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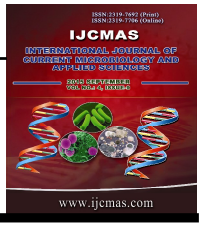

Original Research Article

Evaluation of Antioxidant Activity in Different Parts of *Syzygium cumini* (Linn.)

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K e y w o r d s

Antioxidants, *Syzygium*, Anti- microbial activity

A B S T R A C T

The present study was aimed at evaluation of antioxidant activity in methanolic extract of leaves, fruit pulp and seeds of *Syzygium cumini* (L.). Another aspect of the study was to evaluate the anti microbial activity of the different parts of the plant, keeping in view its pharmacological potential. The quantitative determination of compounds viz., phenolics and flavonoids supposed to be antioxidants was made and overall antioxidant activity was measured using standard methods. The results demonstrate that the total phenolic and flavonoid content of *S. cumini* leaves is greater than the content found in pulp and seed extracts. A linear correlation between total phenolic content and antioxidant activity (r2= 0.464529) has been reported. The results suggested that the phenolic compounds contribute effectively to the antioxidant activity. The highest antioxidant property of leaves is noteworthy as compared to seed and pulp in the present study. Our study showed antibacterial activity against both the gram-negative (*E. coli*) and gram- positive (*Staphylococcus aureus*) cultures grown in the leaf extract of *S. cumini*.

Introduction

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. Much attention has been focused on the activity of the natural antioxidants present in fruits and vegetables, because potentially these components may reduce the level of oxidative stress. Dietary antioxidants include ascorbate, tocopherols, carotenoids and bioactive plant phenols. Amongst the plant sources *Syzygium cumini* fruit is one of those which contain a variety of important nutritional compositions. *Syzygium cumini* (syn. *Eugenia jambolana*) commonly known as a "Jamun" contains various phytoconstituents such as tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, phenols, minerals, carbohydrates and vitamins.

Syzygium cumini (L.) is an evergreen tropical tree belonging to the family Myrtaceae and native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, Philippines and

Indonesia. *Syzygium cumini* is an evergreen tree to a height of 25 m, with grayish white young stems and lower bark coarse and discolored. Leaves are exstipulate, petiolate, simple, elliptic to broadly oblong, smooth, glossy, somewhat leathery, 5–10 cm long, acuminate tip with entire margin and on opposite phyllotaxy. Flowers white to pinkish, about 1 cm across, in branched clusters at stem tips, calyx cuplike and 4 petals fused into a cup with many stamens. Fruits are dark purplish red, shiny, with white to lavender flesh, ovoid, single seeded berry measuring 2.2 –4.5 cm length and 1.5–3cm in diameter. The seed weighs1–3gms and an average sized fruit is said to contain 68–86mg of pulp.

Phytochemical constituent

The principle component Jambolan contains chemical constituents like anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. Seed alkaloid, jambosine, and glycoside jambolin or antimellin are said to have retarding effect to the diastatic conversion of starch into sugar. Ellagic acid of the seed extract has property to control the blood pressure levels (Morton, 1987). The seeds have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes (Ravi *et al.*, 2004).

Total phenolics of the seeds said have an important antioxidant activity (Bajpai *et al.*, 2005). The fruit is rich in sugar, mineral salts, vitamins C. Fruit of *Syzygium cumini* contain malic acid and a small quantity of oxalic acid is also reported to be present. Gallic acid and tannins account for astringency of the fruit. The purple color of the fruit is due to presence of cyaniding diglycosides. Fruit contain sugar (8.09%), non reducing sugar (9.26%) and sulfuric acid (1.21%) Glucose, Fructose, mannose and galactose are the principal sugars.

Jamun has also been reported to be protective in liver disease which could play an important role in prevention of liver damage. In addition, studies also show an anti-cancer potential of fruit extract. These could be possibly due to several bioactive phytochemicals including polyphenols which have the purple pigment called anthocyanin. Many scientists have studied the pharmacological activity of *Syzygium cumini* like antidiarrhoel, antioxidant, gastro-protective, anti-allergic, astringent, analgesic, anti-inflammatory, anti-plaque, antimicrobial and the most important anti-diabetic activity.

Most pharmacological work on diabetes with seeds was carried out but pharmacological potential on other parts of the plant needs to be explored for discovery of safer drugs keeping in view the multi-dimensional functionalities of the plant. The present study was aimed at evaluation of antioxidant activity in methanolic extract of leaves, fruit pulp and seeds. Another aspect of the study was to evaluate the anti microbial activity of the different parts of the plant.

Material and Methods

Plant material used

The fully mature fruits and leaves of *Syzygium cumini* L., were collected from a single tree during the study period August 2013-14 (Hyderabad, India). The methanolic extracts of the pulp, seed and leaves were the samples used for the study.

Determination of total phenolics

The total phenolics of the different sample extracts were determined by the Folin –

Ciocalteu method. The diluted aqueous solution of extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2N, 2.5ml). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2ml) was added. After 2 hr of incubation, the absorbance was measured at 760 nm against water blank. A standard calibration curve was plotted using Gallic acid**.**

Estimation of total flavonoids

Total flavonoid content was estimated following aluminium chloride colorimetric method (Chang *et al.,* 2002). 2 ml of each extract (1:10 g ml-1) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415nm with a double beam UV/Visible spectrophotometer. The calibration curve was plotted by preparing the quercetin solutions at concentrations 40 to 200 µg/100 µl in methanol.

Estimation of total antioxidant activity

The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu, M. (1986). In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron.

Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability. Ascorbic acid at various concentrations (10–100µg/ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Thin layer chromatography of crude extracts of leaf, pulp and seed in *S. cumini*

The separation and identification was performed using TLC. Samples for TLC on Silica gel were prepared with vaporization of 5 ml of sample up to 1ml. Solutions of Standard substance tannic acid, ascorbic acid, salicylic acid, benzoic acid, catechol, resorcinol were prepared by dissolving 10mg in 1ml of distilled water.TLC separation was carried out on silica gel. Solvent system used was benzene: glacial acetic acid: water (125:72:3)

Determination of antimicrobial activity

The antibacterial activity was measured by Agar well diffusion assay (Perez *et al.*, 1990). The plant extracts were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. All the plates are incubated at 37°C for 24hrs. The test cultures used were *Escherichia coli* (Gram negative rods) & *Staphylococcus aureus* (Gram positive cocci). The pure cultures were obtained from National collection of Industrial Microorganisms (NCIM), NCL, CSIR lab, Pune. The bacteria were maintained on nutrient Agar plates (Himedia, India) slopes at 4°C and sub cultured as per the requirement. The antibacterial spectrum of the test sample was determined in terms of zone sizes around each well i.e. diameter of inhibition zones. Each result is a mean of three replicates.

Statistical analysis

Results are presented as the mean± SD. Correlation between analysis of antioxidant activity and the total phenolic and flavonoid contents were carried out using the correlation and regression applications in the Microsoft Excel.

Results and Discussion

Phenols are very important plant constituents because of their antioxidant activity. The antioxidant activities of the plant extracts are often explained by their total phenolics and flavonoid contents. There is a wide range of total phenol content in the extracts of the plants under study. *Ocimum sps* showed high phenolic content according to the results by Veeru *et al.,* (2009) and *Asparagus racemosus* also had comparable high phenolic contents contributing to the medicinal uses. Ghafar *et al.* (2010) found the flavonoid content of Citrus to be the highest. The standard curve generated with gallic acid for total phenol content determination is presented in (Fig. 1). The total phenolic content in the methanolic extract of *S. cumini* leaf was 17.6mg/g, seed 16.1.mg/g and the pulp 8.7mg/g respectively. While the flavonoid content in leaf 43.24mg/g, seed 19.1/g and pulp was 12.27mg/g (Fig. 2). These results demonstrated that the total phenolic content of *S. cumini* leaves were greater than the content found in pulp and seed extracts. A positive correlation was observed between total antioxidant activity and total phenolic content in a study made by Iuliana *et al.* (2011) in the herbal plants. In our present study a similar linear correlation between total phenolic content and antioxidant activity (r^2= 0.464529) has been reported. The results suggested that the phenolic compounds contribute effectively to the antioxidant activity. The antioxidant capacity of a compound can be measured by the ability of the compound to intercept free radicals by scavenging or trapping methods (Huang *et al.,* 2005).

Reducing power assay value (RPA) expressed in ascorbic acid equivalents, was used to determine the antioxidant ability of different extracts in the present study. The RPA value for *S. cumini* leaf extract was the highest (324.67 mg ASE/ml (Fig.3). Seed extract value was 292.5 mg ASE/ml was higher than pulp extract value of 249.5 mg ASE / ml. The highest antioxidant property of leaves is noteworthy as compared to seed and pulp in the present study. The presence of polyphenolic compounds in methanol extracts of seed, leaf & pulp of *S. cumini* might be responsible for the antioxidant activity.

Phenols exhibit variable absorption in the UV or UV/VIS region (Constantine D. Stalikas *et al.,* 2007). Phenolic acids with benzoic acid carbon framework have their maxima in the 200-290 nm range. Only exception is Gentisic acid which has an absorbance at 355nm. The cinnamate derivatives due to additional conjugation show a broad absorbance band in the region, 270 to 360nm.

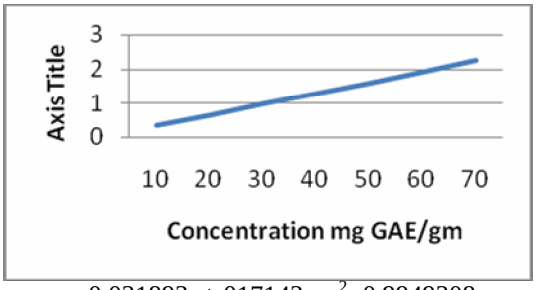
All flavonoid *g*lycones contain at least one aromatic ring and efficiently absorb UV light. The first maximum, which is found in the 240-285 nm is due to the A ring and second maxima which is in 300–550nm range is attributed to the substitution pattern and conjugation of the C ring.

It is evident that phenolics absorb well in UV range and UV detection is therefore convenient method to localize a phenol in the effluent of a column. However no single wavelength is sufficient for their simultaneous monitoring in various natural plant extracts. Detection at 280nm is often used for simultaneous separation of mixtures of phenolic acids. In our study the leaf extract showed six peaks (Fig. 4) with the first two peaks having maximum absorption in the range 365–450nm. The first peak at 365 nm could be referred to as presence of Quercetin and it was reported that chalcones absorb at 365–390nm (Pawar and Salunkhe, 2013).

Table.1 Antibacterial activity in different parts of the plant

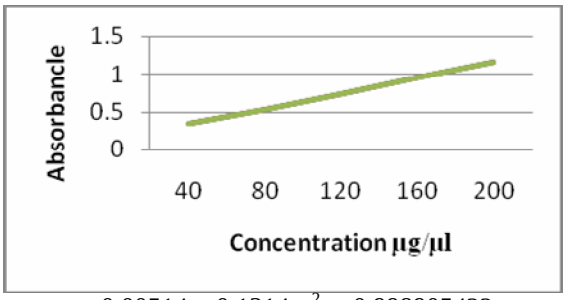
S.No	Type of Extract	E.coli Zone of inhibition(mm)	Staphylococcus Zone of Inhibition(mm)
1	Leaf Extract	15	21
2	Pulp Extract	-	-
3	Seed Extract	-	-

Fig.1 Standard graph of gallic acid



y=0.031893x+.017143 r^2=0.9949308

Fig.2 Standard graph of quercetin



y=0.00514x+0.1314 r^2 = 0.998905422

Fig.3 Ferric reducing power determination of standard ascorbic acid

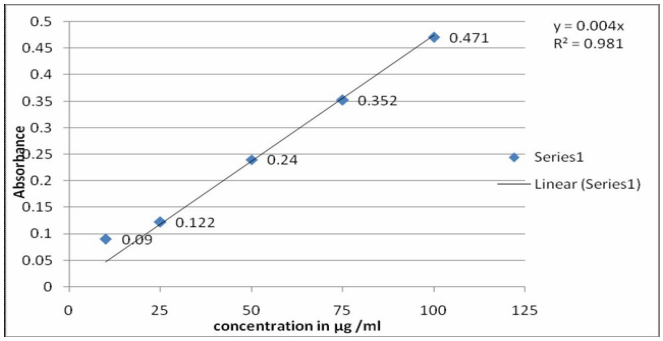


Fig.4 UV Spectrum of leaf sample

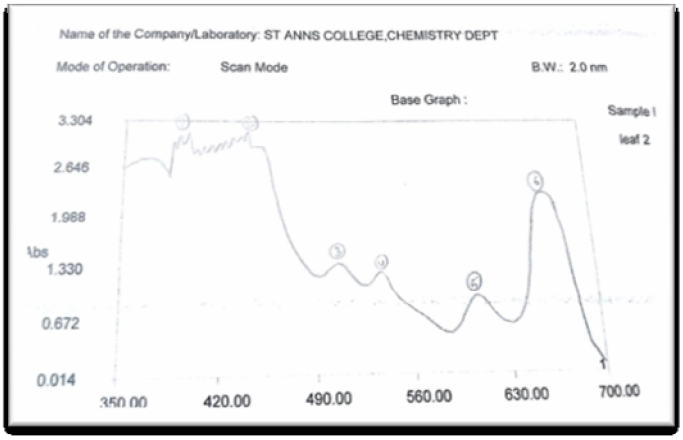


Fig.5 UV Spectrum of seed sample

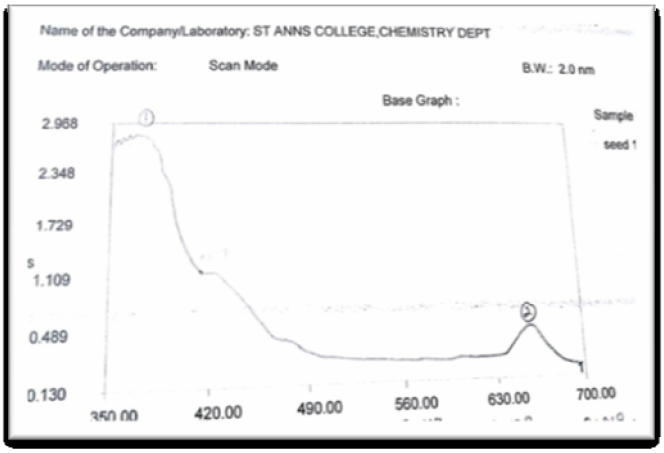


Fig.6 UV Spectrum of pulp sample

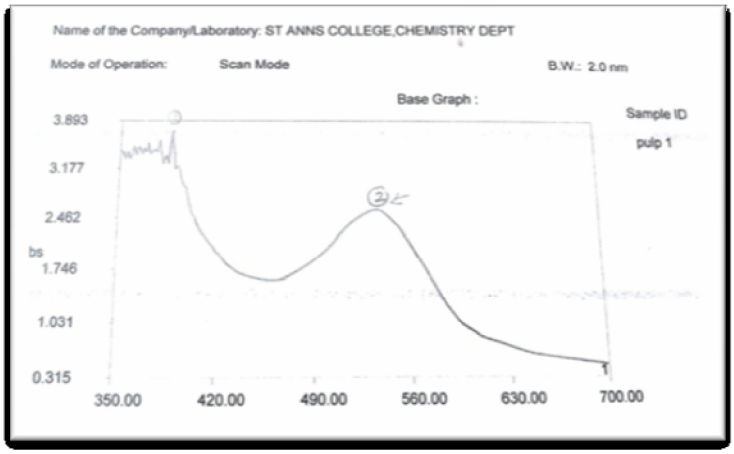
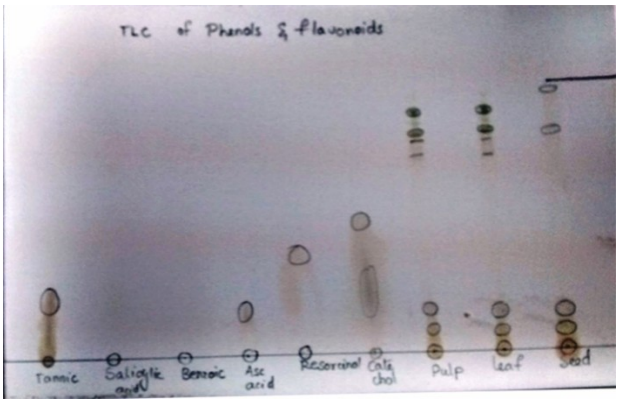


Fig.7 TLC of phenols and flavinoids



The third peak at 508 nm, fourth one at 536nm, fifth one at 607nm, and sixth one at 663.6 nm. UV Spectra was obtained by scanning the sample in the range, 200-400nm. The spectra showed gradual rise in absorbance from 280nm to 400nm.

The seed extract showed maximum absorbance at 369 nm (Fig. 5) in visible spectrum which indicates the presence of rutin in the sample and also at 417nm where polyphenolic acids show absorbance. The UV spectrum shows gradual rise in absorbance from 280nm to 365 nm. The peak value of absorbance at 365 nm could be referred to presence of Quercetin (Pawar and Salunkhe, 2013).

The Pulp extract showed two major absorption peaks in visible spectrum with first peak at 386nm (Fig. 6). This could be referred to presence of catechol. The second absorption band was obtained at 532nm and is characteristic of anthocyanins. Also maximum absorption between 365 to 390nm suggests presence of chalcones as they are reported to absorb in this range. The UV spectra showed gradual rise in absorption in the range 280-400nm.

The TLC chromatogram of phenols and flavonoids in our study (Fig. 7) showed the presence of tannic acid and ascorbic acid in the leaf, seed and pulp extracts. The other standards could not be detected in the extracts but the extracts showed other bands, one among which was common in all the extracts (Rf value 0.77) corresponding to flavonols.

Determination of antimicrobial activity

The antimicrobial activity of the *S. cumini* leaves may be due to tannins and other phenolic constituents. *S. cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives. Most antibacterial medicinal plants are more effective against gram-negative bacteria (Lin *et al.*, 1999; Srinivasan *et al.*, 1989). Our results showed antibacterial activity against both the gram-negative (*E. coli*) and gram- positive (*Staphylococcus aureus)* cultures grown in the leaf extract of *S. cumini* but pulp and seed extracts did not show any antibacterial activity. The results of the extract of the samples are presented in the table 1.

In conclusion, *Syzygium* is widely used by the traditional healers for the treatment of various diseases especially diabetes and related complications. The plant has many important compounds which confer most characteristics of the plant. High radical scavenging activity was observed in all parts of the plant, especially leaves. Further

investigations are needed from HPLC data, to support with specific identifiable fractions of compounds, so that this plant could be exploited as an antioxidant additive or a nutritional supplement.

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