are complex metabolites of plants. Even they show a large group of activities they are difficult to isolate individually. There were several analytical procedures have been reported in literature for determination of total phenolic compounds such as Jerumanis[13], liquid chromatography [14, 15, 16] electrophoretic [17, 18] spectrophotometry with Diode Array Detection [19]. Most simple and widely used methods are butanol-acid treatment, HPLC, HPTLC, hide powder adsorption etc [20]. In this study the folin-denis method was used because of ease of sample preparation and less time consuming. The experimental study showed that hydrolysable tannins found in fruit shell that was 21.42±0.22% was greater than that of free tannins in fruit shell which was 14.26±0.25% in contrast hydrolysable tannins found in fruit pulp that was 3.77±0.13% were less in concentration than that of the free tannins found in fruit pulp which were 5.16±0.42%. Ultimately total tannin found in fruit pulp and fruit shell was calculated and it was found that fruit shell contents very large quantity of total tannins that was 35.68±0.39% than that of fruit pulp which was 8.93±0.44%. The studies on seasonal variation of tannin contents in fruit shell and fruit pulp are in process.

CONCLUSION

Many plants have been studied and reported the importance of tannins and its variation such as wattle, oak, eucalyptus, birch, willow, pine [21]. This research study reveals the comparative account of concentration of tannins in the fruit shell and pulp of *Couroupita guianensis*. In research finding we found that concentration of tannins in shell was more than that of pulp in that hydrolysable tannins are found in high amount. This plant may found the major source of tannins in future. Further study is necessary for isolation and production of tannins from plant source.

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