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Study of phytoconsistuents, nutritional profile, anti-bacterial properties, assay and isolation of phenolic compounds in the extract of pulp, seed, leaves and bark of *Syzygium cumini*

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Abstract : *The present investigation was undertaken to screen methanol extract of leaf, seed, bark and pulp of the plant Syzygium cumini for analysis of phytochemical constituents, anti-diabetic and anti-bacterial property. The study revealed that carbohydrate, protein, phenol, tannin, alkaloid, flavonoid, terpenoid were present in the plant samples whereas steroid and saponin were not detected. The result revealed significant amount of anti-bacterial and anti-diabetic property of the plant extract. The leaf extracts showed maximum anti-bacterial activity against Streptococcus aureus with zone of inhibition 6 mm. The FTIR analysis of the samples and the diabetic drugs revealed presence of some common bioactive functional groups like carboxylic compound. The study provided scientific basis of use of the plant extract as a source of nutrition and potential anti-bacterial and anti-diabetic drug.*

Keywords : *Functional group, Anti-bacterial property, Anti-microbial property, UV-vis-spectrometer, FTIR spectrometer and zone of inhibition.*

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Introduction :

Syzygium cumini commonly called as Black plum or Jamun is an important medicinal plant in various traditional system of medicine belonging to Myrtaceae family. The plant is rich in compound containing alkaloid, flavonoid, terpenoid, carbohydrate, phenol and tannin

It help in controlling diabetics, specifically jamun has an action on pancreas, the main organ responsible for causing diabetics. The fruit, the seed and even the juice of the jamun all play an important role in the treatment of diabetics. The jamun contain a type of glucose called as jamboline, which check the conversion of the starch into sugar in cases of increased production of glucose, the main reason behind the high sugar level. The antibiotic activity of the black berry extract

has been widely studied and found useful against a number of microbial agents (Oliveria et al., 2007). The present study deals with phytochemical analysis, nutritional profile, functional group detection, anti-bacterial, anti-diabetic property of the leaf, seed, pulp and bark of *Syzygium cumini.*

Material and Methods :

The plant sample pulp, seeds, leaves and bark of *Syzygium cumini* collected from the campus of Patna Women's College. The plant samples were washed thoroughly in running tap water and then were dried in Hot in oven (35°C). The methanolic extracts were prepared of each dried samples using method described by Imelda et. al. (2017).

The extracts were subjected to preliminary phytochemical screening tests, described by Irondi et. al. (2013) and Abiodum and Adeleke (2010).

To 3 ml of each extract sample 1 ml Fehling A and Fehling B was added. Appearance of first yellow and then brick red coloration indicate the presence of reducing sugar.

To 3 ml of each extract sample 1ml of alcoholic ferric chloride was added, dark blue coloration of the solution indicated the presence of tannin and phenolic in the sample.

3 ml of methanolic extract of the plant samples were taken separately in the test tube and few pieces of magnesium turning and 1ml of concentrated HCL were added to test tube. Pink red coloration to the solution indicates the presence of flavonoid in the sample.

3 ml of methanolic extract of the plant samples were taken separately in the test tube with wagner's reagent which includes 1.27gm of iodine (I), 2gm of potassium (K) and 100 ml of distill water was added. Reddish-brown precipitate indicated the presence of alkaloid in the sample.

To 3 ml of each methanolic extracts, 3ml of chloroform, 3ml of acetic anhydride and 2 drops of concentrated sulphuric acid were added. No dark green coloration of the solution indicated the absence of steroid in the sample.

To 3 ml of the methanolic extract of each sample, 3ml of chloroform, 3ml of acetic anhydride and 2 drops of concentrated sulphuric acid were added. Red coloration indicated the presence of terpenoid in the sample.

3 ml of each extract of the samples were vigorously shaken with 5ml of distilled water in a test tube, then allowed to strand for a while at a room temperature. No persistent frothing indicates the absence of saponin.

3 ml of extract of each samples were drawn in the test tube and few drops of 2,6-dicholophenol-indophenol was added. The solution turns colorless which indicates the presence of vitamin-c in the sample.

To 3ml of each sample, 2 drops of concentrated HNO_3 was added. The solution was boiled for 1min and then cooled. To this 1 ml of 5% ammonium thiocynate solution was added. Formation of blood red color of the solution indicates the presence of iron in the sample.

To 3ml of each sample, few drops of glacial acetic acid were added. To this solution 3ml saturated ammonium oxalate solution was added. Formation of white precipitate indicates the presence of calcium in the sample.

To 3ml of each sample, 2ml ammonium hydroxide was added. A gelatinous white precipitate indicates the presence of magnesium in the sample.

To 3ml of each sample, 3ml of potassium permanganate solution was added. A dark brown colour indicates the presence of manganese in the sample.

To 3ml of each sample, 3ml of potassium ferrocyanid was added. A cream precipitate indicates the presence of zinc in the sample.

To estimate the phytoconstituents which is one of the necessary steps to find out the chemical consistuents which lead to the isolation of the compound for the biologically active secondary metabolite such as alkaloid, flavonoid, steroid, terpenoid, saponin, tannin and carbohydrate. Phytochemical screening revealed the presence of terpenoid, steroid and tannin in the plant extract.

UV-vis-spectroscopy is an analytical method used to measure the absorbance of ultra-violet or visible radiation through an analayte. It is based on the Lambert-beer principle which states that the absorbance of a solution is directly proportional to its concentration when the wavelength of the incidence light remains fixed.

Estimation of total soluble carbohydrate was done by Anthrone method described by Kayano et. al. (2011). Absorbance of the samples were taken at 620 nm.

100 ug/ml of quercetin standard and appropriately diluted sample in 80% ethanol were taken in different test tubes and made up to 2ml with 95% ethanol followed y the addition of 0.1 ml of 10 % aluminium chloride. 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water and incubated in room temperature (30-34 ^{0}C) for 30 minute (Peolozo et al., 2010). The intensity of color developed was read at 415 nm against reagent blank.

5ml amount of the extract was taken and the pH was maintained at 2.0-2.5 with dilute HCL. A 2ml amount DR (solution of 4.0 gmof potassium iodide in 10ml of distilled water), was added to it, and the precipitate formed was centrifuged at 2500 rpm for 10 minutes. Afifi et al. (2012) were followed to estimate the absorbance at 435 nm.

Total phenolic composition using Folins-Ciocalteu reagent and expressed as gallic acid equivalent were estimated by the method described by Afifi et. al. (2012). The absorbance of the sample of the taken at 760 nm.

It is suggested that the anti-microbial activity is mainly due to the presence of alkaloid, tannin and other phenolic compound or due to free hydroxyl groups. Chromatography is a technique used to separate mixtures of substance into their components. Different components travel at different rates. Wagner et al. (2009) were followed to the mobile phase for thin layer chromatography contains a substance which is detected through the $Fecl_3$- $K_3Fe(CN)_6$ spray reveal all the phenolic compounds as a Blue spot.

Silica gel and small amount of calcium sulphate was mixed with water. This mixture was spread on glass plate, it is activated at 110 ^{0}C. A loading point was made at about 2.0 cm from the lower surface of the plate. Typical solvents are butanol, acetic acid and water in 14:1:15. The relative position or R_f values of phenolic spots can be used as first indication of phenolic compounds.

FTIR spectroscopy was used to detect the presence of different functional groups.

Pure culture of bacterial strain *Staphylococcus aureus* was obtained from the Microbiology department, PMCH, Patna. The strain used for the study was sub-cultured in the laboratory of the department of Microbiology Patna women's college, Patna. Esteban et al. (2005) were followed for anti-bacterial assay through disc-diffusion method. Nutrient agar plates was prepared and incubated by spread plate method. The test was performed in triplicate for plant sample. Tetracycline antibiotic drugs were used as a control agent.

Results and Discussion :

The sample subjected to the different tests reveals it property as a crude drug. Results were tabulated.

Table 1. Preliminary screening for the phytocompounds

Test	Pulp	Bark	Seed	Leaf
Carbohydrate	+	+	+	+
Tannin and Phenol	+	+	+	+
Zinc	+	+	+	+
Flavanoid	+	+	+	+
Alkaoid	+	+	+	+
Terpenoid	+	+	+	+
Steroid	-	-	-	-
Saponin	-	-	-	-
Vitamin-c	+	+	+	+
Iron	+	+	+	+
Calicum	+	+	+	+
Magnesium	+	+	+	+
Manganese	+	+	+	+

(+) Positive; (-) Negative

In Table 1 the methanolic extract of pulp, seed, leaf and bark showed the presence of carbohydrate, tannin, phenol, zinc, flavanoid, alkaloid, terpenoid, vitamin c, iron, calcium, magnesium and manganese were detected while steroid and saponin were absent in *Syzygium cumini*.

Table 2. Estimation of phytoconstituents by UV-vis-spectophotometer

Phytoconstituents	Wavelength	Absorbance				Concentration (ug/ml)			
		Pulp	Leaf	Seed	Bark	Pulp	Leaf	Seed	Bark
Carbohydrate	630 nm	0.21	0.47	0.32	0.18	3.0 ug/ml	4.5ug/ml	3.5ug/ml	1.2 ug/ml
Flavanoid	415 nm	0.37	0.12	0.30	0.21	2.0ug/ml	1.2ug/ml	1.6ug/ml	1.8 ug/ml
Alkaloid	435 nm	0.15	0.18	0.16	0.19	2.0ug/ml	2.5ug/ml	2.4 ug/ml	2.7 ug/ml
Phenol	760 nm	0.19	0.23	0.21	0.20	2.0ug/ml	2.7 ug/ml	2.5 ug/ml	2.4 ug/ml

In Table 2 the methanolic extract of pulp, seed, bark and leaf were subjected to a particular wavelength to estimate the value of phytochemical in the plant extract. It provided accurate value of phytoconstituents present in the plant extract of the plant sample carbohydrate (630 nm) was highest in the seed, flavonoid (415 nm) was highest in pulp, alkaloid (435 nm) was highest in bark and phenol (760 nm) was highest in leaf.

Table 3. Assay and isolation of phenolic compound silica gel TLC

Plant extract	R_f value	Phenolic compound
Pulp	0.83	Delphinidin
Leaf	0.95	Quercetin
Seed	0.93	Gallic acid
Bark	0.93	Gallic acid

In Table 3 shows through TLC technique, Isolation of phenolic content from the plant extract such as pulp, leaf, seed and bark take place in the silica plate. By comparing $R_f$value with the standard phenolic value, specific phenol present in the plant extract were isolated i.e delphinidin, quercetin and gallic acids in different plant extract. These compounds impact resistance and tolerance to plants against invasion by various micro-organisms. According to literature the anti-bacterial property of leaf, seed, bark and pulp is due to presence of tannin and other phenolic constituent. Agarwal et al. (1998) showed similar results.

Table 4. FTIR absorption of plant extract

Peak value of methanolic extract	Functional group
332294	Alcoholic/ Phenolic group (-OH)
294474,283304	Carboxylic group (-COOH)
165874	Amide group (-C=O)

In Table 4 shows alcoholic / phenolic group (-OH), carboxylic group (-COOH) and amide (-C=O) were detected by FTIR in the plant extract which might play role in treatment of diabetes. According to the literature *Syzygium cumini* result in significant role in decreasing the blood sugar, Villasenor et al. (2006) have shown the treatment of diabetic mellitus.

Table 5. FTIR absorption of the drugs

Peak value of the drug	Functional group
2928	C-H,COOH (alkane, carboxylic acid)
1550	NO_2 (Nitro)
1445	NO_2 (Nitro)
1413	NO_2(Nitro)
1058	CO (Carbonyl)
1036	CO(Carbonyl)
936	C=C(alkane)
734	C= C (alkyne)

In Table 5 shows the presence of carboxylic acid, nitro, carbonyl, alkene, alkyne and alkane as a functional group in the plant extract, which were compared to functional group of plant extract and it has been observed that both the sample is consists of carboxylic acid (-COOH) which might play role it has in treating diabetes. On the basis of FTIR analysis it is been suggested that the plant sample can be used as anti-diabetic drugs although further research is required.

Table 6. Antibacterial activity of *Syzygium cumini*

Sample	Control	*Staphylococcus aureus* (Zone of inhibition (mm))
Bark	0	4.0mm
Leaf	0	6.0mm
Pulp	0	2.0mm
Seed	0	4.5mm

In Table 6 the methanolic extract of *Syzygium cumini* such as pulp, leaf, bark and seed showed an extensive anti-bacterial activity against *Staphylococcus aureus.* Leaf extract of *Syzygium cumini* showed maximum zone of inhibition(6.0mm) followed by seed(4.5mm), bark(4.0mm) and pulp (2.0mm). According to Aymann et al. (2013) the high property of the plant extract were easily observed by calculating its zone of inhibition against *Staphylococcus aureus*, therefore pulp, seed, leaf and bark can be used as efficient against bacteria. They suggested that the activation of the phenolic pathway is known to be the part of defence response against phytopathogen microbes.

Conclusion :

In the present study the phytochemical screening, nutritional profile, anti-bacterial, assay and isolation of the phenolic compound in the pulp, seed, leaf and bark of *Syzygium cumini*. The extract of plant is rich in flavonoid, alkaloid, tannin, phenol, carbohydrate, terpenoid, calcium, vitamin c, magnesium, manganese, iron and zinc, the various phytoconstituents were detected are known to have beneficial importance in medicinal sciences. Flavanoid, alkaloid and tannin shows anti-bacterial property while calcium, vitamin c, magnesium, manganese , zinc and iron are important nutrition which is necessary for the metabolic activity. Isolation of phenolic content by silica gel TLC technique as it is suggested that the anti-bacterial property is mainly due to tannin, alkaloid and other phenolic compound, the R_f value of the plant extract were compared with the standard phenol value to detect the phenolic group present in the plant extract. According to the literature *Syzygium cumini* has anti-diabetic property, the functional group of the plant extract were detected by the FTIR which further compared with functional group of anti-diabetic drugs. As from the FTIR both the sample consists –COOH functional group which might cure the diabetes. The FTIR analysis suggests that the plant sample can be used as anti-diabetic drugs although further research is required

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