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Isolation and Identification of Tannins from *Syzygiumcumini (Linn.)* Bark and Investigation of its Antimicrobial Properties

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A B S T R A C T

The bark of Syzygiumcumini was extracted using aqueous acetone, yielding 10% tannins. The isolated compound was identified as closely resembling tannic acid through organoleptic evaluation, chemical tests, thin-layer chromatography (TLC), and UV-IR spectroscopy, with spectroscopic peaks consistent with those of standard tannic acid. The antimicrobial activity of the isolated compound was evaluated using the agar disc diffusion method against Escherichia coli and Candida albicans. It exhibited inhibition zones ranging from 19.0 mm to 30.3 mm for E. coli and 18.6 mm to 30.3 mm for Candida albicans. A protein precipitation assay using albumin confirmed the presence of tannins, with opaque precipitate discs measuring 23 mm at 10 mg/ml and 40 mm at 100 mg/ml. The total tannin content was determined to be 75.1 mg/g using the Folin-Denis method, while protein concentration, measured by the Lowry assay, was 140.8 mg/ml. These results confirm the successful isolation and characterization of tannic acid from Syzygiumcumini bark, highlighting its antimicrobial potential and strong protein-precipitating properties. This study emphasizes the potential of Syzygiumcumini tannins for applications requiring antimicrobial and astringent activity.

Keywords: Syzygiumcumini, Tannic acid, Antimicrobial activity, Isolation, Spectroscopic analysis

1. Introduction

Plants are rich sources of bioactive chemicals with uses in food, medicine, and cosmetics, making them essential to human health and industry. Plant-based chemicals have been used for their medicinal properties, industrial applications, and culinary uses since ancient times, demonstrating the extraordinary adaptability of botanical resources. These plant-derived constituents are valuable because they may be extracted from different plant parts, such as bark, roots, leaves, flowers, fruits, and seeds. Each of these sections may contain different active ingredients that are useful for different purposes. [1]

The medicinal qualities of plants are primarily due to their secondary metabolites, a diverse group of compounds synthesized through pathways distinct to each species or family, thereby conferring unique therapeutic and

protective roles. These secondary metabolites include tannins, alkaloids, flavonoids, saponins, phenolics, and terpenes, among others, each playing a role in the plant's defense against environmental challenges like microbial pathogens, pests, and climatic stresses, such as drought and salinity. [2]

In addition to protective functions for plants, many secondary metabolites offer substantial health benefits to humans. For instance, tannins are well-known for their antioxidant and astringent properties, which make them valuable in skin care and wound healing applications [3]. The presence of these compounds in fruits, vegetables, and herbs highlights the significance of plant-based diets in promoting health and preventing disease.

Plant secondary metabolites are widely used in modern medicine, cosmetics, and the food industry for their health-promoting properties. *Syzygiumcumini* (Jamun), in particular, has gained recognition due to its rich tannin content in the bark, making it a promising source of natural antioxidants and antimicrobials. The plant is known to contain bioactive compounds like vitamin C, alkaloids, saponins, gallic acid, anthocyanins, glucosides, and other phenolic compounds, with nearly all parts—leaves, seeds, fruit pulp, kernels, and bark—exhibiting therapeutic benefits. [4,5] The isolation and detailed characterization of tannins from *Syzygiumcumini* bark could, therefore, provide valuable insights into its potential applications in pharmacognosy and the development of natural therapeutic agents.

2. Materials and Methods [6]

2.1 Isolation of tannin:

About 60 g of dried and powdered bark was defatted with petroleum ether using Soxhlet for 24 hours at 20 -80' C. The defatted bark was then extracted with aqueous acetone (70% acetone) for 60 min at 60 °C in a water bath with constant stirring. The mixture was filtered and centrifuged at 3000 rpm for 10 minutes. The supernatant was allowed to evaporate at room temperature. The acetone-free extract was kept for dry at room temperature The resulting powder was collected and weighed. Percentage yield was calculated. The powder was stored in sterile bottles at 4 °C in a refrigerator for further studies.

2.2 Identification of Isolated compound.

A. Chemical test

I. **Ferric Chloride test:** Extracts were treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

II. **Gelatin test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

III. **Lead-acetate test:** Test solution was treated with 10% lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

B. Thin layer chromatography.[7]

Various solvent systems were tried to develop a TLC system for identification of constituents. The one showing maximum separation was selected as mobile phase for study.

Sample preparation: 1mg of isolated compound were dissolved in 1ml of water in Eppendorf tube.

Solvent System: Toluene: Acetone: Formic acid. (6:6:1)

Method: The precoated TLC plates were kept for activation at 105ºC in hot air oven for 20 minutes before use and also mobile phase was prepared and kept for chamber saturation. Sample solutions were applied in form of spots on activated TLC plates using capillary tubes. The plates were, air dried and developed in TLC chamber. After development, the spots were reported. Then $R_{f\ value}$ was calculated using the formula.

$$\mathbf{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

The developed TLC plates were observed in UV at 254 nm and 366 nm.

C. Melting point determination [8]

To determine the melting point of a substance using a digital melting point apparatus, the sample was first ensured to be dry and finely powdered. A capillary tube was filled with about 2-3 mm of the sample by pressing the tube into the powder and tapping it to pack the sample tightly. The digital melting point apparatus was turned on, allowing it to calibrate if necessary, and a slow heating rate of 1-2°C per minute was set. The capillary tube was inserted into the apparatus, ensuring it was properly positioned and visible through the viewing window. Heating was started, and the temperature display was monitored, noting the temperature at which the sample first began to melt and when it was completely melted.

D. UV-Visible Spectrophotometry. [9,10]

UV-Visible spectroscopy was conducted to determine the Absorption maxima (λ-max) by preparing a solution of the isolated compound in water at a concentration of 10 µg/mL, within the Beer-Lambert range of 2-20 µg/mL. The solution was scanned over a wavelength range of 200-400 nm using a UV-Visible spectrophotometer version 1.02 (Shimadzu UV-1900i). The λ-max was recorded to provide preliminary structural insights. Tannins typically show absorption maxima in the range of 204-284 nm, and if the compound exhibits absorbance within this range, it indicates the possible presence of tannins.

E. FTIR Spectroscopy. [9,10]

For FTIR identification, the sample was prepared using the KBr pellet method. A small amount of the isolated compound (1-2 mg) was finely ground with spectroscopic-grade potassium bromide (KBr) in a 1:100 ratio to ensure proper dilution. The mixture was ground thoroughly in an agate mortar and pestle to achieve a homogenous blend. This mixture was then pressed under high pressure (around 10 tons) to form a thin, transparent KBr pellet. The pellet was placed in the sample holder of the FTIR spectrometer, and the spectrum was recorded in the range of 4000-400 cm^{-1}. Key absorption peaks were analysed to identify functional groups, with particular attention to hydroxyl (-OH) groups (3200-3600 cm^{-1}), carbonyl (C=O) groups (1600-1700 cm^{-1}), and aromatic rings (1450-1600 cm^{-1}), which could suggest the presence of tannins. The results were compared to reference spectra for confirmation.

F. Hagerman's radial diffusion method [11]

"The Hagerman's radial diffusion method offered a reliable means to identify tannins.

• **Gel Preparation:** A solution containing 50 mM acetic acid and 60 mM ascorbic acid was prepared, and the pH was adjusted to 5 by adding sodium acetate. 1% agarose (type I) was dissolved in the prepared solution. The mixture was heated while stirring until it reached the boiling point, ensuring complete homogenization. The mixture was then allowed to cool to approximately 45 °C.

• **Gel Casting and Sample Loading:** Fatty acid-free bovine serum albumin (BSA) fraction V was added to the agarose solution at a concentration of 0.1%. 50 ml aliquots of the agarose-BSA mixture were poured into petri dishes placed on a level surface to form uniform layers. After the gel completely solidified, wells spaced 2 cm apart were created on the gel surface. Approximately 100 µl of the tannin extract was added directly into the wells. The petri dishes were sealed with parafilm to prevent evaporation.

• **Incubation:** The sealed petri dishes were incubated at 30 °C for 120 hours to allow for the reaction between tannins and albumin.

2.3 Estimation of total tannin content. [12]

Reagents

1) Folin-Denis reagent
2) Sodium Carbonate solution- Dissolve sodium carbonate (35g) in distilled water (upto 100 ml. Allow to stand overnight and filter through glass wool.
3) Working Standard solution of tannic acid.

Procedure:

- 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of the working standard solution of tannic acid were taken in separate test tubes. To each test tube, 0.5 ml of Folin-Denis reagent and 1 ml of sodium carbonate were added.
- The volume of each test tube was made up to 10 ml, forming a blue-colored solution.
- The absorbance was measured at 700 nm within 30 minutes, against a blank preparation made in a similar manner without the tannic acid.

2.4 To Estimate protein by lowry assay method.[12]

Reagent A: (2 g sodium potassium tartrate and 100 g sodium carbonate were dissolved in 500 ml 1N NaOH, and the volume was made up to one Liter with water),
Reagent B: (2 g sodium potassium tartrate and 1 g copper sulphate were dissolved in 90 ml water, then 10 ml 1N NaOH was added).
Reagent C: (1 ml Folin-Ciocalteu reagent was diluted with 15 ml water).

Procedure:

The concentration of standard protein (Bovine serum albumin) was prepared between 30 to 150 µg/ml.

1.0 ml of each dilution of standard, sample, or buffer (for the reference) was taken in separate test tubes and mixed with 0.90 ml of reagent A.

The tubes were incubated for 10 minutes in a water bath at 50°C and then cooled to room temperature. 0.1 ml of reagent B was added to each tube, mixed, and incubated for 10 minutes at room temperature.

Rapidly, 3 ml of reagent C was added to each tube, mixed, and incubated for 10 minutes in a 50°C water bath before cooling to room temperature.

The final assay volume was 5 ml, and the absorbance was measured at 650 nm. A standard calibration curve of the concentration of standard versus absorbance was plotted.

The same procedure was repeated for the sample, and the concentration of the sample was determined from the standard calibration curve.

2.5 Evaluation of Antimicrobial Activity by Agar Well Diffusion Method [13]

Microorganisms Used:

Bacteria: *Escherichia coli* (ATCC 8739), procured from DTL, Bengaluru.

Fungi: *Candida albicans* (ATCC 10231), procured from DTL, Bengaluru.

Chemicals and Apparatus

Antibacterial standard: Amoxicillin (from HI Media Laboratories)

Antifungal standard: Miconazole (from Karnataka Antibiotics & Pharmaceuticals Ltd)
Other reagents: Distilled water, 5% DMSO, Ethanol, Alcohol, Nutrient agar, Sabouraud dextrose agar
Apparatus: Petri dishes, conical flask, glass rod, inoculation loop, sterile cork borer, cotton, ruler/scale

Instruments: Autoclave, incubator, laminar airflow cabinet, hot air oven

Preparation of Standards and Samples

Standards: Amoxicillin and Miconazole were dissolved in 5% DMSO to prepare concentrations of **10, 20, 30, 40, and 50 µg/ml**.

Samples: Ethanolic extract, water extract, and the isolated compound were prepared in concentrations of **250, 500, 750, and 1000 µg/ml**.

Procedure: Nutrient agar (for antibacterial testing) and Sabouraud dextrose agar (for antifungal testing) were prepared using distilled water and sterilized in an autoclave at 121°C for 1 hour. Petri plates were thoroughly washed and sterilized in a hot air oven at 160°C for 1.5 hours. Sterile molten agar (50 ml) was poured aseptically into the plates and allowed to solidify at room temperature. Wells were made using a sterile cork borer, and different concentrations of the isolated compound (250, 500, 750, and 1000 µg/ml) were added to the respective wells, with 5% DMSO as the negative control. Standards—Amoxicillin for bacteria and Miconazole for fungi—were prepared in 5% DMSO at concentrations of 10, 20, 30, 40, and 50 µg/ml and added to separate wells. The plates were incubated at 37°C for 24 hours in a BOD incubator. After incubation, the zones of inhibition were measured in millimetres using a ruler, and the average values from triplicate experiments were recorded.

3. RESULTS & DISCUSSION

3.1 Isolation of tannin:

The percentage yield of the aqueous acetone extract of the bark of *Syzygiumcumini* was found to be 10 %.

3.2 Identification of Isolated compound.

Table 1: Identification of Isolated compound.

CHARACTERS		**ISOLATED**	**TANNIC ACID**
ORGANOLEPTIC CHARACTERS	**Color**	Dark brown	Light brown or yellowish
	Odor	Characteristic, mild	Slightly astringent
	Taste	Astringent	Strongly astringent
	Texture	Crystalline or fine powder	Fine powder
	Solubility	Soluble in water and alcohol	Soluble in water and alcohol
CHEMICAL TEST	**Ferric Chloride Test**	Positive	Positive
	Gelatin Test	Positive	Positive
	Lead Acetate Test	Positive	Positive
MELTING POINT		204.7°C.	218°C
UV SPECTROSCOPY (λmax)		279 nm (λ1) and 252 nm (λ2).	250-280 nm
IR SPECTROSCOPY	**Types of Vibration**	**Observed Peak**	**Frequency Range**
	OH Stretching	3208	3500-3050
	C=O Stretching	1628	1740; 1625; 1720
	0H Bending	1603	1625
	Ring Skeletal Vibration	1537; 1444	1540; 1440
	Ester groups	1314; 1331 1198	1370-1300 1250-1150
	OH Deformation	1097; 1024	1080; 1030

All these results were conducted and compared with standard tannic acid, indicating that the isolated compound closely resembles tannic acid.

Figure 1: Ultraviolet spectrum of Isolated compound

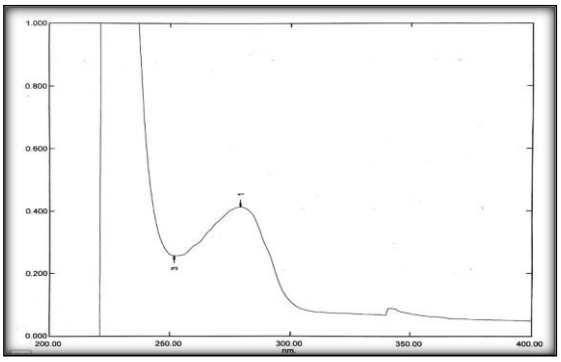
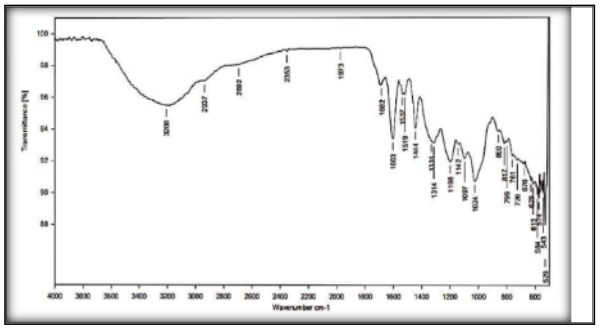


Figure 2: FTIR spectroscopy



3.3 Thin layer chromatography of standard and isolated compound

Optimization of the mobile phase was conducted, and it was found that the Toluene: acetone: formic acid (6:6:1) provided better separation than another mobile phase.

TLC confirmed the presence of tannic acid in isolated compound of *Syzygiumcumini*. The isolated compound has displayed a spot with a similar Rf value.

Figure 3: TLC of Standard and Isolated compound

[T-Tannic acid, I-Isolated compound]

In the chromatographic analysis, the tannic acid standard exhibited an Rf value of 0.18, while the isolated compound showed a Rf value of 0.17.

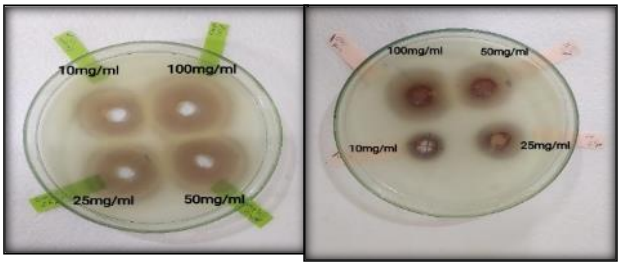
3.4 Assessment of Tannin concentration by Hagerman's radial diffusion method

The Hagerman's radial diffusion method utilizes a protein precipitation test with albumin to visually assess tannin concentration.

After incubation of tannin extracts and tannic acid standards with albumin in the gel wells, opaque precipitate discs were visibly formed. The diameter of the precipitate discs was measured using a ruler.

Table 2: Zone of Precipitation

Extracts	Concentration (mg/ml)	Zone of Precipitation (mm)			Average (mm)
		Trail 1	Trail 2	Trail 3	
Isolated Compound	10	23	23	23	23
	25	30	31	31	30
	50	34	34	35	34
	100	41	40	40	40
Tannic acid standard	10	34	35	34	34
	25	36	35	36	35
	50	38	39	39	34
	100	42	41	42	41



Tannic acid **Isolated compound**

Figure 4: Photographs of Zone of precipitation

The protein precipitation test performed on the stem bark of *Syzygiumcumini*, an opaque precipitate formed as a ring around the wells treated with both the extract and tannic acid standard, confirming the presence of tannins. The ability of tannins to precipitate proteins is due to their polymeric nature, which allows them to cross-link with fibrous proteins. This observation highlights the astringent nature of the tested samples.

The isolated compound exhibited consistent protein precipitation across different concentrations, with average zones of 23 mm at 10 mg/ml, 30 mm at 25 mg/ml, 34 mm at 50 mg/ml, and 40 mm at 100 mg/ml. The tannic acid standard showed slightly higher precipitation, with averages of 34 mm at 10 mg/ml, 35 mm at 25 mg/ml, 38 mm at 50 mg/ml, and 41 mm at 100 mg/ml. The results indicate that the isolated compound from *Syzygiumcumini* stem bark has significant tannin-like properties, although its protein precipitation efficiency is slightly lower than that of the tannic acid standard.

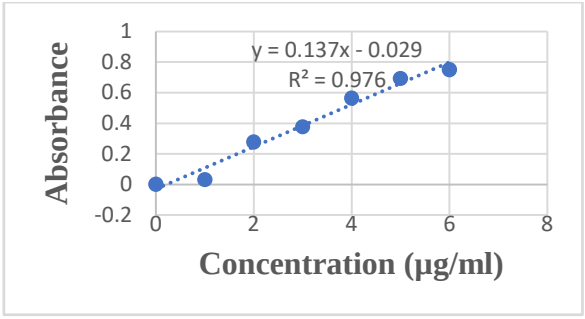
3.5 Estimation of total tannin content by Folin-Denis method

Tannic acid was used as a standard and the total tannin content were expressed as tannic acid equivalents (TAE). Absorbance was measured using a spectrophotometer at 700 nm.

Table 3: Absorbance of Standard Tannic acid and extracts for estimation of Total Tannin content.

Sl/ no	Tannic acid (std) (ml)	Concentration	Folin Denis (ml)	Sodium Bi carbonate (ml)	Water (ml)	Absorbance (700nm)
1	0	0µg	0.5	1	8.5	0.000
2	0.1	1µg	0.5	1	8.4	0.031
3	0.2	2µg	0.5	1	8.3	0.277
4	0.3	3µg	0.5	1	8.2	0.376
5	0.4	4µg	0.5	1	8.1	0.563
6	0.5	5µg	0.5	1	8.0	0.692
7	0.6	6µg	0.5	1	7.9	0.750
8	Sample	3µg	0.5	1	8.2	0.473

Graph 2: Standard curve of Tannic acid for the estimation of Total Tannin Content



T=CV/M

T= Total tannin content in terms of mg/g of plant extract

C= Concentration of tannin acid from the calibration curve

V= Volume of the extract in ml

M= Weight of the extract in grams

Table 4: Total Tannin content

Sl. No	**Extracts (3 µg/ml)**	**Tannin content -Tannic acid equivalent (mg/g)**
1.	Sample	75.1 mg /g

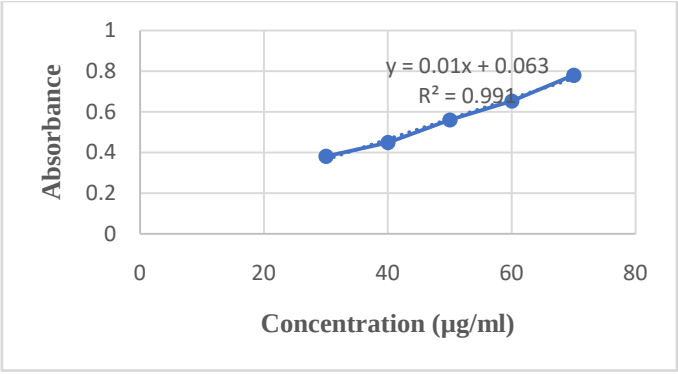
3.6 ESTIMATION OF PROTEINS BY LOWRY ASSAY

The protein concentrations were determined using the Lowry assay method, in which Bovine Serum Albumin was used as standard.

Table 5: Estimation of Protein by lowry assay

Sl.No	**Bovine serum Albumin (ml)**	**Reagent (A) (ml)**	**Reagent (B) (ml)**	**Reagent (C) (ml)**	**Concentration (µg/ ml)**	**Absorbance (650nm)**
1	1	0.9	0.1	3	30	0.381
2	1	0.9	0.1	3	40	0.449
3	1	0.9	0.1	3	50	0.560
4	1	0.9	0.1	3	60	0.653
5	1	0.9	0.1	3	70	0.780
6	Sample 1 ml	0.9	0.1	3	30	0.486

Graph 3: Standard curve of Bovine serum albumin for the Estimation of Protein content



T=CV/M

T= Total Protein content in terms of mg/g of plant extract

C= Concentration of tannin acid from the calibration curve

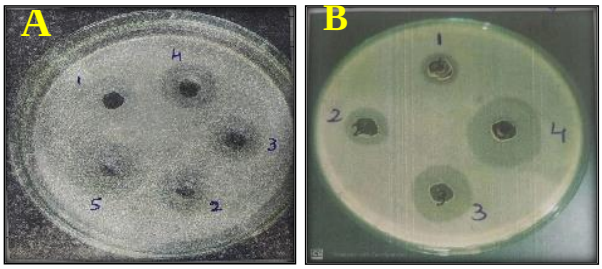
V= Volume of the extract in ml

M= Weight of the extract in grams

Table 5: Protein content of different extracts of *Syzygiumcumini*

Sl. No	Extracts	Protein content (mg/ml)
1.	Sample	140.8 mg/ml

3.7 Evaluation of antimicrobial activity Activity on bacteria i.e., e. Coli



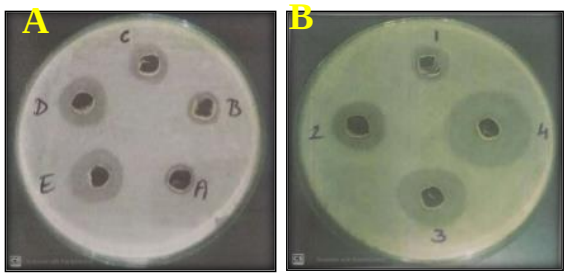
A-Amoxicillin [1-10µg/ml, 2-20µg/ml, 3-30µg/ml, 4-40µg/ml, 5-50µg/ml.]

B-Sample [1-250µg/ml, 2-500µg/ml, 3-750µg/ml, 4-1000µg/ml.]

Table 6: Zone of Inhibition against *E. coli*

STD/TEST	CONC (µg/ml)	ZONE OF INHIBITION (mm)			
		Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Average (mm)
AMOXICILLIN	10	12	10	11	11.1±1.0
	20	17	16	17	16.6±0.5
	30	20	20	21	20.3±0.5
	40	25	26	26	25.6±0.5
	50	33	32	32	32.3±0.5
SAMPLE	250	20	19	18	19.0±1.0
	500	23	24	24	23.6±0.5
	750	27	26	27	26.6±0.5
	1000	30	31	30	30.3±0.5

Activity on fungal i.e., *candida albicans*



A-Miconazole [1-10µg/ml, 2-20µg/ml, 3-30µg/ml, 4-40µg/ml, 5-50µg/ml.]

B-Sample [1-250µg/ml, 2-500µg/ml, 3-750µg/ml, 4-1000µg/ml.]

Table 7: Zone of Inhibition against *Candida Albicans*

STD/TEST	**CONC (µg/ml)**	**ZONE OF INHIBITION (mm)**			
		Trial 1(mm)	**Trial 2(mm)**	**Trial 3 (mm)**	**Average (mm)**
MICONAZOLE	10	12	12	13	12.3±0.5
	20	16	16	17	16.3±0.5
	30	18	19	20	19.0±1.0
	40	24	23	24	23.6±0.5
	50	29	30	29	29.3±0.5
SAMPLE	250	19	20	17	18.6±1.5
	500	22	23	23	22.6±0.5
	750	26	26	28	26.6±1.1
	1000	31	30	30	30.3±0.5

4. Conclusion

The isolation of tannins from the bark of *Syzygiumcumini* yielded a 10% aqueous acetone extract, confirming a significant presence of these bioactive compounds. The isolated compound exhibited characteristics closely resembling tannic acid, including organoleptic properties, positive reactions in Ferric Chloride, Gelatin, and Lead Acetate tests, and matching melting point, UV, FTIR, and TLC profiles with standard tannic acid.

The compound demonstrated strong astringent activity through Hagerman's radial diffusion method, although with slightly lower precipitation efficiency compared to the tannic acid standard. The quantified total tannin content of 75.1 mg/g highlights the extract's potential for various applications, particularly in formulations requiring astringent or protein-interactive properties.

The Lowry assay confirmed a high protein concentration of 140.8 mg/ml, further supporting its applicability in protein-based formulations. Additionally, the isolated tannins exhibited notable antimicrobial activity, with inhibition zones against *E. coli* and *Candida albicans* comparable to standard antibiotics, reinforcing their antibacterial and antifungal potential.

These findings validate the pharmacological significance of *Syzygiumcumini* tannins, supporting their traditional medicinal use and highlighting their potential for drug formulation and development. Further research is warranted to explore the compound's therapeutic applications and optimize its use in modern medicine.

5. References:

1. Cragg GM, Newman DJ. Natural product drug discovery in the next millennium. Pharmaceutical biology. 2001 Jan 1;39(sup1):8-17.
2. Wink M, editor. Biochemistry of plant secondary metabolism. CRC Press; 1999 Sep 21.
3. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The scientific world journal. 2013;2013(1):162750.

4. Suradkar NG, Pawar VS, Shere DM. Detection of Phytochemicals in *Jamun (Syzygiumcumini L.)* Pulp Powder. *J Env Bio-Sci*. 2015;29(1):197-199.
5. Akhila H, Hiremath US. Physico-Chemical Properties of *Jamun* fruits and Its Processed Products. *Int J Pure App Biosci*. 2018;6(6):1317-1325.
6. Helen LR, Jyothilakshmi M, Latha MS. Isolation and quantification of tannins from the root bark of *clerodendruminfortunatumlinn*. and assessment of their antioxidant potential and antiproliferative effect on hct-15 cells. Int J Pharm Pharm Sci. 2015;7(10):170-5.
7. Tripathi AK, Kohli S. Pharmacognostical standardization and antidiabetic activity of *Syzygiumcumini (Linn.)* barks (Myrtaceae) on streptozotocin-induced diabetic rats. Journal of Complementary and Integrative Medicine. 2014 Jun 1;11(2):71-81.
8. Young JC. True melting point determination. Chem Educator. 2013;18:203-8.
9. Sari Y. Tannin determination in young coconut coir *(Cocos nucifera L.)* by FTIR and UV-Vis spectroscopy. JurnalMatematika Dan IlmuPengetahuanAlamLldikti Wilayah 1 (Jumpa). 2023 Mar 30;3(1):08-13.
10. Arshad M, Beg A, Siddiqui ZA. Infrared spectroscopic investigation of tannins. Die AngewandteMakromolekulareChemie: Applied Macromolecular Chemistry and Physics. 1969 Jun 30;7(1):67-78.
11. Helen LR, Jyothilakshmi M, Latha MS. Isolation and quantification of tannins from the root bark of *clerodendruminfortunatumlinn*. and assessment of their antioxidant potential and antiproliferative effect on hct-15 cells. Int J Pharm Pharm Sci. 2015;7(10):170-5.
12. Dr. S SKhadabadi. Experimental Pharmacognosy: A Comprehensive Guide,1st Edition :4.4-4.12.
13. Sen A and Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach L*. International Journal of Current Pharmaceutical Research. 2012;4(2):67-73.