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Phytochemical Screening and Antioxidant Potential of *Syzygium cumini* Leaves

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ABSTRACT

Background: As a source of alternative medicines, plant derived drugs are of great potential and can be used to cure various health related ailments. One of the most common medicinal plant, *Syzygium cumini* commonly known as Jamun is well known for its various pharmaceutical properties. In the traditional medicine, the entire plant has been widely used in the treatment of various diseases. For the treatment of diabetes, the preparation of tea from the leaves of *Syzygium cumini* is known for its hypoglycaemic effect. Leaves are also used in the treatement of various skin diseases.

Methods: Keeping in view the above concerns, the investigation of this plant aims to assess the phytochemical and antioxidant content of the ethyl acetate extract of locally available *Syzygium cumini* leaves samples acquired from the campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar. The proposed study was conducted in Department of Chemistry, CCS HAU, Hisar during 2019-2021. Samples were collected and moisture content was estimated. Various chemicals and phytonutrients like alkaloids, tannins, minerals, crude protein, crude fibre, flavonoids and total phenolics were investigated in shade dried samples.

Result: The total phenolic and flavanoid content were 11.48 mg GAE/g and 5.17 mg CE/g, respectively. The antioxidant activity of *Syzygium cumini* leaves extract was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and total antioxidant capacity using phospho-molybdenum assay. The result showed that ethyl acetae extract of Jamun leaves had potential antioxidant activity which justifies their medicinal applications for the treatment of various ailments.

Key words: Antioxidant activity, Ethyl acetate extract, Hypoglycaemic, Phytonutrients.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs in the traditional system of medicine, chemical entities for synthetic drugs, food supplements and pharmaceutical intermediates (Ncube *et al.,* 2008). These are the local heritage with global importance and world is endowed with a rich wealth of medicinal plants (Suriyavathana *et al.,* 2010). The non-nutritive secondary metabolites are known as phytochemicals that are naturally occurring in the medicinal plants and have defensive as well as disease preventive properties (Tan *et al*., 2010 and Suhas *et al*., 2014). The major secondary metabolites include alkaloids, carbohydrates, flavonoids, tannins, terpenoids and steroids (Edoga *et al*., 2005). Having a great pharmacological relevance, phytochemicals have been of great interest to human for long time. Plant based drug constitute a major share of medicine in India, China *viz* ayurveda, yoga, unani, siddha, homeopathy and naturopathy, except allopathy (Vaidya and Devasagagam, 2007). Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal products to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases (Joselin and Jeeva, 2014).

Syzygium cumini also known as *Syzygium jambolanum* and *Eugenia cumini* belongs to family Myrtaceae. It is commonly known as “Jamun” and is a traditional medicinal plant native to India having several medicinal properties such as hypoglycaemic, antidiarrhea, antibacterial and anti-HIV activity (Ravi *et al.,* 2004). Leaves and barks of *Syzygium cumini* are known for their anti-inflammatory activity (Muruganandan *et al.,* 2001). Its medicinal properties may be due to its ability to synthesize various phytochemicals. As a remedy for diabetes mellitus in many countries, *S. cumini* leaves have been used since ancient time (Texixeri *et al.,* 2000). The plant possesses acetyl-oleanolic acid, triterpenoids, ellagic acid, isoquercetin, quercetin, kaempferol and myricetin in different concentrations (Rastogi and Mehrortra 1990). Keeping in view, wide medical applications of Jamun, the current experiment was designed to investigate the phytochemical

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compounds and antioxidant potential of ethyl acetate extract of *Syzygium cumini* leaves.

MATERIAL AND METHODS

Material

Syzygium cumini (Jamun) leaves samples were acquired from the campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar. The proposed study was conducted in Department of Chemistry, CCS HAU, Hisar during 2019-2021. The plant materials were brought and before processing, the materials were kept under the shade at room temperature. Taxonomical description of juman is presented in Table 1.

Methods

Prepartion of plant extract

Extraction plays an important role as it helps in the recovery of desired medicinally bioactive constituents from plants by using selective solvents and leaving out those non-desired with an aid of the solvents (Dhanani *et al*., 2017). The powdered sample of Jamun leaves was percolated by using conventional soxhlet apparatus using ethly acetate as solvent. The extracts was collected and kept for further studies.

Qualitative screening for phytochemicals

For the presence of phytoconstituents such as flavanoids, phenols, alkaloids, tannins, saponins, terpenoids and steroids, ethyl acetate extract of *Syzygium cumini* leaves was investigated. Standard methods suggested by Harborne (1998) Kokate (2001) were used for qualitaitive screening of phytochemicals.

Test for alkaloids (Mayer's test)

1.36 gm of mercuric chloride and 5 gm of potassium iodide were dissolved in 60 ml, 10 ml distilled water, respectively. Prepared solvents were mixed and volume was made upto 100 ml using distilled water. To 1 ml of acidic solution of samples few drop of reagent was added. Formation of white or pale precipitates shows the presence of alkaloids.

Test for tannins (Lead-acetate test)

To a test tube containing a small amount of leaves extract, few drops of 1% lead acetate were added. Formation of yellow precipitate indicates the presence of tannins.

Test for saponins

In the test tube containing 50 ml leaves extract, a drop of sodium bicarbonate was added. The mixture was vigorously shaken and kept for two minutes. A honey comb like froth formation indicates the presence of saponins.

Test for flavonoids

To a test tube containing about 0.5 ml of alcoholic extract of sample, few drops of diluted HCl and small amount of Mg or Zn were added and the solution was boiled for 5 minute. Appearance of reddish pink colour supports the presence of flavonoids.

Test for phenols

To 1 ml of the alcoholic solution of the sample, 2 ml of distilled water followed by few drops of the 10% aqueous solution of ferric chloride were added. Development of blue or deep green colour indicates the presence of phenols.

Test for terpenoids

In a test tube containing 1 mL of extract, 2 ml of chloroform and 5-10 drops of concentrated H_2SO_4 were added and appearance of reddish brown colour suggests the presence of terpenoids in leaf sample.

Test for steroids

0.1 g of plant sample was dissolved in 2 ml of $CHCl_3$. H_2SO_4 was added carefully to form a lower layer. A reddish brown colour at the interface was an indicative of steroidal ring.

Proximate composition

1. Moisture content
2. Ash content
3. Crude fat content
4. Crude fiber content
5. Crude protein content
6. Total carbohydrate content

Determination of moisture content

Two gram of powdered leaves sample of Jamun in triplicate was taken and method of AOAC (1995) was used to calculate the percentage of moisture content.

$$\text{Moisture content (\%)} = \frac{\text{Powdered wt. (before drying)- Powdered wt. (after drying)}}{\text{Powdered wt. (before drying)}} \times 100$$

Determination of ash content

Two grams of powdered sample of Jamun leaves was weighed in triplicates and transferred into previously ignited and weighed crucible which is placed for 2 hours in a muffle furnace. From the furnace it was transferred into a desiccator and allowed to cool and their weight was taken.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of crude fat content

In a thimble, two gram of the dried powdered sample of jamun leaves was taken and placed in a soxhlet extractor. A

Table 1: Taxonomical description of jamun

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	Cumini

250 mL dried and pre-weighted round bottomed flask was connected to the soxhlet assembly and petroleum ether was added up to one and a half siphons. The assembly was heated and extraction was performed for 8 hr. After extraction, petroleum ether was evaporated and weight of the round bottomed flask with the residue was determined again. The crude fat (%) contents were calculated as follows:-

$$\text{Crude fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Determination of crude fibre content

Moisture and fat free three gram powdered sample of jamun leaves was taken. Thereafter, the percentage of crude fibre content was calculated by using modified method of Maynard (1970).

Determination of crude protein content

Micro-Kjeldahl method (AOAC, 1990) was used for the determination of nitrogen content. By multiplying % of nitrogen with 6.25 factor, crude protein was calculated.

Determination of total carbohydrates content

Total carbohydrates content was calculated by difference as follows:

Total carbohydrates content (%) =

100 - [Moisture (%) + Ash (%) + Crude fat (%) + Crude fibre (%) + Crude protein (%)]

Mineral analysis

The mineral content of Jamun leaves samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after Microwave-assisted acid digestion.

Quantitative estimation of phytochemical parameters

Quantitative determination of tannin content

Vanillin-HCl method of Burns (1971) was used for estimation of tannin content as catechin equivalent.

Quantitative determination of alkaloid content

Method of Harborne (1973) was used for estimation of alkaloid content in Jamun leaves sample.

Quantitative determination of total phenolics

Analysis of total phenolics were done by the Folin-Ciocalteu method (Singleton and Rossi, 1965) and expressed as milligrams of Gallic acid equivalent per gram (mg GAE/g). 0.2 mL of extract was taken in a test tube and to adjust the optical density with in calibration limits were diluted with respective solvents.

Quantitative determination of total flavonoids

The amount of total flavonoids present was calculated using catechin as standard. Analysis of total flavonoids was done by aluminium chloride colorimetric assay, as explained by Marinova *et al.* (2005). Using the standard curve, the total flavanoids contents in ethyl acetate extract was calculated and the results obtained are expressed as mg catechin equivalents per gram (mg CE/g).

Evaluation of DPPH free radical scavenging activity

Method of Hatano *et al.* (1988) with slight modifications was used for the evaluation of DPPH free radical scavenging activity.

Evaluation of total antioxidant capacity

Modified phosphomolybdenum method by Prieto *et al*., (1999) was used for the evaluation of total antioxidant capacity of Jamun leaves powder.

Statistical analysis

For statistical analysis, triplicates of each sample were taken and the resulting values are expressed as mean ± standard error (S.E.). To assess any significant differences between the means of sample, one way analysis of variances (ANOVA) were carried out in Online Statistical Analysis (OPSTAT). IC_{50} values of DPPH free radical scavenging activity were calculated by regression analysis in Microsoft Excel 2016. All other measurements were also carried out in Microsoft Excel 2016.

RESULTS AND DISCUSSION

Ethyl acetate extract of *Syzygium cumini* leaves was screened by qualitative analysis for the presence of various phytochemicals. The result of qualitative analysis is depicted in the Table 2.

In proximate composition, the leaves part of jamun consist the moisture content (6.06%), crude fibre content (16.15%), ash content (5.25%), crude protein content (8.79%), crude fat (0.26) and total carbohydrates (63.49%).

The minerals (Fe, Mn, Zn and Cu) content was estimated and data is presented in Table 3. The mineral content in leaves part of Jamun, Fe (39.15 ppm), Mn (2.52 ppm), Zn (49.35 ppm) and Cu (2.05 ppm).

In chemical analysis, leaves part of Jamun contains the tannin content (1.59 mg CE/g) and alkaloid content (6.78%). In phytochemical parameters, the content of total phenolics and total flavonoids were estimated. Phenols are very important plant constituents because of their antioxidant activity. The antioxidant activities of the plant extracts are

Table 2: Phytochemical screening of ethyl acetate extract of *Syzygium cumini* leaves.

Phytoconstituents	Ethyl acetae extract
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Phenols	+
Terpenoids	+
Steroids	-

(+)= Presence of phtoconstituents.

(-)= Absence of phytoconstituents.

often explained by their total phenolics and total flavanoids content. The standard curve obtained using gallic acid for total phenol content determination was depicted in Fig 1. The total phenolics in ethyl acetate extract of Jamun leaves was (11.48 mg GAE/g) and the total flavanoid content in jamun leaves was calculated using standard curve of catechin as depicted in Fig 2. The total flavanoids content in ethyl acetate extract of jamun leaves was 5.17 mg CE/g .

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free-radical scavenging activities of antioxidants. The percentage of DPPH free radical scavenging activity kept on increasing when the concentration of ethyl acetate extracts of jamun leaves was increased (Table 4). Total antioxidant capacity estimation using phosho-molybdenum assay is based on the principle that antioxidants present in sample reduce the Mo (VI) to Mo (V). Mo (V) react with the phosphate group of sodium phosphate to form a green coloured complex *i.e*. Mo (V)- phosphate complex (phosphomolybdenum complex) in acidic medium which is measured using UV-Vis spectrophotometer. IC_{50} value for DPPH scavenging and phospho-molybdenum assay is 42.64 and 31.32 µg/mL,

Table 3: Proximate composition, mineral content and chemical analysis of *Syzygium cumini* leaves.

Proximate composition	Moisture content (%)	6.06
	Crude fibre content (%)	16.15
	Ash content (%)	5.25
	Crude protein content (%)	8.79
	Crude fat content (%)	0.26
	Carbohydrate (%)	63.49
Mineral analysis	Fe (ppm)	39.15
	Mn (ppm)	2.52
	Zn (ppm)	49.35
	Cu (ppm)	2.05
Chemical analysis	Tannin content (mg CE/g)	1.59
	Alkaloid content (%)	6.78

Table 4: DPPH free radical scavenging activity and total antioxidant capacity of ethyl acetae extract of *Syzygium cumini* leaves.

Concentration of ethyl acetate extract (µg/mL)	% DPPH free radical scavenging activity	Total antioxidant capacity (%)
5	5.25	8.23
15	19.93	25.60
25	33.26	42.83
35	42.67	56.56
45	51.44	62.4
55	58.75	75.9
IC_{50} Value (µg/mL)	42.64	31.32

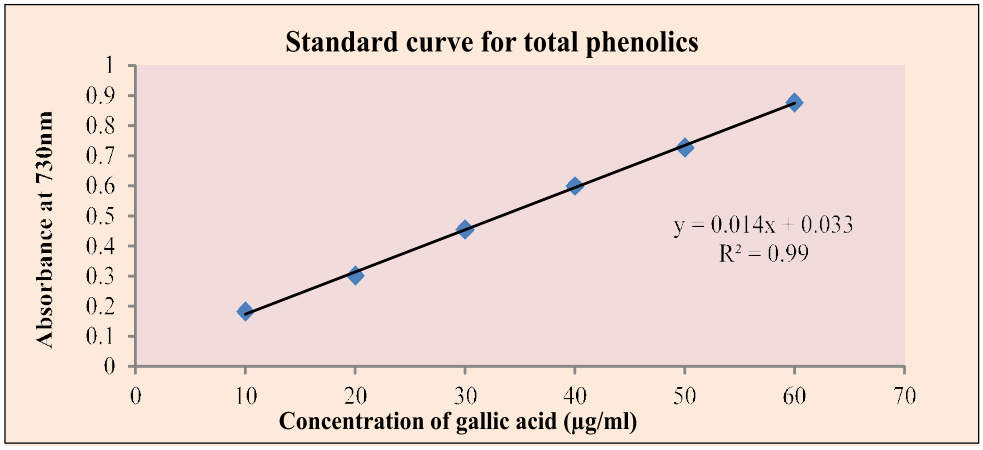

Fig 1: Standard curve for total phenolics using gallic acid as a standard.

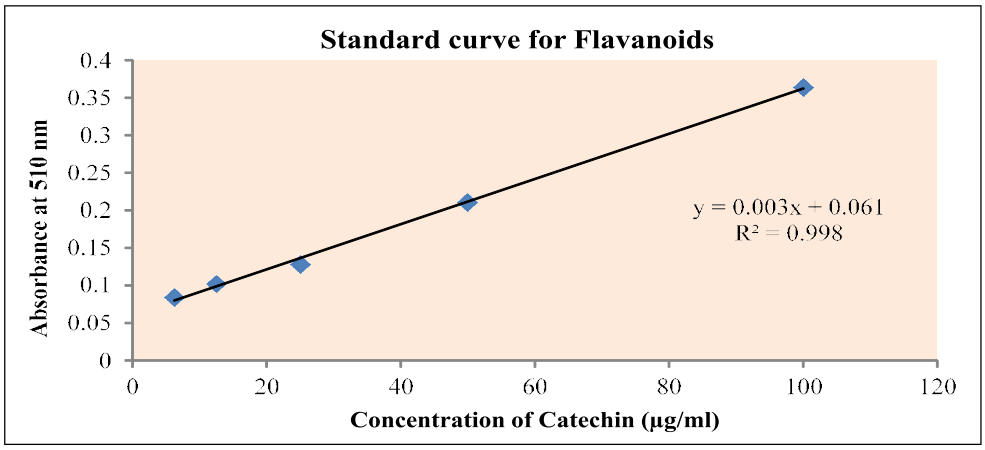

Fig 2: Standard curve of total Flavanoids using catechin as a standard.

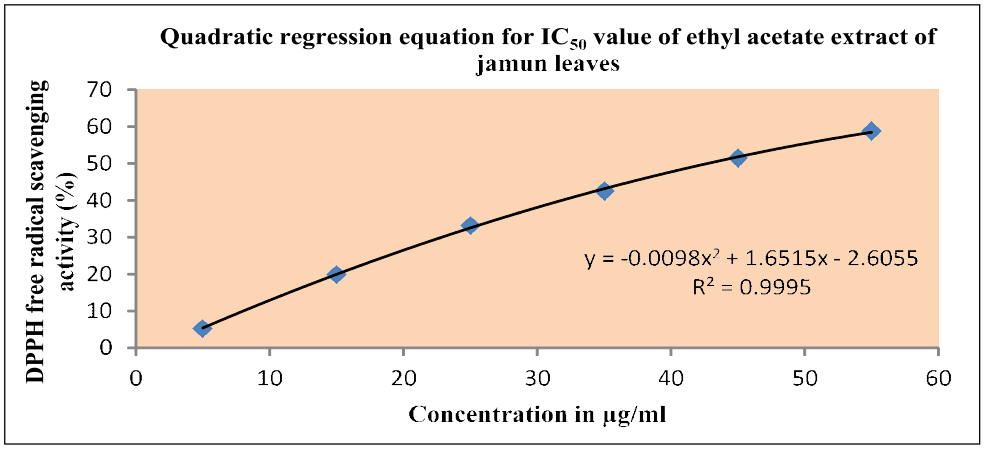

Fig 3: Quadratic regression equations for IC_{50} (µg/mL) value of DPPH free radical scvenging assay.

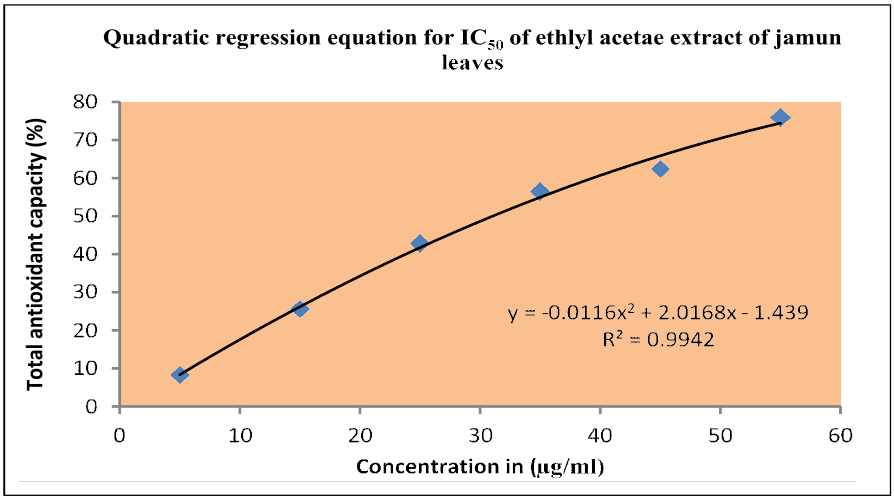

Fig 4: Quadratic regression equations for IC_{50} (µg/mL) value of total antioxidant capacity (%).

respectively was calculated using the quadratic regression equation for IC_{50} value as depicted in Fig 3 and 4. The value suggests that ethyl acetate extract of Jamun leaves have appreciable antioxidant activity.

CONCLUSION

The result indicates the presence of medicinally important phytochemicals in the ethyl acetate extract of *Syzygium cumini* leaves. The present findings have a great importance in the field of dietary supplements, drugs and pharmaceutical companies. The present study suggests that Jamun leaves powder is a potential source of antioxidants enriched with phenolic and flavanoid content in the shade dried sample.

Conflict of interest: None.

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