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Research Article

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Phytophenolics and functional group analysis of *Syzygium cumini* L. seeds for antibacterial activity

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Abstract

Indian traditional medicinal systems analysed the different parts of the plant proved to have medicinal properties. Plants have been a source of drugs for human health management. Such plant drugs are considered to be less toxic and free of side effects than synthetic drugs. Jamun species are widely distributed in Alagar Kovil Hills of Madurai district of Tamil Nadu. Such medicinally important *Syzygium cumini* (jamun) seeds were collected from such areas which were selected for the present study to analyse the antibacterial activity of powdered seed extract under laboratory condition.

Plant phenolics screening was carried out for *Syzygium cumini* wild variety and the crude juice of Jamun seed were extracted by using distillation method with various solvents such as acetone, butanol, ethanol, methanol, petroleum ether, and distilled water. Such result showed that Jamun seed extract have flavonoids, alkaloids, phenols, saponins, terpenoid, amino acids, anthraquinone glycosides and tannins. The methanolic seed extract of *S. cumini* have active functional groups –OH, -CH, -C=O, -C=C, -S=O, -C-O, -C-Cl, -C-Br and C-I of various compounds which was analyzed by FTIR. Then the bacterial inhibition activity of the above seed extract was tested on the enteropathogenic bacteria like *Escherichia coli, Enterobacter aerogens, Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis*. The zone of inhibition was measured and it showed that the butanol extract of seed had maximum inhibition activity (1.6±0.2 cm) on all selected pathogens and it was least (1.2±0.1 cm) in acetone extract of seed on such pathogens. Thus, the present work would be applied in traditional system of medicine to analyze the plant phenolics and functional group which is responsible one to control the enteropathogens in human.

Keywords: Jamun, Phytophenolics screening, Enteropathogens, FTIR - functional groups and antibacterial activity.

Introduction

Syzygium cumini (Jamun) is a large ever green tree belongings to the family Myrtaceae and grow up to above 30 meter height which are distributed throughout India up to an altitude of 1800 m (Kumar et al. 2009). According to the World Health Organization, up to 90% of human population in developing countries used plants and its products as traditional medicine for primary health care. It is applied in Ayurvedic and Unani system of medicine having therapeutic properties for curing lot of disorders related to throat, liver and digestive problems, heart ailments and wound healing in human. India is the second largest producer of the jamun seeds in the world and contributes 15.4% in the world production of 13.5 million tonnes. Amongst the Indian states, Maharashtra is the largest jamun producer followed by Uttar Pradesh, Tamil Nadu, Gujarat, Assam and others (Patil et al. 2012). Phytochemicals are naturally occurring in the medicinal plants and have defence property like protection against from various diseases (Krishnaiah et al. 2007).

The significant source of commercial medicines and leading drug derived from natural products. Screening of crude plant extracts pave the way for discovery of novel bioactive compounds and elucidation of their structures can open the door for new synthetic preparations (Colegate and Molyneux 2008). Many investigations have reported the various phytochemicals extracted from the different parts of the plant. The phytochemical analysis of ethanol extract of Jamun stem, bark, leaf, seed and fruit pulp showed the presence of alkaloids, anthroquinone glycosides, flavonoids, tannins, saponins, phenols, cardiac glycosides, terpenoids, phytosterols, steroids and amino acids (Mubassara et al.2015).

FTIR spectroscopic analysis is the powerful technique applied as a tool for analyze functional groups of the phytochemicals in plant samples. The infrared radiation passes through the sample material which is the proportion of absorbed intensity over the total intensity that enters the material is in direct relation to the concentration of absorbing molecules. It is a flexible approach to provide qualitative and quantitative information for the plant sample analysis (Kacurakova et al. 1999). The present study focused on the plant phenolics and functional groups of seed extract of *Syzgium cumini* L. for analysing the antibacterial activity.

Materials and Methods

Sample collection

The fresh fruits of wild variety of *Syzygium cumini* were collected from Eastern Ghats of Alagar hill located near northeast of Madurai (12˚18'N; 76˚ 42'E;altitude 275 m) in Tamil Nadu, India. The collected fruits were washed with tap water, then the fruit pulp was separated and seeds were cleaned thoroughly. They were dried at room temperature for 1-2 weeks and finally crushed as fine powder using pulverizer. The powdered seeds were packed in small glass containers for further analysis.

Preparation of *Syzygium cumini* seed extract

The one gram of *Syzygium cumini* seed powder of wild variety was extracted with using 100 ml of different solvent such as acetone, methanol, ethanol, butanol, petroleum ether and distilled water separately. It was stirred manually and incubated under room temperature for 12 hours. After incubation the suspension was centrifuged at 3000 rpm for 15 minutes. The extracts were separated and analysed.

Antibacterial assay

The seed extract of *Syzygium cumini* was used for checking the antibacterial activity using various bacterial pathogens such as *Escherichia coli*, *Enterobacter aerogens, Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis* by sterilized agar plate method. Sample wells were made on sterilized nutrient agar plates using sterile cork borer with diameter 8 mm. The 0.1 ml of each solvent extract of *Syzygium cumini* seed

was transferred aseptically in each well of agar plate. They were incubated at room temperature for 24 hours. The diameter of inhibition zone was measured for each solvent extracted sample.

Phytophenolics screening of *Syzygium cumini* seed extract

The seed extracts of *Syzygium cumini* were analysed for the presence of phytophenolic constituents such as alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, amino acids and anthraquinone glycosides (Harborne 1998 and Kokate, 2001).

Test for alkaloids (Mayer's Test)

One millilitre of acidic aqueous (methanolic solution acidified with dilute hydrochloric acid) solution of sample was mixed with1-2 drops of Mayer's reagent was added. The formation of white or pale precipitate showed the presence of alkaloids.

Test for Tannins (Lead acetate Test)

A test tube containing 5 ml of sample was mixed with 1-2 drops of 1% solution of lead acetate solution. The formation of bulky white precipitate indicated presence of tannins.

Test for Saponins

A drop of sodium bicarbonate was added in the test tube containing 50 ml extract of the sample. The mixture was vigorously shaken and kept for two minutes. A honey comb like froth was formed and it showed the presence of saponins.

Test for Flavonoids

The test tube containing about 0.5 ml of alcoholic extract of sample, 5-10 drops of diluted Hydrochloric acid and trace amount of Mg or Zn were added. The solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

Test for Phenols

One millilitre of the alcoholic solution of the sample mixed with 2 ml of distilled water which is followed by the addition of few drops of the 10% aqueous solution of ferric chloride. The formation of blue or deep green colour indicated the presence of phenols.

Test for Terpenoids

One milligram of extract was added with 2 ml of 2 ml of chloroform and 5-10 drops of Conc. H_2SO_4. Reddish brown colour was appeared which indicated the presence of terpenoids.

Test for Amino acids

Two milli litre of sample extract was treated with the 1-2 drops of ninhydrin reagent. The appearance of violet or purple colour indicated the presence of amino acids.

Test for Anthraquinone Glycosides (Borntrager's Test)

Five milli litre of sample extract was mixed with 2 ml of dilute $H_2SO_4$and it was boiled for 5 min then filtered. The collected filtrate was mixed with equal volumes of $CHCl_3$. Organic layer was separated and 10% ammonia solution was added. The appearance of brick pink colour of the ammonia layer which confirmed the presence of anthraquinone glycosides.

Functional Group analysis

FTIR (Fourier Transform infrared Spectrometry) was applied for the detection of functional groups in the powdered mass of Jamun seeds.

FTIR sample preparation: The dried seed of *Syzygium cumini* was powdered by using pestle and mortar. Each sample for FTIR analysis was prepared by mixing the fine ground powder of sample with 2% KBr. The FTIR spectra of each variety were recorded in the range of 500 cm^{-1} to 4000 cm^{-1} at a resolution of 400 cm^{-1}. The spectral data of seed sample was measured.

Results and Discussion

The Myrtaceae is a large family which consists of trees and shrubs distributed in the tropic and subtropical regions. It comprised about 150 genera and 3,600 species (Cronquist 1981). Among these *Syzygium cumini* L. is an important plant which is commonly known as Jamun and distributed widely in all over Indian sub-continent. The fruits and seeds of *S. cumini* are used to treat diabetes mellitus for several centuries in folklore medicine of south Asia. In India, Jamun is cultivated for the edible fruits (Black Plum) and reported to contains vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components (Martinez and Del Valle1981).

Syzgium cumini (L) is also known as *Syzgium jambolanum* and *Eugenia cumini*. The original home of *Syzgium cumini* is in India and the East Indies. It is found in Thailand, Philippines, Madagascar, West Indies, East and West Africa and some subtropical regions including Florida, California, Algeria and Israel. The entire part of the plants has been widely used in the treatment of various diseases in the traditional and folk medicine. The seeds are additionally used as an anesthetic in South American cultures. In association to its dietary use, all parts of the tree and, importantly the seeds are used to treat a range of ailments, the most important being diabetes mellitus (Sagrawat et al. 2006).

In the present study, the wild variety of *Syzygium cumini* seeds were selectively collected from the Algarkoil hills of Madurai district. Powdered seed was applied for screening the phytophenolics and analysed the functional groups of seed biomass. Such extract was used for checking the antibacterial activity on selective bacterial species. This approach has been utilized for the discovery of antibacterial agents from natural sources which is based on the evaluation of traditional plant extracts.

The plant components are non-phytotoxicity, biodegradable and stimulatory in nature. Such products possess the potentials and applied in pest management (Dubey et al 2008). A large proportion of world population, especially in the developing countries depends on traditional system of medicine for various diseases. Plant based drugs constitute a major share of medicine in India, China viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy (Vaidya and Devasagayam2007).

In the present study, phytochemical screening was performed with the acetone, methanol, ethanol, butanol, petroleum ether and aqueous extracts of the seed of *Syzgium cumini* collected from Alagarkovil Hills of Madurai district. Methanolic and ethanolic extract of seeds from wild variety contains alkaloids, tannins, phenols, amino acid, tannins and terpenoids. The butanol extract contained alkaloids, tannins, saponins, aminoacids and anthraquinone glycosides and flavonoids, phenols, terpenoids are moderately present. Petroleum ether extract consists of alkaloids, tannins, saponins, aminoacids and anthraquinone glycosides. The aqueous extract (Distilled water) composed of saponins, flavonoids, phenols, terpenoids, amino acids and anthraquinone glycosides but in which alkaloids and tannins are moderately present. The acetone extract have alkaloids, tannins, flavonoids, phenols, terpenoids, amino acids and anthraquinone glycosides (Table: 1). The study reported that presence of phyto chemicals in selective the plant (*Syzygium cumini* seed extracts have been considered as active medicinal chemical constituents which are responsible for curing the different ailments in human by traditionally.

Table: 1Phytophenolics screening of *Syzygium cumini* (Wild variety) seed extract

Phytochemical constiuents	Acetone	Methanol	Ethanol	Butanol	Petroleum ether	Distilled Water
Alkaloids	+	+	+	+	+	++
Tannins	+	++	++	+	+	++
Saponins	-	-	-	+	+	+
Flavonoids	+	+	+	++	-	+
Phenols	++	+	+	++	-	+
Terpenoids	+	+	+	++	-	+
Amino acids	+	+	+	+	+	+
Anthraquinone glycosides	+	-	-	+	+	-

(+Presence , ++ Moderately present, - Absence)

Fourier Transform Infrared Spectrometry (FTIR) could identify the structure of unknown composition or its chemical groups, and the intensity of absorption spectra associated with molecular composition or content of the chemical group (Mc Cann et al. 1992; Surewicz et al. 1993). According to them, the IR spectra from plant samples could detect the minor changes of macromolecule compound, such as carbohydrate, protein, lipid and cell wall pectin. It is more quick and convenient than other techniques for detecting physiological indicators. The entire process of plant growth could be determined with this method require only small quantity of sample.

FTIR would be used extensively in the research on plant physiology due to its virtues of simple and efficient on manipulation. It provided more detailed chemical information on the samples composition because it measured the fundamental vibrations. In such research, it has been used to identify the concrete structure of certain plant secondary metabolites (Ivanova and Singh 2003). In the present study, the presence of functional groups of bio-compounds in methanolic seed extract of *Syzygium cumini* (Table: 2 and Fig:1) was analysed by FTIR. According to the result, the absorption spectrum between 4000 and 400cm^{-1} revealed the presence of various bio compounds in the seed sample of wild variety of *Syzygium cumini*. Strong- broad and weak-broad bands around 3451.38 cm-1 to 3129.29cm-1 represented –OH group of alcohol. The band between 2931.6 cm-1 and 2886.27 cm-1 represented-CH group of alkanes and the peak around 1735.81 cm-1 showed strong C=O stretching of aldehyde. Spectrum region at 1619.13 cm-1 revealed that the presence of C=C stretching of conjugated alkenes have medium di substitute (trans) groups in the seed sample.

The peak at 1460.01 cm-1 showed the medium –CH bending of alkanes which represented the presence of methylene groups. But the region at 1400.01 cm-1 represented the presence of strong -S=O stretching of sulphate in the sample. The band between 1191.93 cm-1 to 1103.21 cm-1 showed the presence of strong –C-O stretching of secondary and tertiary alcohol. The peak at 993.27 cm-1 showed strong more substituted -C=C bending of alkane and 921.91 cm-1 region showed the presence of string -C=C bending of alkene. The absorption spectrum at 862.12 cm-1 and 780.87 cm-1 showed the Strong band with C-Cl stretching of halo compounds. But 710.72 cm-1 peak showed the strong –C=O bending of di substituted (cis) alkane. The region of peak at 604.64 cm-1 showed the presence of strong =C-Br compounds and 525.57 cm-1 region of peak represented the seed extract sample containing halo compounds with strong stretching of –C-I groups.

Such FTIR study for the seed extract of *Syzygium cumini* reported that the presence of various functional groups in bio- active compounds analysed based on the absorption mechanism of compounds at different intensity.

Table: 2 Functional groups analysed by FTIR spectroscopy for the methanolic seed extracts of *Syzygium cumini*.(Wild)

Sl.No	Absorption band/Peak region	Functional group of molecules or compounds
1	525.57	C-I Strong stretching of Halogen compound
2	604.64	C-Br strong stretching of Halogen compound
3	710.72	C=O strong bending of di substituted (cis) alkane
4	780.87	C-Cl strong stretching of halogen compound
5	862.12	C-Cl strong stretching of halogen compound
6	921.91	C=C strong bending of alkene
7	993.27	C=C strong more substituted stretching of alkane
8	1103.21	C-O strong stretching of secondary alcohol
9	1154.32	C-O strong stretching of tertiary alcohol
10	1191.93	C-O strong stretching of secondary alcohol
11	1400.22	S-O strong band stretching of sulfate
12	1460.01	C-H medium bending of alkane-methylated groups
13	1619.13	C=C strong stretching of conjugated alkene have medium di substituted(trans) groups
14	1735.81	-C=O strong stretching of aldehyde
15	2886.27	-CH stretching of alkane
16	2931.6	-CH stretching of alkane
17	3129.29	Weak broad –OH stretching of alcohol
18	3451.38	Strong broad –OH stretching of alcohol

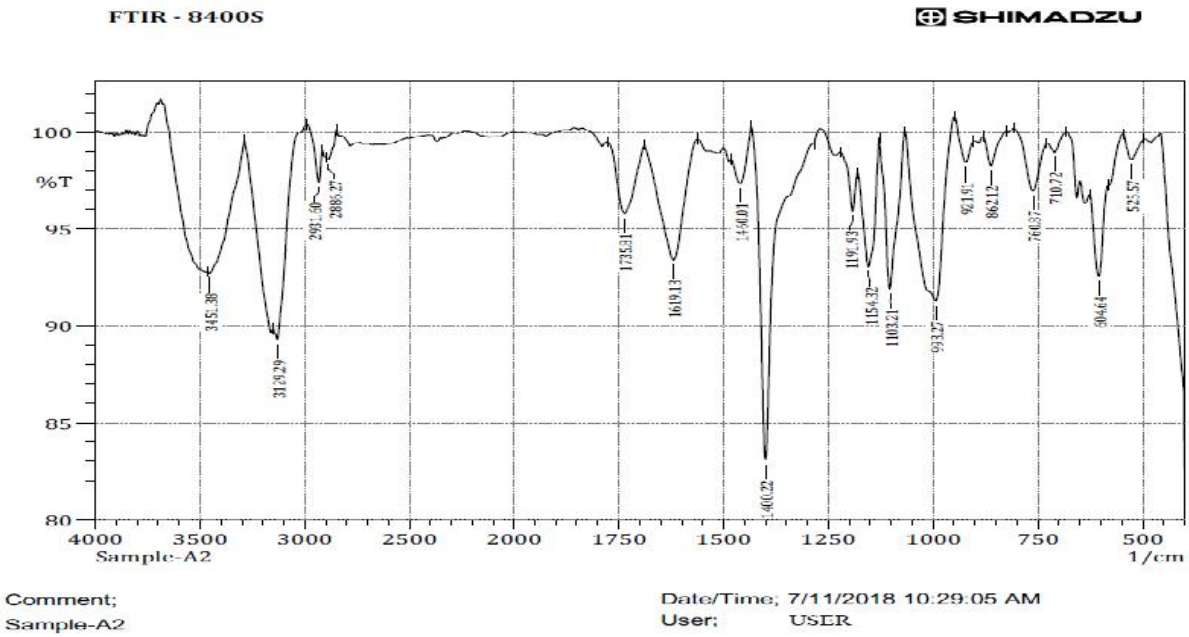


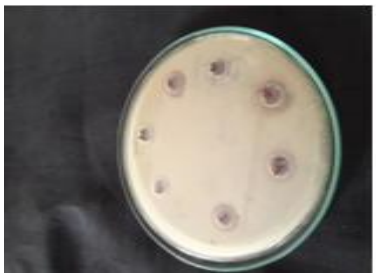
Figure 1: FTIR analysis for the methanolic extract of *Syzygium cumini*.(Wild) seed

The presence of phenolics compound and functional group analysis of the seed extract of *Syzgium cumini* in this study was tested for checking antimicrobial activity on the selective bacterial species. The inhibition activity of seed extract from each solvent on enteropathogenic bacteria (*Escherichia coli, Enterobacter aerogens, Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis*) was measured and tabulated (Table: 3 and Plate:1). The butanol extract of seed had maximum inhibition activity (1.6±0.2 cm) on all selected pathogens and it was least (1.2±0.1 cm) in acetone extract of seed on such pathogens. But petroleum ether and water extract of seed does not show any inhibition effect on pathogens. Thus, it confirmed that the seed extracts by using other organic solvents except petroleum ether and water have the ability to control the growth of enteropathogens. Similar observation with methanolic extracts were reported earlier regarding antibacterial activity of *Eugenia jambolana* against two strains of *Staphylococcus aureus* (Recio 1989). The present study was reported that the different components are extracted from the seeds which had different levels of inhibition against the growth of enterobacterial pathogens.

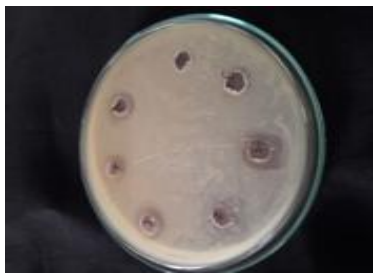
Table: 3Antibacterial efficiency of *Syzygium cumini*, L. Wild seed extract on the selective bacterial enteropathogens

S.no	Solvent	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterococcus faecalis*	*Enterobacter aerogens*
1	Acetone	1.1 cm	1.2cm	1.2cm	1.1cm	1.2cm
2	Methanol	1.5 cm	1.5cm	1.2cm	1.4cm	1.4cm
3	Ethanol	1.3cm	1.4cm	1.3cm	1.5cm	1.4cm
4	Petroleum ether	No effect	No effect	No effect	No effect	No effect
5	Butanol	1.5cm	1.6	1.6	1.6cm	1.5cm
6	Water	No effect	No effect	No effect	No effect	No effect

Enterobacter aerogens



Escherichia coli



Enterococcus faecalis

Klebsiella pneumoniae

Proteus mirabilis

Plate: 1 Antibacterial activity of *Syzygium cumini* seed extract on the selective bacterial enteropathogens

Aziz and Banerjee (2018) studied the phytochemical screening and antibacterial activity of *Syzygium cumini* seed extract with methanol, petroleum ether and ethanol extracts of the seeds of *Syzygium cumini*. Such study reported that the *Syzygium cumini* seeds were rich in alkaloids, tannins, saponins, flavonoids, phenols, terpenoids, steroids and amino acids. The antibacterial activity of methanolic seed extract of Jamun showed potential inhibitory activity over gram negative bacteria- *Salmonella typhi* (19.3mm) and *Escherichia coli* (22.6 mm) and gram positive bacteria-*Bacillus subtilis* (17.2 mm) and *Staphylococcus aureus* (23.6 mm).

The major phytoconstituents were reported to contain vitamin C, gallic acid, tannins, anthocyanins, cyaniding, petunidin, malvidin glucoside and other components (Annon, 1976, Martinez and De Valle 1981). According to Kuncha et al. (2012) the preliminary phytochemical analysis showed the presence of phenols, terpenoids, tannins, saponins, phytosterols, carbohydrates, flavonoids, amino acids in the stem bark of *Syzgium cumini* (L).

The current study supported the usage *Syzygium cumini* seeds which could be applied against multidrug resistant pathogenic bacteria and actively applied as herbal medicine alternative to the antibiotics.

Conclusion

The seeds of wild variety of *Syzygium cumini* have lot of phytophenolic compounds (alkaloids, tannins, phenols, amino acid, tannins and terpenoids) and functional groups (halogen compounds, compounds with aromatic ring, polysaccharide, ester carbonyl, nitrosamine, diketones, aldehyde , alkaline and alcohol groups) which have high potent to control *Escherichia coli, Enterobacter aerogens, Enterococcus faecalis, Klebsiella pneumoniae* and *Proteus mirabilis* under laboratory culture condition. The seed extract of *Syzygium cumini* had high therapeutic value against gram negative pathogenic bacteria. Presence of such significant bioactive compounds that make the plant as a potential antioxidant, anti-diabetic and applied in other therapeutic medicine. This work have suggested that the wild variety of *Syzygium cumini* seeds are more potential one for curing the many ailments in human.

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References

Annon (1976) The Wealth of India: Raw materials, CSIR, New Delhi 10: 100-106

Aziz A, Banerjee S(2018) Phytochemical Screening and Antibacterial Activity study of *Syzygium cumini* (Myrtaceae) Seed Extracts. Pharma Tutor 6(4): 70-73

Colegate S, Molyneux R(2008) An introduction and overview. In *Bioactive Natural Products-Detection, Isolation and Structural Determination* (2nd edition), Eds. Boca Raton: CRC Press, pp 1-9

Cronquist A (1981) An Integrated System of Classification of Flowering Plants. New York: Columbia Univ Pr p 642

Dubey NK, Srivastava B, Kumar A (2008) Current status of plant products as botanical pesticides in storage pest management. Journal of Biopesticides 1(2): 182-186

Harborne JB (1998) Phytochemical methods. A guide diabetes in rats, biological trace element to modern techniques of plant analysis. 3rd edn., chapman and hall int. Ed., New York

Ivanova DG, Singh BR, (2003) Nondestructive FTIR monitoring of leaf senescence and elicitin-induced changes in plant leaves. Biopolymers 72(2):79-85

Kacurakova M, Wellner N, Ebringerova A, Hromadkova Z, Wilson RH, Belton PS(1999) Characterisation of xylan-type polysaccharides and associated cell wall components by FT-IR and FT-Raman Spectroscopies. Food hydrocolloids 13(1):35-41

Kokate CK (2001) Pharmacognosy. 16thedn., Nirali Prakasham, Mumbai, India

Krishnaiah D, Sarbatly, R, Bono, A(2007) Phytochemical antioxidants for health and medicine - a move towards nature. Biotechnology and Molecular Biology Review 1 (4):97-104

Kuncha J, Ayyachami M,Mariapan M(2012) *In vitro* evaluation of nitric oxide scavenging activity of methanolic and aqueous extract of *Syzygium cumini*. International Journal of Pharmaceutical Science and Research 3: 615-619

Martinez SB, Del Valle, MJ (1981) Storage stability and sensory quality of duhat *Syzygium cumini* Linn. anthocyanins as food colorant. UP Home Economic Journal9:1

McCann MC, Hammouri M, Wilson R, Belton P, Roberts K (1992) Fourier transform infrared microspecroscopy is a new way to look at plant cell walls. Plant Physiol 100:1940-1947

Mubassara S, Biswas KK, Hasan MM, Hossain MI, Paul S (2015) *In vitro* phytochemical, antibacterial and antioxidant analyses in different plant parts of *Syzygium cumini*. Int J Pharmacog Phytochem Res7(1):150–155

Patil SS, Thorat RM, Rajasekaran P (2012) Utilization of jamun fruit (*Syzygium cumini*) for the production of red wine. Journal of Advanced Laboratory Research in Biology. 3:234-238

Recio MC(1989) A review of some antimicrobial compounds isolated from medicinal plants reported in the literature. Phytotherapy Research3:1445-1453

Sagrawat H, Mann AS ,Kharya MD (2006) Pharmacological potential of *Eugenia jambolana*: a review. Pharmacog mag2: 96-105

Surewicz WK, Mantsch HH, Chapman, D, (1993) Determination of protein secondary structure by Fourier Transform Infrared Spectroscopy: A critical Assessment. Biochemistry 32(2):389-393

Vaidya ADB, Devasagayam TPA(2007) Current status of herbal drugs in India: An Overview. Journal of Clinical Biochemistry and Nutrition. 41(1): 1-11

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