WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 14, 734-741. Research Article ISSN 2277– 7105

TOTAL PHENOLICS & FLAVONOID CONTENT OF THE LEAVES OF *CARICA PAPAYA & SYZYGIUM CUMINI*

Swati Kabra* and Shivam Patel

Department of Biotechnology, Sir Padampat Singhania University, Bhatewar, Udaipur-Rajasthan (313601).

Article Received on
22 May 2018,

Revised on 12 June 2018,
Accepted on 02 July 2018

DOI: 10.20959/wjpr201814-12858

***Corresponding Author**
Swati Kabra
Department of Biotechnology, Sir Padampat Singhania University, Bhatewar, Udaipur-Rajasthan (313601).

ABSTRACT

The present study is aimed to determine the total phenolics & total flavonoid content in the aqueous leaf extracts of two plants namely *Carica papaya* (papaya) & *Syzygium cumini* (black plum). To determine the flavonoid content Aluminium chloride colorimetry method was used and to determine phenolics content Folin-Ciocalteu assay was used. Both the plants revealed significant amount of phenolics & flavonoids and black plum leaves have shown more amount of phenolics and flavonoids compared to papaya leaves. As both plants leaves have significant phenolics and flavonoids content they can be beneficial to mankind and can be further explored for their medicinal properties.

KEYWORDS: *Carica papaya, Syzygium cumini,* flavonoid, phenolics, Aluminium chloride, Folin-Ciocalteu.

INTRODUCTION

Indian subcontinent is a home to diverse species of plants and they are known to be the source of various medicines since ages. They have a variety of bioactive compounds which can provide different medicinal value to human beings. One of the bioactive compounds present in plants comes in the category of secondary metabolites. Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism; they aid a plant in important functions such as protection, competition, and species interactions, but are not necessary for survival. They are active compounds (phenolics, flavonoids, alkaloids, terpenoids, steroids, saponins, etc.), which are produced by plant in response to stress.[1]

They have important biological and pharmacological activities too which can be beneficial to humans, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic.[2] [3] [4]

Plant phenolics & flavonoids are very important secondary metabolites.[5] Flavonoids are the most abundant phenolics in plants. Such phenolics compounds are very useful and they can be beneficial to humans. Flavonoids are helpful as they inhibitor kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. Their most important property is low toxicity in animals, and hence are used to treat many important common diseases. Further, they can stimulate some hormones and neurotransmitters, and they can also show anti-oxidative properties.[6]

Syzygium cumini, commonly known as black plum, is an evergreen tropical tree in the flowering plant family Myrtaceae. It is native to the Indian subcontinent, adjoining regions of Southeast Asia, China and Queensland. It can reach heights of up to 30 meters and can live more than 100 years. Different parts of this plant, such as seeds, bark, fruit, and leaves have been used in medicine as a remedy for *Diabetes mellitus* in many countries.[7] The leaves of this plant have an aroma similar to turpentine, are pinkish when young, changing to a leathery, glossy dark green with a yellow midrib as they mature and have good nutritional value. The leaves are also rich in acylated flavonol glycosides.[8] They are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy,[9] and their leaf extract also possess antimicrobial activities.[10]

Carica papaya, commonly known as papaya is a small, sparsely branched tree, usually with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 cm (20–28 in) in diameter, deeply palmately lobed, with seven lobes. It is indigenous to the tropical region of Mexico, Central America and Northern South America. Papaya is distributed throughout the tropics and subtropics where it is extensively cultivated. Leaves of this plant is rich in flavonoids, tannins, alkaloids and organic acids and phenolics.[11] *Papaya l*eaf extract can be considered as a potential candidate for increase in platelet count in patients of dengue.[12] The hypoglycemic activities in the leaves of *Carica papaya* has also been reported previously.[13]

Above functional attributes of both the plants may be due the phytochemicals present in them, so in this study we have determined total phenolics and total flavonoid content of the leaves of these two plants: *Carica papaya* (papaya) & *Syzygium cumini* (Black plum).

MATERIALS AND METHODS

Materials

Ferric Chloride, Potassium Iodide, Iodine, Mercuric Chloride, Ammonium Hydroxide, Aluminium Chloride, Quercetin, Potassium Acetate, Folin-Ciocalteu Reagent, Gallic Acid, Sodium carbonate.

Methods

Sample collection

Fresh leaves of around 8-year old plants namely *Carica papaya* (papaya) & *Syzygium cumini* (Black plum) were collected from Sir Padampat Singhania University campus, Bhatewar, Rajasthan, India.

Drying of leaves & Preparation of extracts

Leaves of both plants were washed with water and kept for shade drying for 10 days. After complete drying, leaves were pulverised into powder. The powdered leaves (around 8 gms) were then subjected to hot extraction method using water as solvent. After completion of extraction the contents were filtered and concentrated, and the aqueous extracts obtained were stored in refrigerator for later use.

Qualitative Analysis of crude aqueous extracts

Crude extracts (leaf extract) of both plants were then subjected to different phytochemical tests to determine the presence of phenolics, flavonoids & alkaloids. Ferric Chloride test for phenolics, alkaline reagent test for flavonoids & Mayer's as well as Wagner tests for alkaloids were performed to detect the presence of phytochemicals using standard procedures.

Estimation of total Phenolics content in Extracts

The total phenolic content of crude extract was determined by using the Folin-Ciocalteu assay. Gallic acid was used as standard in this assay.

To perform the assay, an aliquot (1ml) of extract solution & 1 ml of different concentration gallic acid solutions (200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml & 1000µg/ml) were taken

in test tubes and to them 9ml of distilled water was added.1 ml of Folin Reagent was then added to mixture and shaken well. After 5 mins 10ml of 7% sodium carbonate solution was added to the mixture and all the tubes were incubated for 1.5 hour at room temperature, after that the absorbance was determined at 765 nm in an UV-Visible spectrophotometer. All assays were carried out in triplicates and results are expressed as mean value.

Quantitative Estimation of Flavonoids

To estimate total flavonoids content Aluminium Chloride colorimetric method was used. In this assay quercetin was used as a standard. The assay was performed using 0.5ml of leaf extract and each concentration of standard quercetin (6.25ug/ml, 12.5ug/ml, 25ug/ml, 100ug/ml, 200ug/ml, 250ug/ml) taken separately in test tubes. To each test tube 1.5ml methanol, 0.1ml aluminium chloride solution, 0.1ml potassium acetate solution and 2.8ml distilled water were added and mixed well. After incubation, absorbance was taken at 415 nm against the suitable blank.[14] All assays were carried out in triplicates and results are expressed as mean value.

RESULTS AND DISCUSSION

Phytochemical Qualitative Analysis

Preliminary phytochemical analysis conducted on the aqueous leaves extracts of *Carica papaya* (papaya) & *Syzygium cumini* (Black plum) revealed the presence of phenolics, flavonoids and alkaloids. Table 1 shows the presence of some active constituents in the aqueous extracts of the above plants.

S. No.	Test	*Carica papaya*	*Syzygium cumini*
1	Phenolics	+	+
2	Flavonoids	+	+
3	Alkaloids	+	+

(+ indicates presence)

Phenolics, Flavonoids & Alkaloids were present in aqueous extracts of both plants. Further quantification of total phenolics and total flavonoids were done.

Total phenolics content

The total phenolic contents of crude extracts were determined by using the Folin-Ciocalteu assay. Generally, gallic acid is used as the reference standard compound and results are expressed as mg gallic acid equivalents per gm of sample (GAE/gm).The principle involved

in this assay is as follows; Folin-Ciocalteu reagent contains phosphomolybdic/ phosphotungstic acid complexes. In this method, using alkaline medium electrons are transferred from phenolics compounds to form a blue chromophore constituted by a phosphotungstic/ phosphomolybdenum complex where the absorption depends on the concentration of phenolic compounds. The reduced Folin-Ciocalteu reagent is detectable with a spectrophotometer at 765 nm.[15]

In this first standard curve was obtained using gallic acid as shown in Fig.1. From the standard curve total phenolics concentration in both the plant extracts was calculated.

Table 1 Shows the total phenolic content in both the plants.

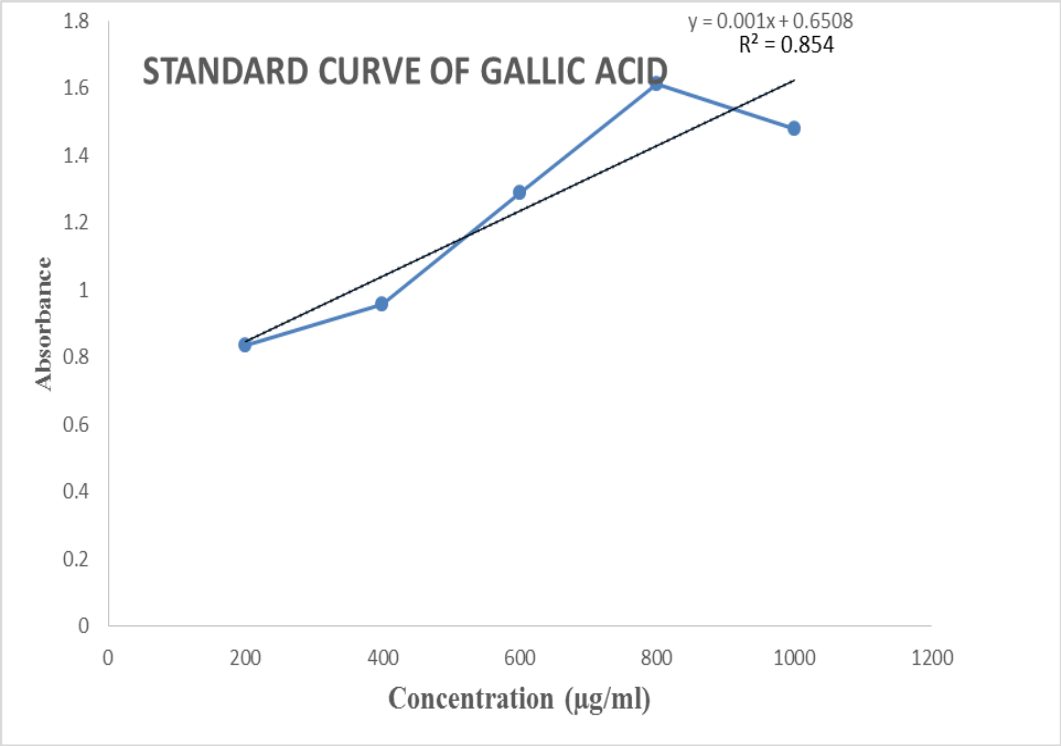

Fig. 1: Standard curve for the quantification of phenolics content.

Table 1: Total phenolics content.

Leaf Extracts	Total phenolics Content (mg GAE/gm)
Syzygium cumini	16.511
Carica papaya	14.532

Total flavonoid content

Principle involved in flavonoids estimation is formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition

with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids.[16]

To estimate flavonoid content in the studied plants, a standard curve is needed which is obtained from a series of different quercetin concentrations. After that standard curve of quercetin was obtained as shown in Fig. 2 and from the standard curve, flavonoids concentration in both the plant extract was calculated in mg quercetin/gm of sample. Total flavonoid content in both the plants is shown in Table 2.

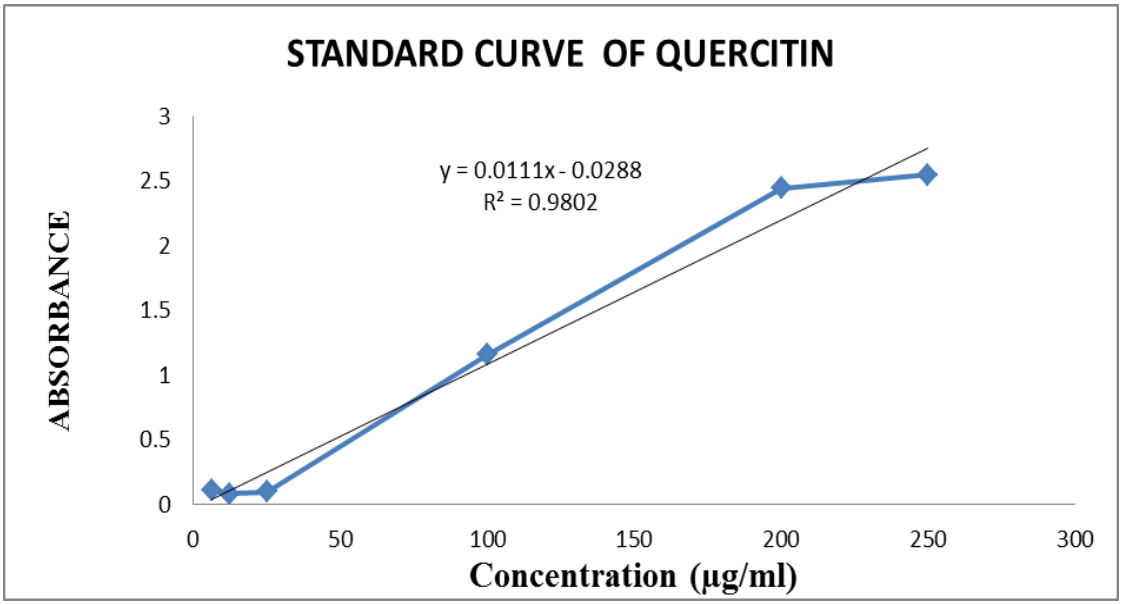

Fig. 2: Standard curve for the quantification of flavonoids content.

Table 2: Total flavonoid content.

Leaf Extracts	Total flavonoid content (mg quercetin equivalent/gm)
Syzygium cumini	8.746
Carica papaya	5.469

CONCLUSION

Leaves of both Carica papaya (papaya) & *Syzygium cumini* (Black plum) have many medicinal uses in Ayurveda. Preliminary phytochemical analysis conducted on the leaves of above plants revealed the presence of phenolics, flavonoids & alkaloids. They had shown good phenolics & flavonoids content. Total phenolics as well as flavonoid content in *Syzygium cumini* leaves is found to be more than *Carica Papaya* leaves. Phenolics and flavonoids in plants are widely used as source of medicine, and as above study suggests that aqueous extracts of both the leaves have significant phenolics and flavonoids content, so they can be further checked for their medicinal properties in-vitro.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to Prof P. C Deka, Vice Chancellor, and Dr Archana Gajbhiye, Head, Department of Biotechnology, for their suggestions in carrying out the experiments. We are thankful to Sir Padampat Singhania University to provide laboratory facility for carrying out this work.

REFERENCES

1. Keeling CI, Bohlmann J. Genes, enzymes, and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. New Phytologist, 2006; 170(4): 657-675.
2. Borneo R, Leon EA, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and there in vitro testing in model food system. Food Chem, 2008; 112(3): 664-70.
3. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem, 2004; 94(4): 550-57.
4. Mulabagal V, Tsay H. Plant cell cultures-an alternative and efficient source for the production of biologically important secondary metabolites. Int J Appl Sci Eng Technol, 2004; 2(1): 29-48.
5. Dai J, Mumper RJ. Plant Phenolics: Extraction, analysis and their antioxidant and anticancer Properties. Molecules, 2010; 15(10): 7313-52.
6. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacolgy & Pharmaceutics, 2002; 96(2-3): 67-202.
7. Rahman A.U., Zaman K. Medicinal plants with hypoglycemic activity. *J. Ethnopharmacol*., 1989; 26(1): 1–55.
8. Ayyanar M, Subash-Babu P. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. Asian Pac J Trop Biomed, 2012; 2(3): 240-6.
9. Warrier, P.K., Nambiar V.P.K., Ramankutty C. *Indian Medicinal Plants*. Orient Longman Ltd, 1996; 5: 25–228.
10. Oliveira, Guilherme Ferreira de, Furtado, Niege Araçari Jacometti Cardoso, Silva Filho, Ademar Alves da, Martins, Carlos Henrique Gomes, Bastos, Jairo Kenupp, Cunha, Wilson Roberto, & Silva, Márcio Luís de Andrade e. Antimicrobial activity of Syzygium cumini (Myrtaceae) leaves extract. *Brazilian Journal of Microbiology*, 2007; *38*(2): 381-384.

11. Juárez-Rojop, Isela E., A.Tovilla-Zárate, Carlos, Aguilar-Domínguez, Dora E., Fuente, Luis F. Roa-de la, Lobato-García, Carlos E., Blé-Castillo, Jorge L., López-Meraz, Leonor, Díaz-Zagoya, Juan C., & Bermúdez-Ocaña, Deysi Y. Phytochemical screening and hypoglycemic activity of Carica papaya leaf in streptozotocin-induced diabetic rats. *Revista Brasileira de Farmacognosia*, 2014; *24*(3): 341-347.
12. Charan, J., Saxena, D., Goyal, J. P., &Yasobant, S. Efficacy and safety of *Carica papaya* leaf extract in the dengue: A systematic review and meta-analysis. *International Journal of Applied and Basic Medical Research*, 2016; *6*(4): 249–254.
13. Aruoma, O.I., Hayashi, Y., Marotta, F., Mantello, P., Rachmilewitz, E., Montagnier, L., 2010. Applications and bioefficacy of the functional food supplement fermented papaya preparation. Toxicology, 2010; 278(1): 6-16.
14. Bag G.C., Grihanjali D. & Bhaigyabati T. Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley. Int. J. Pharm. Sci. Rev. Res, 2014; 30(1): 154-159.
15. Singleton, V. L., Rossi Jr., J. A. & Rossi J A Jr. Colorimetry of Total Phenolics with Phosphomolybdic- Phosphotungstic Acid Reagents. Am. J. Enol. Vitic, 1965; 16: 144–158.
16. KalitaP, Tapan B K., Pal T K., Kalita R. Estimation of total flavonoids content (tfc) and anti oxidant activities of methanolic whole plant extract of Biophytumsensitivumlinn. J. Drug del. & Pharmaceutics, 2013; 3(4).