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# Phytochemical Screening, *in-vitro* Anti-Diabetic and Anti-Microbial Activities of Hydro-Alcoholic Leaf Extract of *Syzygium cumini*

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## Abstract
The plant under investigation (*Syzygium cumini*) was a large evergreen dicotyledonous plant and belongs to the family Myrtaceae. *Syzygium cumini* is a medicinal plant broadly used in conventional therapeutic preparation of many pharmacological activities. The goal of our investigation was to determine whether the leaf extracts of this plant held any significant anti-diabetic, anti-bacterial and anti-fungal activities. The Phytochemical screening of hydro alcoholic extract of *Syzygium cumini* leaves revealed that the extract is rich in phenols, flavonoids, triterpenoids, tannins and carbohydrates. In the present study, the In-vitro anti-diabetic property of *Syzygium cumini* leaves extracts was analyzed by using standard methods so as to ensure the biological potency of the plant. An *In vitro* anti-diabetic study was done by glucose uptake by yeast cells & inhibition of α-amylase enzymes. The results of the present study concluded that the hydro alcoholic extract of *Syzygium cumini* exhibited between 44.44% - 98.83% in glucose uptake by yeast cells when compared to metformin & 18.36% - 93.65% in α-amylase activity while compared to acarbose in dose dependent manner. Anti-microbial activity was evaluated employing the disc diffusion and agar well method. The extract of all the fractions and Amoxycillin and Fluconazole (standard) exhibited significant anti-bacterial & anti- fungal activity. The hydro alcoholic leaf extract produced significant effects as evaluated and Zone of Inhibition (ZOI) was measured.

**Keywords**
*Syzygium cumini*, *In vitro*, Anti-diabetic, Anti-microbial, yeast cells, α-amylase, ZOI.

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## INTRODUCTION
Plants have served mankind since ages as they are reservoirs of important medicinal components and help to alleviate chronic diseases. The past was considered the synthetic era due to the commercial production of large varieties of synthetic drugs by pharmaceutical industries (Gershell *et al.,* 2005). Over time the continuous use of synthetic drugs caused severe side effects and led to resistance of microbes. Also, synthetic drugs are expensive and large populations cannot afford to get benefit from these drugs. During the last decades a global trend with focus on green medicines due to minimum side effects and cost effectiveness. Medicinal plants play an appreciable role in the development of modern herbal medicines as many diseases like cancer, liver diseases and arthritis find no complete cure in allopathy. The bioactive compounds of medicinal plants are used as anti-diabetic, chemotherapeutic, anti-inflammatory, anti-arthritic agents where no

satisfactory cure is present in modern medicines (Tanko *et al*., 2012). Many plants have shown their immense potential to fight against dreadful diseases including cancer.

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of carbohydrates, proteins, fat, electrolytes and water and includes a group of metabolic diseases characterized by hyperglycemia. Currently, there is growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents for the treatment of diabetes mellitus (Bhalodi *et al*., 2008). Hence the traditional herbal medicines are mainly obtained from plants are used in the management of diabetes mellitus. In recent years herbal medicines have started to gain importance as a source of hypoglycemic agents. It is estimated that more than thousand plant species are being used as folk medicine for diabetes (Bhandari *et al*., 2008). Biological actions of the plant products used as alternative medicines to treat diabetes are in relevance to their chemical composition. Herbal products or plant products are rich in flavonoids, phenolic compounds, coumarins, terpenoids and other constituents which help to reduce blood glucose levels (Jung et al., 2006). Several species of herbal drugs with potential antidiabetic activity have been described in the scientific literature. Herbal drugs are prescribed due to their good effectiveness, fewer side effects in clinical experience and relatively low costs (Ren et al., 1997). Medicinal and natural herbal plant products are traditionally used from long time in many countries for the treatment of diabetes mellitus.

Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. Our nature has provided us with rich wealth of compounds and hence can be considered as a store house of remedies as they can cure all ailments of mankind. Traditional plants as a source of medicines in treating various diseases has been in use from ancient times. Researchers are continuously engaged in studying the medicinal plants as they are rich source of novel drugs which form the basis in traditional medicine, nutraceuticals, pharmaceutical intermediates and lead compounds in synthetic drugs. Roughly 50,000 species of higher plants have been used medicinally. These herbs or plants and their active ingredients are used in traditional herbal remedies. The major benefits of using herbal medicines lie in their efficacy, low cost and low incidence of serious adverse effects.

*Syzygium cumini* is native to the tropics and belongs to the family Myrtaceae. It has a worldwide distribution. It is commonly known as jamun, jambolan, black plum, Indian black berry etc. Plants belonging to this family are reported to be rich in volatile oils and as well for their use in medicine. *Syzygium cumini* is well known for its anti-diabetic values. The various parts of this plant have wide applications in traditional medicines throughout tropical and sub-tropical regions. The fruits of this plant have been used for a variety of ailments that includes diabetes, inflammations, cough, ring worm infections, dysentery etc. As well, the other parts of this plant like - leaves, bark etc were found to have antioxidant, anti-bacterial, anti-HIV, anti-leishmanial and anti-fungal activities. The main objective of the current study was to investigate the antimicrobial, anti-diabetic activities of hydro-alcoholic extract of leaf of *Syzygium cumini*. The anti-microbial activities are done by disc diffusion and agar well method. The anti-diabetic activity was studied using invitro glucose uptake by yeast cells and inhibition of α-amylase method.

## MATERIALS AND METHODS

### Materials:

Leaves of *Syzygium cumini,* Potassium iodide, Sodium Iodide, Bismuth carbonate, Glacial acetic acid, Aqueous picric acid, Benedict's reagent, Fehling's reagent, Sulphuric acid, Ferric chloride, Pyridine, Sodium Nitroprusside, sodium Hydroxide, Benzene, Ammonia Solution, Lead Acetate, aqueous Potassium Hydroxide, Ninhydrin solution, Chloroform, Metformin, Acarbose, Beef extract, peptone, NaCl, Peptone, Dextrose, Agar, Amoxycillin, Fluconazole.

### Materials and Methods:

The fresh Leaves of *Syzygium cumini* (Jamun), was collected in August from Atkuru, Vijayawada, Krishna district, Andhra Pradesh. The collected leaves were washed with distilled water, pierced into small pieces and dried in dark place over one week. The dried materials were powdered using a grinder (Hammer mill) and passed through no. 40 and no. 120 mesh sieve for phytochemical analysis study and pharmacognostic study respectively. The obtained powder is around 300grams.The powders were packed in sealed plastic bottles for storage.

**Figure 1: *Syzygium cumini* tree**

**Preparation of hydro-alcoholic extract:**
100grams of leaf powder was taken in a Round Bottom Flask (RBF) and macerated with ethanol and water in the ratio of 70:30 v/v with constant stirring after one week filter the solution. The extracts of are combined and centrifuged at 3500rpm for 10min at Room temperature. The supernatant was collected and concentrated by using rotary evaporator. The residue formed is the Hydro-alcoholic extract of *S.cumini.* And the extract obtained from extraction is used for experiment. (Johann *et al*., 1998).

**Phytochemical Screening:**

**Macroscopic and Organoleptic characters:** of leaves were examined thoroughly. **Pharmacognostic Studies**: The powdered leaves were passed through sieve # 120 and then mounted on clean grease - free glass slides for microscopic observations.

| Characters | Observation Of Leaves | |
|---|---|---|
| | **Fresh** | **Powder Form** |
| **Colour** | Pink to dark green | Light green |
| **Texture** | Smooth, leathery | - |
| **Odour** | Aromatic (turpentine-like) | Aromatic |
| **Taste** | Astringent | Astringent |
| **Shape** | Ovate | - |
| **Apex** | Acuminate | - |
| **Surface** | Smooth and glossy | - |
| **Venation** | Pinnately reticulate | - |
| **Length** | 7-11cm | - |
| **Width** | 5-8 cm | - |
| **Type** | Simple | - |

**Table 1: Macroscopic and organoleptic characteristics of *S.cumini***

**Powder Characteristics**: Powder microscopy study of leaves of *Syzygium cumini* revealed the presence of lignified cork cells, single-celled trichome, simple and compound starch grains, lignified fibres, tracheids with narrow lumen and tapering ends, xylem vessels with scalariform, reticulate and spiral thickenings, calcium oxalate crystals and stomata. The findings have been described.

| Powder Characters | Observations |
|---|---|
| **Cork cells** | - |
| **Trichomes** | - |
| **Starch grains** | Simple |
| **Fibre** | Short, parenchyma cells attached |
| **Tracheid** | Short |
| **Vessel element** | Short, scalariform thickening |
| **Stomata** | Anisocytic |

**Table 2: Powder characteristics of *S.cumini***

**Phytochemical Analysis**: 5 g of dried and powdered form of leaf sample of *Syzygium cumini* (Kalajam) was mixed separately with 25 ml of different solvents viz. methanol and water. The different extracts were used for standard phytochemical studies. The methanolic and aqueous extracts of different plant parts were used to evaluate the presence of phytoconstituents such as alkaloids, flavonoids, phenols, saponins, tannins, etc. This study was carried out by using standard procedures.

| TEST | OBSERVATION | INFERENCE |
|---|---|---|
| **TESTS FOR ALKALOIDS**: To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed. | | |
| **Mayer's Reagent Test**: To 2 ml of filtrate, few drops of Mayer's reagent were added along sides of the tube. | Formation of creamy precipitate. | Presence of alkaloids |
| **Wagner's Test**: To 2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. | Formation of reddish-brown precipitate. | Presence of alkaloids. |
| **Hager's Test**: To 2 ml of filtrate, few drops of Hager's reagent were added in a test tube. | Formation of yellow colour precipitate. | Presence of alkaloids. |
| **TESTS FOR FLAVONOIDS**: | | |
| **Lead Acetate Test**: The extract was treated with a few drops of lead acetate solution. | Formation of yellow colour precipitate. | Presence of flavonoids. |
| **TESTS FOR CARBOHYDRATES**: | | |
| **Molisch Test**: 2 ml of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube, and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. | No Formation of violet ring at the junction of two layers. | Absence of carbohydrates. |
| **Barfoed's Test**: 1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on a water bath for 2 min. | No formation of red colour. | Absence of monosaccharides. |
| **TESTS FOR REDUCING SUGARS**: | | |
| **Fehling's Test**: To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated on a water bath for 10 min. | Formation of red precipitate. | Presence of reducing sugars. |
| **Benedict's Test**: Equal volume of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5 - 10 min. | Formation of light yellowish green colour. | Presence of reducing sugars. |
| **TEST FOR SAPONIN**: | | |
| **Froth Test**: The extract was diluted with distilled water and shaken in a graduated cylinder for 15 min. | Thin layer of foam is formed. | Presence of saponins. |
| **TESTS FOR TANNIN AND PHENOLIC COMPOUNDS:** | | |
| **Ferric Chloride Test**: A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5%, ferric chloride solution was added. | Formation of blue, green or violet colour. | Presence of tannins and phenolic compounds. |
| **Lead Acetate Test**: A small amount of extract was dissolved in distilled water. To this solution, a few drops of lead acetate solution were added. | Formation of white precipitate. | Presence of tannins and phenolic compounds. |
| **TESTS FOR PROTEINS AND AMINO ACIDS**: | | |
| **Ninhydrin Test**: 3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 min. | No Formation of blue colour. | Absence of proteins. |

| | | |
|---|---|---|
| **Biuret Test**: The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. | Formation of dark blue colour. | Presence of proteins. |
| **TEST FOR GLYCOSIDES**: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous sodium hydroxide was added. | Formation of a yellow colour. | Presence of glycosides. |
| **Steroids [Salkowski's test]:** About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. | A reddish-brown colour at the interface was an indicative of the presence of steroidal ring. | Presence of steroidal ring. |
| **Cardiac glycosides [Keller killiani's test]:** About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. | A brown ring obtained at the interface. | Presence of de-oxy sugar characteristic of cardenolides. |
| **TEST FOR TERPENOID:** 2ml of chloroform and 1ml of conc. H2SO4 was added to 1mg of extract. | Formation of reddish-brown colour. | Presence of terpenoids. |

**Table 3: Phytochemical screening of *S.cumini.***

**Figure 2: Test for alkaloids, flavonoids, carbohydrates, reducing sugars.**

**Figure 3: Test for Saponins, Tannins & Phenolic Compounds, Proteins and Amino Acids**

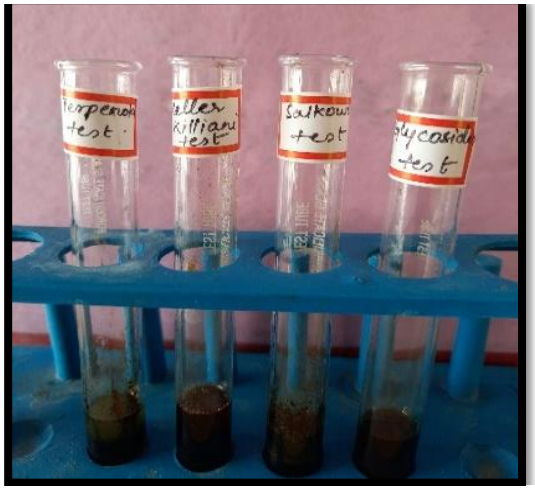



**Figure 4: Test for Glycosides, Cardiac Glycosides, Steroids & Terpenoids.**

***IN-VITRO* METHODS FOR ANTI-DIABETIC ACTIVITY:**

**Glucose uptake in yeast cells:** The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3,000×g, 5 min) until clear supernatant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50- 2000 µg/mL)) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding 100 µL of yeast suspension followed by vertexing and further incubation at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and amount of glucose was estimated in the supernatant (Cirillo, 1962). Metformin was used as standard drug of concentration 0.25mg/ml. Measure the absorbance at 520nm. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

**Increase in glucose uptake (%) = [(Abs sample – Abs control) ÷ Abs sample] X 100**

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

**Figure 5: Concentrations of solutions with 1-5mg/ml leaf extract.**

**Inhibition of alpha-amylase enzyme:** A starch solution (0.1% w/v) was obtained by stirring 0.1g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both control and plant extracts were added with starch solution and left to react with α-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm.

**METHODS FOR ANTI- MICROBIAL ACTIVITIES**

The petriplates and the nutrient agar medium were sterilized for 20 minutes at 120˚C. The rest of the procedure was carried out in laminar air flow. Approximately 20ml of the media was poured into the sterile petriplates and allowed to get solidified. After the media gets solidified the organisms were swabbed on the medium using cotton swabs.

**Disc diffusion method:** Sterile nutrient agar plates were prepared for bacterial & fungal strains and inoculated by a spread plate method under aseptic conditions. The filter paper disc of 5 mm diameter (Whatman's No. 1 filter paper) was prepared and sterilized. The leaf extracts to be tested were prepared various concentrations of 20 µg/ml, 40 µg/ml, 100 µg/ml were added to each disc of holding capacity 10 µl. The sterile impregnated disc with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Filter paper discs soaked in solvent were used for negative controls. The standard drugs are taken in the concentrations of Amoxycillin 20µg/ml and fluconazole 30µg/ml. All the plates were incubated at 37˚C for 24 hours. The bacterial cultures are placed in incubator and observed after 24 hours. The fungal cultures are placed in BOD incubator and observed after one week. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

**Figure 6: Stock solutions of samples and standard drugs, Gram +ve S. aureus and Gram -ve P. aeruginosa**

**Figure 7: Disc Diffusion method after inoculation of microorganisms**.

**Agar well diffusion method:** The antimicrobial activity was screened by using agar well diffusion method. The plates containing agar medium were spread with 0.1 ml of the bacterial inoculum. Wells (8 mm diameter) were cut out from agar plates using sterilized stainless-steel cork borer and filled with 0.1 ml of the sample. The leaf extracts to be tested were prepared various concentrations of 20 µg/ml, 40 µg/ml, 100 µg/ml and the standard drugs are taken in the concentrations of Amoxycillin 20 µg/ml and fluconazole 30 µg/ml were added to each well. The plates were incubated at 37˚C in case of bacteria for 24 hours and 25˚C in case of fungi for one week. The diameter of resultant zone of inhibition (ZOI), if any, was measured. DMSO was used as negative control. Experiments were run in triplicates for each

combination of extract and microbial strains. A significance zone of inhibition has been observed around each well and the diameter/radius of such zone was measured in millimeters.

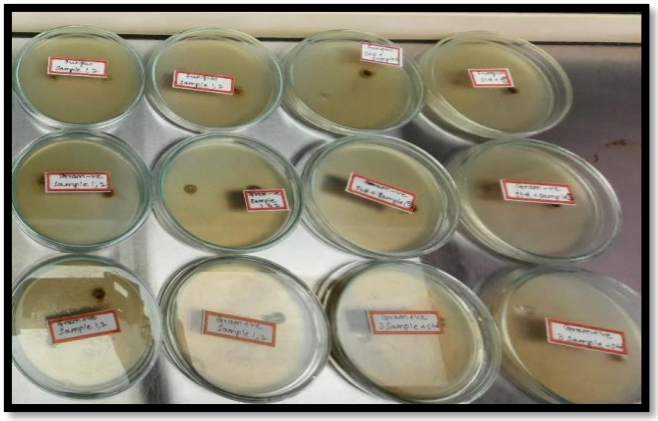


**Figure 8: Agar Well Diffusion method after inoculation of microorganisms.**

**RESULTS & DISCUSSION:**

**Phytochemical Analysis:**

The preliminary Phytochemical analysis of hydro alcoholic extract of leaves of *S.cumini* showed the presence of the following:

| S. NO. | PHYTOCHEMICAL CONSTITUENTS | HYDROALCOHOLIC LEAF EXTRACT |
|---|---|---|
| 1 | Alkaloids | ++ |
| 2 | Flavonoids | +++ |
| 3 | Carbohydrates | + |
| 4 | Reducing sugars | + |
| 5 | Saponins | + |
| 6 | Tannins & phenolic compounds | +++ |
| 7 | Proteins and amino acids | + |
| 8 | Glycosides | + |
| 9 | Cardiac glycosides | ++ |
| 10 | Steroids | +++ |
| 11 | Terpenoids | + |

+ = present, ++ = moderately present, +++ =Appreciable amount

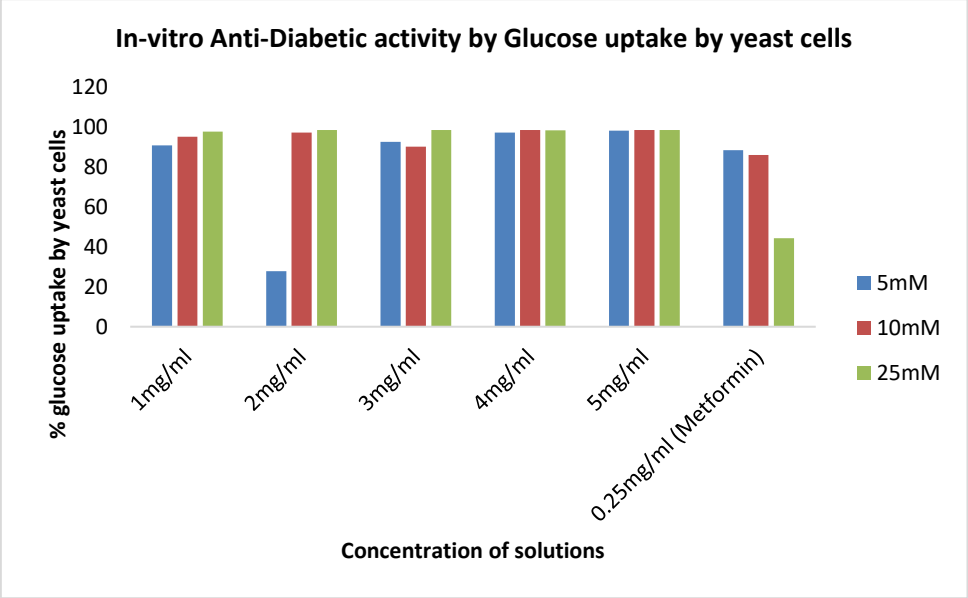
**Table 4:** Phytochemical investigation of *S.cumini* leaf extract

***IN VITRO* ANTI-DIABETIC ACTIVITY**

**Glucose uptake by yeast cells:** The percentage inhibition of glucose uptake by yeast cells with extract concentration ranging from 1mg/ml to 5mg/ml. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration i.e., 1mg/ml the increase in percentage inhibition was somewhat linear but as concentration increases higher till 5mg/ml it tends to become somewhat more exponential.

| S. No. | Concentration | % Glucose Uptake by Yeast Cells | | |
|---|---|---|---|---|
| | | 5mM | 10mM | 25mM |
| 1 | 1mg/ml | 91.05 | 95.34 | 97.91 |
| 2 | 2mg/ml | 27.89 | 97.446 | 98.722 |
| 3 | 3mg/ml | 92.76 | 90.322 | 98.68 |
| 4 | 4mg/ml | 97.45 | 98.833 | 98.631 |
| 5 | 5mg/ml | 98.49 | 98.679 | 98.79 |
| 6 | 0.25mg/ml (Metformin) | 88.65 | 86.25 | 44.44 |

**Table 5: Results of Glucose uptake by yeast cells**

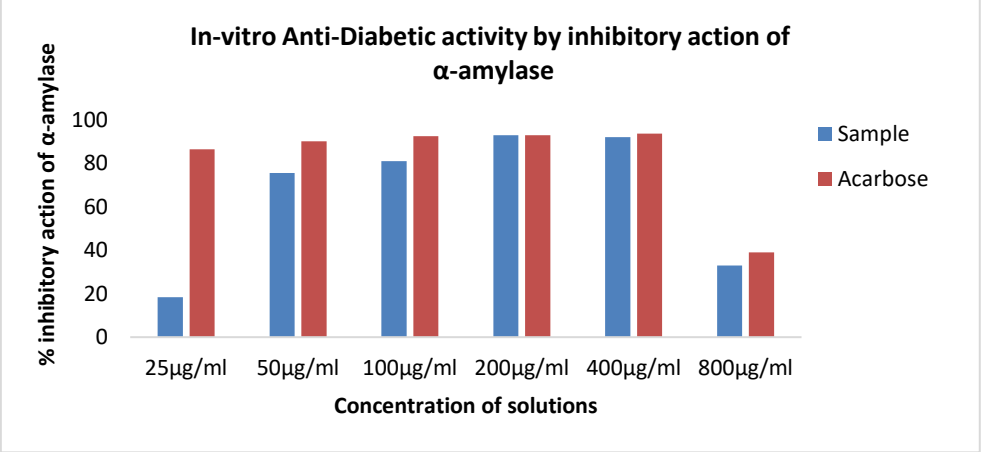



**Graph 1: Percentage of Glucose Uptake by Yeast Cells**

**Inhibition of α-amylase activity:** Alpha-amylase is a digestive enzyme found in the secretions of the intestinal mucosa, pancreas, and the saliva. It is responsible for the breakdown of α-1, 4- glycosidic bonds in starch. Thus, the catalytic activities of the enzyme (especially in the small intestine), increase the availability of glucose in the blood, since the pH of the intestine is around 6.9, α–amylase has access to starch at this pH and catalyses the breakdown of this polysaccharide into monosaccharide and disaccharide. In this study *S.cumini* leaf extract revealed a significant inhibitory action of alpha-amylase enzyme. The percentage inhibition at 0.2-1.0 ml concentrations of *S. cumini* extract showed a dose dependent increase in percentage inhibition. The percentage inhibition varied from 38.6% - 95.4% for lowest concentration to the highest concentration when compared to acarbose. There was a dose dependent increase in percentage inhibitory activity against α–amylase enzyme.

| S. No. | Concentration of Solutions | % Inhibitory Action of A-Amylase | |
|---|---|---|---|
| | | Sample | Acarbose |
| **1** | 25 µg/ml | 18.367 | 86.5 |
| **2** | 50 µg/ml | 75.609 | 90.136 |
| **3** | 100 µg/ml | 81.012 | 92.467 |
| **4** | 200 µg/ml | 93.016 | 92.936 |
| **5** | 400 µg/ml | 92.125 | 93.65 |
| **6** | 800 µg/ml | 32.96 | 38.955 |

**Table 6: Results of Inhibition of α-Amylase Activity**




**Graph 2: Percentage Inhibition of α-Amylase Activity**

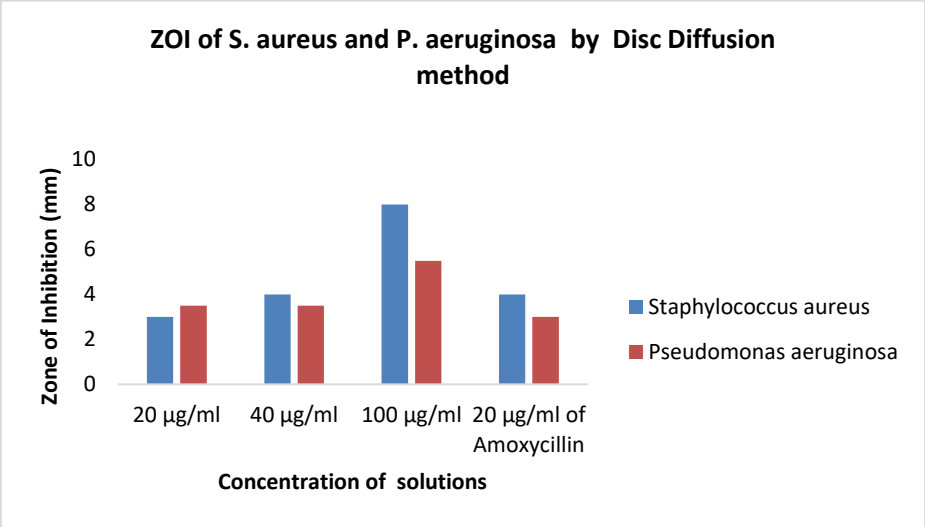
**ANTI-MICROBIAL ACTIVITY:**

**Anti-Bacterial Studies:**

**1. Disc Diffusion Method:**

| S. No. | Name of The Organism | Concentration | Zone of Inhibition | |
|---|---|---|---|---|
| 1 | *Staphylococcus aureus* (Gram +ve) | 20 µg/ml | 3 mm | 3 mm |
| | | 40 µg/ml | 4 mm | 4 mm |
| | | 100 µg/ml | 8 mm | 8 mm |
| | | 20 µg/ml of Amoxycillin | 4 mm | 4 mm |
| 2 | *Pseudomonas aeruginosa* (Gram -ve) | 20 µg/ml | 5 mm | 2 mm |
| | | 40 µg/ml | 3 mm | 4 mm |
| | | 100 µg/ml | 6 mm | 5 mm |
| | | 20 µg/ml of Amoxycillin | 2 mm | 4 mm |

**Table 7: Disc Diffusion method results for bacterial species.**




**Graph 3: ZOI of *S. aureus* & *P. aeruginosa* by Disc Diffusion method**

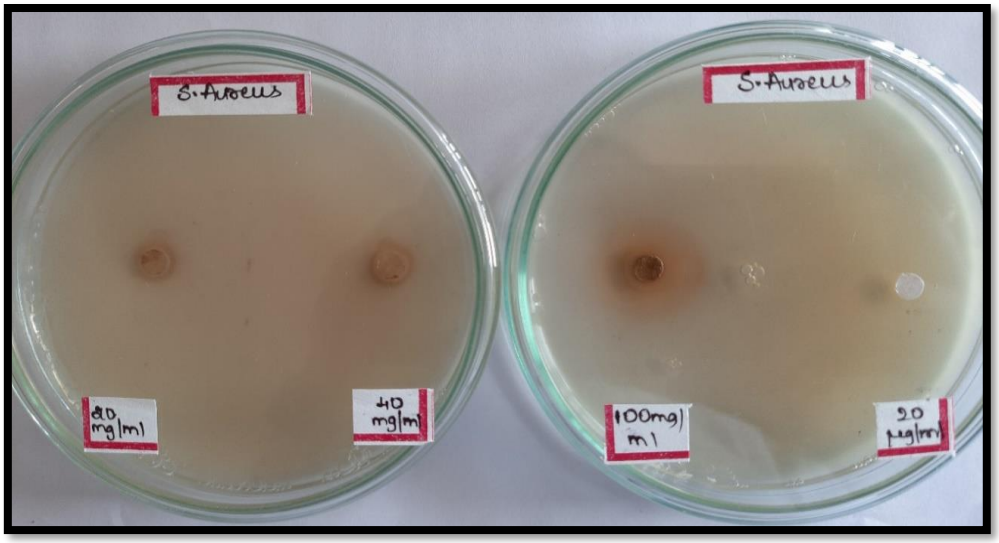



**Figure 9: ZOI of *S. aureus* by Disc Diffusion method**

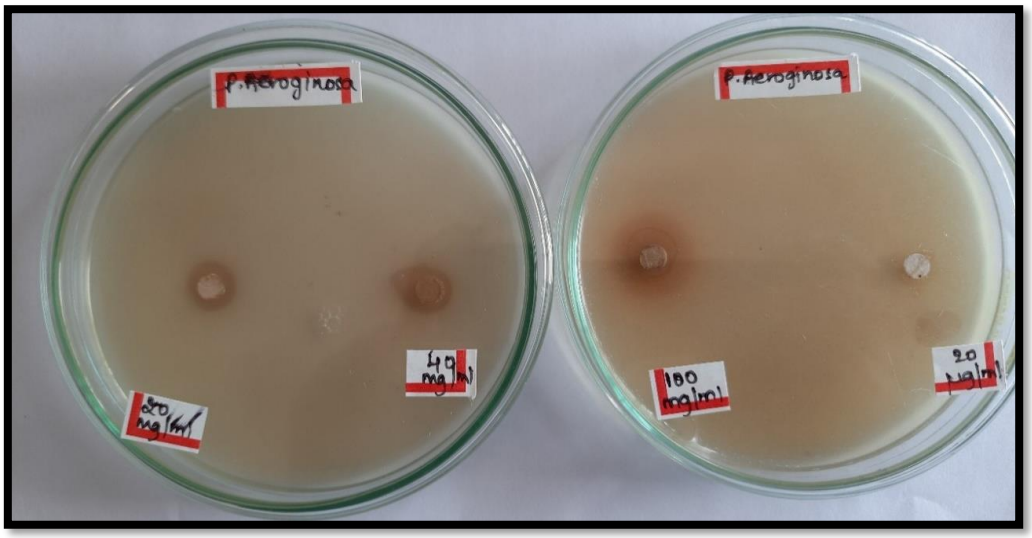
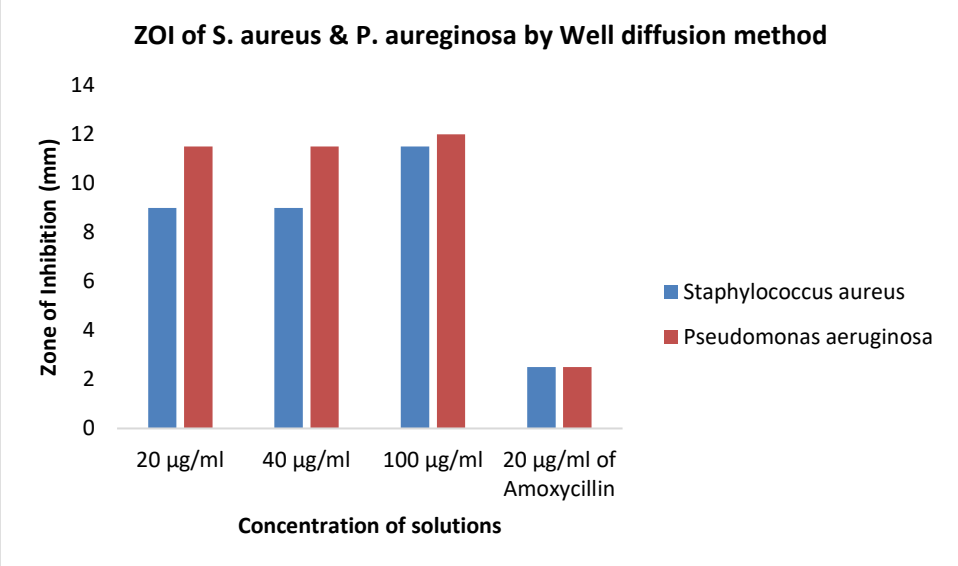



**Figure 10: ZOI of *P. aeruginosa* by Disc Diffusion method**

**2. Agar well diffusion method:**

| S. No. | Name of The Culture | Concentration | Zone of Inhibition | |
|---|---|---|---|---|
| 1 | *Staphylococcus aureus* (gram +ve) | 20 µg/ml | 10mm | 8mm |
| | | 40 µg/ml | 8mm | 10mm |
| | | 100 µg/ml | 11mm | 12mm |
| | | 20 µg/ml of Amoxycillin | 2mm | 3mm |
| 2 | *Pseudomonas aeruginosa* (gram -ve) | 20 µg/ml | 10mm | 11mm |
| | | 40 µg/ml | 11mm | 10mm |
| | | 100 µg/ml | 12mm | 12mm |
| | | 20 µg/ml of Amoxycillin | 2mm | 3mm |

**Table 8: Agar Well Diffusion method results for bacterial species.**




**Graph 4: ZOI of *S. aureus* & *P. aeruginosa* by Well Diffusion method**

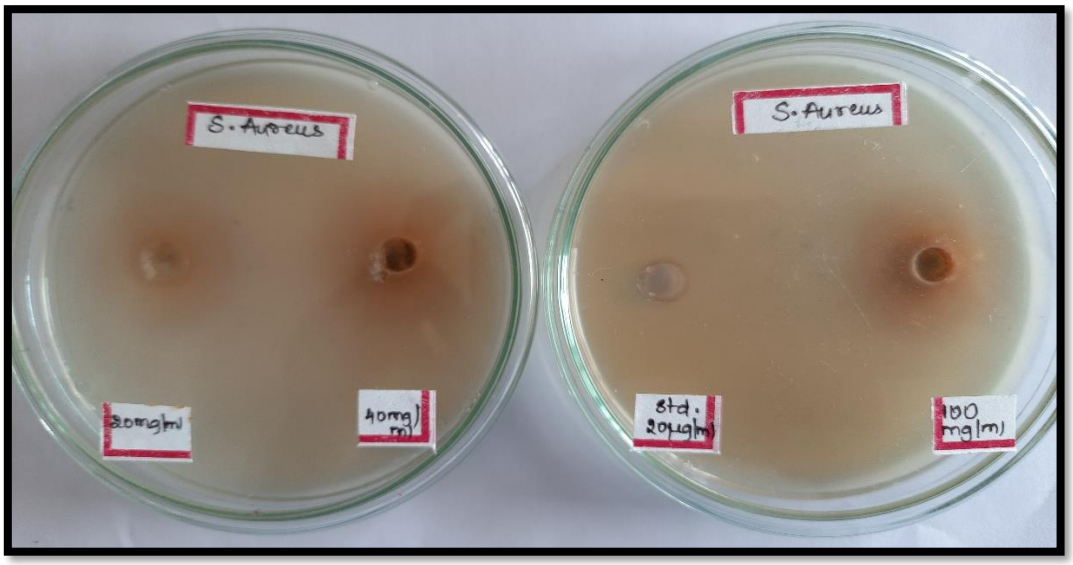



**Figure 11: ZOI of *S. aureus* by Agar Well Diffusion method**

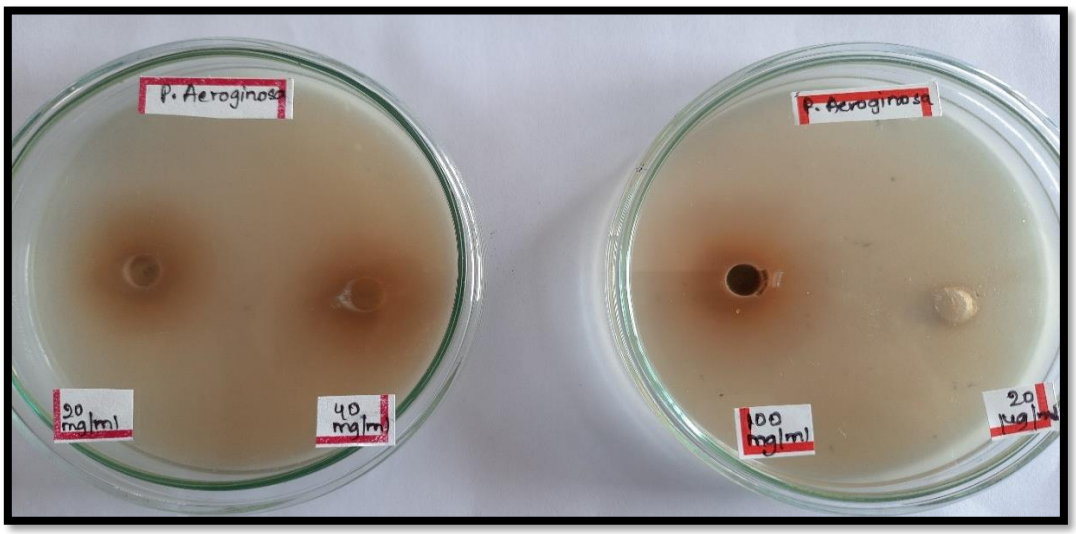
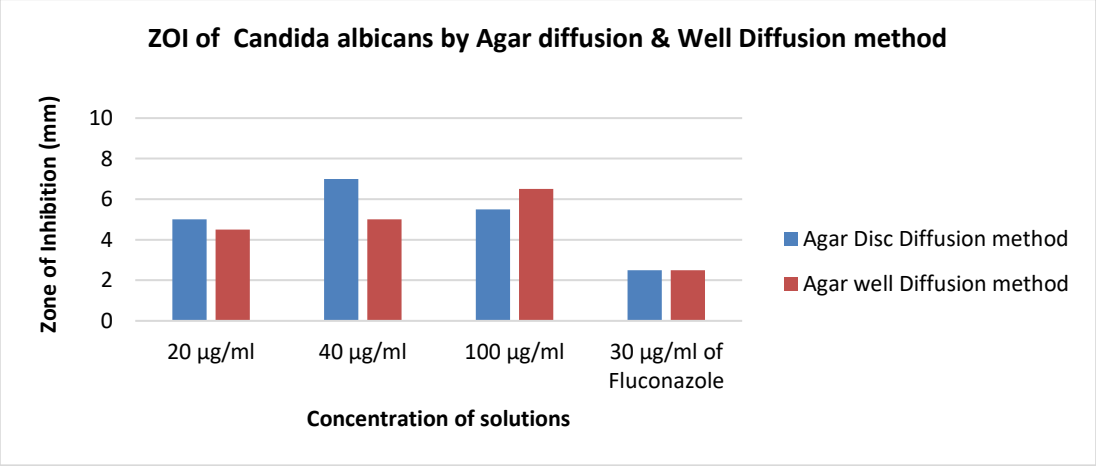



**Figure 12: ZOI of *P. aeruginosa* by Agar well Diffusion method.**

**Anti-Fungal Studies:**

| S. No. | Name of The Method | Concentration | Zone of Inhibition | |
|---|---|---|---|---|
| 1 | Disc Diffusion method | 20 µg/ml | 5mm | 5mm |
| | | 40 µg/ml | 8mm | 6mm |
| | | 100 µg/ml | 6mm | 5mm |
| | | 30 µg/ml of Fluconazole | 2mm | 3mm |
| 2 | Agar well Diffusion method | 20 µg/ml | 6mm | 3mm |
| | | 40 µg/ml | 4mm | 6mm |
| | | 100 µg/ml | 3mm | 10mm |
| | | 30 µg/ml of Fluconazole | 3mm | 2mm |

**Table 9: *Candida albicans* -fungal species results of both Disc Diffusion method and Agar well Diffusion method.**




**Graph 5: ZOI of *Candida albicans* by Disc Diffusion method and Agar well Diffusion method.**

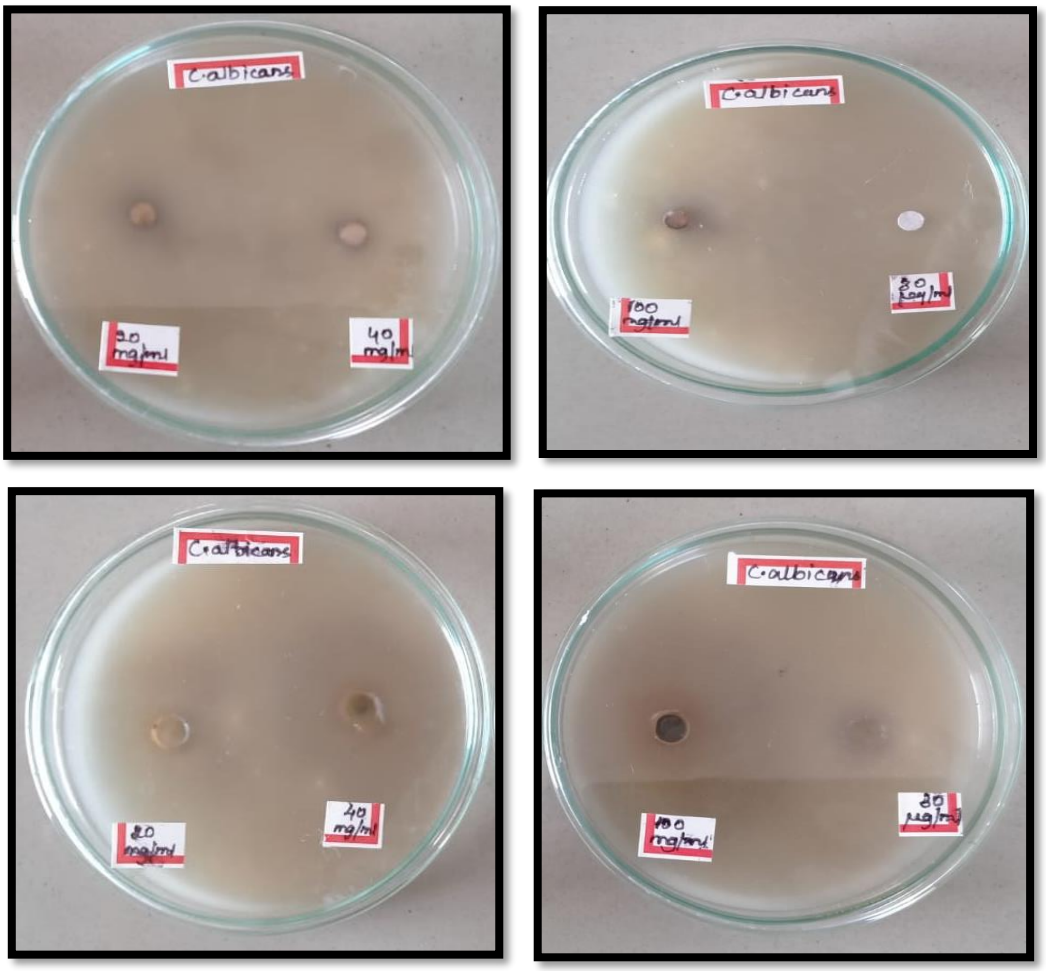



**Figure 13: ZOI of *Candida albicans* by Disc Diffusion method and Agar well Diffusion method.**

The current work is mainly focused to find out the Phytochemical screening, anti-microbial and invitro anti-diabetic activity of hydroalcoholic extract of *S.cumini* leaves.

The phytochemical analysis of hydroalcoholic extract of *S. cumini* showed the presence of phenols, flavonoids, triterpenoids, tannins and carbohydrates. Most of the available literature shows the antidiabetic nature of compounds isolated from *S. cumini*. But *Syzygium cumini* is also a good resource of bioactive compounds due to its content of various phytochemicals. In support of this our study focused on the extraction of antimicrobial compounds from the leaves of *S. cumini* using Hydroalcoholic solution. Glucose uptake in yeast cells (Saccharomyces cerevisiae) is rapid and occurs down the

concentration gradient. Glucose uptake reaches equilibrium and is not accumulative. Phosphorylation accompanies with glucose entry into the cell. The percentage inhibition at 1-5mg/ml concentrations of *S. cumini* leaf extract showed a dose dependent increase in percentage inhibition of glucose uptake by yeast cells. The percentage inhibition varied from 44.44% - 98.83% for lowest concentration to the highest concentration. There was a dose dependent increase in percentage inhibitory activity against glucose uptake.

In this study *S.cumini* extract revealed a significant inhibitory action of alpha-amylase enzyme. 25 - 400 µg/ ml concentrations of *S. cumini* extract showed a dose dependent increase in percentage inhibition when compared to 800 µg/ ml, where it showed decrease in percentage inhibition. The percentage inhibition varied from 18.36% - 93.65% for lowest concentration to the highest concentration. There was a dose dependent increase in percentage inhibitory activity against α–amylase enzyme as shown in graph 2.

The study was performed on anti-microbial activities by using disc diffusion and agar well method by taking 20µg/ml, 40µg/ml and 100µg/ml of leaf extracts. Amoxycillin(20µg/ml), Fluconazole(30µg/ml) were used as standard drugs on the culture strains of *S.aureus* (gram +ve), *P. aeruginosa*(gram-ve), *Candida albicans*(fungus).

The hydro-alcoholic extracts of *Syzygium cumini* leaves was efficient in inhibiting the growth of bacterial and fungal species. The investigation reported that anti-microbial activity of *S. cumini* leaf extract is due to presence of tannins and other phenolic compounds. The extracts of *Syzygium cumini* showed maximum inhibitory activity against the bacterial and fungal strains. From our observation, it was clear that *S. cumini* extract was active in inhibiting the growth of bacterial and fungal organisms.

## CONCLUSION

The present study demonstrated that the hydro-alcoholic extract of *Syzygium cumini* leaves possesses phytochemicals such as phenols, flavonoids, triterpenoids, tannins and carbohydrates which are of high therapeutic value. This study also demonstrated that the extract has dose dependent increase in percentage inhibition of glucose uptake by yeast cells & a potent α-amylase inhibitor with a higher degree of inhibition. The results suggested that the *S.cumini* leaves possess significant anti-diabetic activity. The result showed potential antibacterial effects of *S.cumini* extracts against bacterial strains *S. aureus* & *P. aeruginosa.* The extract also exhibited antifungal activity against *Candida albicans*. *S.cumini* extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

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