PHYTOCHEMICAL, ANTIOXIDANT, ANTIFUNGAL AND ANTIDIABETIC ACTIVITY OF *SYZYGIUM CUMINI* PULP AND SEEDS

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE

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CHEMISTRY

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MAY, 2024

DECLARATION

We, Jay Krishna Singh [2K22/MSCCHE/16] and Umesh Pal [2K22/MSCCHE/44], students of M.Sc. (Applied Chemistry), hereby declare that the project Dissertation titled "Phytochemical, Antioxidant, Antifungal and Antidiabetic Activity of *Syzygium cumini* Pulp and Seeds" which is submitted by us to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Masters, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi
Date: 31st May 2024

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CERTIFICATE

I hereby certify that the project titled "Phytochemical, Antioxidant, Antifungal and Antidiabetic Activity of *Syzygium cumini* Pulp and Seeds" which is submitted by Jay Krishna Singh [2K22/MSCCHE/16] and Umesh Pal [2K22/MSCCHE/44] of Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the Degree of Masters in Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

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ABSTRACT

The seeds and pulp of *Syzygium cumini* used as traditional medicine to treat various diseases such as ringworm infection, spleenopathy, pharyngitis and mainly diabetes. Therefore, in the present study we deal with the phytochemicals analysis and bioactive compounds, anti-oxidant property, antifungal, anti-diabetic, cytotoxicity of *Syzygium cumini* in the methanolic extract of jamun seeds and pulp extract.

The bioactive components were analysed by GC-MS. The phytochemicals analysis showed the saponins, carbohydrate, flavonoids, glycoside were present in the pulp and tannins, saponins, carbohydrate, glycoside, phenolic compound were present in seeds of jamun. GC-MS showed that the 5- [hydroxy methyl] -2-furaldehyde, pyranone, 2-furoic acid, 5-5-oxy-dimethylene-bis (2-furaldehyde) were major component in the jamun pulp. In jamun seeds the major components were 5-oxymethylfurfurole, pyranone, pyrogallol, cycloisolongifolene observed. Jamun seeds show most active antioxidant property as compared to the pulp. The jamun pulp shows maximum zone inhibition in antifungal activity. In α-glucosidase enzyme inhibition activity, the jamun seed shows most active antidiabetic property. The reactive oxygen species activity production was observed in the jamun seeds. These findings suggest that *Syzygium cumini* seeds and pulp might be potential source of phytochemical, bioactive components may be used in pharmaceutical, cosmetics and food industry.

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LIST OF SYMBOLS AND ABBREVIATIONS

Syzygium cumini	*S. cumini*
Candida albians	*C. albians*
Monoisotopic mass	MM
Degree Celsius	^{o}C
Ultra High Performance Liquid Chromatography-Quadrupole Timing of Flight- Mass Spectrometry	UHPLC-QTOF-MS
Minutes	min
Diabetes Mellitus	DM

CHAPTER 1

INTRODUCTION

Diabetes is a metabolic condition characterised by chronic hyperglycemia and impaired insulin signalling, which causes metabolic abnormalities and an inflammatory state in all body tissues [1]. The pancreatic α-amylase is a major intestinal enzyme that converts starch to maltose and then to glucose. The dietary starch is rapidly digested by pancreatic α- amylase, resulting in postprandial hyperglycemia. The human pancreatic α-amylase (HPA) present in the small intestine correlates positively with post-prandial glucose levels, making regulation of glucose a crucial factor in diabetes treatment. As a result, inhibiting the α-amylase enzyme would play an important role in diabetes control by reducing starch digestion [2]. Currently, the International Diabetes Federation found 425 million people in the world that are affected by diabetes. Symptoms of type 2 DM include polyuria, lethargy, polydipsia and polyphagia among others. Type 2 diabetes is associated with some difficulties such as cardiovascular disease, retinopathy, neuropathy [3]. Due to the increasing uses of synthetic and semisynthetic derivatives for the diabetic treatment, they cause many health problems. In recent studies, we use natural antidiabetic medications which are becoming more popular as a result of the high costs and side effects associated with allopathic treatment options. Herbal preparations are complementary and alternative medicine, and the quest for novel substances obtained from natural sources such as herbs or plants is increasing, owing primarily to acquire resistance, side effects, and adverse events associated with allopathic medicines [4]. In this context, we selected

Syzygium cumini plant that is traditionally used to treat diabetes with hope of supporting global efforts to battle diabetes and its complications.

Syzygium cumini is an important medical plant widely used in the treatment of diabetes. It belongs to the Myrtaceae family. Local names include Jamun, Jambolan, Jambul, Jamblang, Black plum, Blackberry and Java plum. *Syzgium cumini* is an evergreen plant found in Pakistan, Southeast Asia, China, and Queensland. The largest jamun producer in India is Maharashtra. The plant can reach 30 cm in height and can live for more than 100 years. It blooms in March to April and has a 5mm diameter fragrant small flower. Fruits start to appear in May and June, similar to a large barrier, and when they are ripe, the skin turns black. Fruits have a sweet, acidic and soft taste, and tend to purple the tongue. Fruits contain seeds used to treat diabetes in traditional medicine. The plant helps to regulate blood sugar levels by reducing glucose index. In addition to fruits, they purify the blood and benefit the eyes and skin. In Chinese medicine, *Syzygium cumini* is commonly used to treat digestive disorders and has a high level of vitamins A and C. The different parts of plants, including leaves, fruits, seeds, bark and roots, have therapeutic effects. It has biological effects such as antioxidants, anti-diarrheal, antibacterial, anti-viral, anti-inflammatory and possible hypoglycemic effects. *Syzygium cumini* shows anti-hyperglycemic, cardioprotective, and low lipid properties. Additionally, this herb is thought to have anti-diabetic properties. *Syzygium cumini* contains several phytochemicals, including anthocyanins, ellagic acid, glycosides. The seeds contain flavonoids and phenols, which contribute to the plant's antioxidant capabilities [1].

Antioxidants are significant in diabetes, with low levels of plasma antioxidants stated as a risk factor for disease development and circulating levels of radical scavengers mitigated throughout diabetes progression [5]. Antioxidants from both internal and external sources protect against oxidative stress by removing free radicals and maintaining the redox state. In addition to endogenous antioxidants, there are also a variety of non-nutrient derived antioxidants from plants, some of which are powerful free radical decomposers (e.g. gallate, catechins). External antioxidants in biological systems are chemicals (substances) that, when present at lower concentrations than the reactive oxygen species (ROS), significantly reduce or delay tissue damage and are often oxidized. Antioxidants can be used as chain breakers or as mechanisms for eliminating ROS initiators. Antioxidants also show that they affect gene expression due to cell-based redox states. The jamun pulp is commercially used by industries to make value added products such as wines, jam, juice and jellies. Due to nutritional qualities and functional qualities, seeds are used in the different food formulations, cosmetics and pharmaceutical industries [6].

In the present study our aims to evaluate (a) *Vitro* antidiabetic activity of *Syzygium cumini* pulp and seeds by the process of α-glucosidase, DPP-IV activity assay, α-amylase assay, Reactive oxygen activity. (b) Anti-oxidant activity by the method of DPPH radical scavenging assay. (c) Anti-fungal activity by the process, zone inhibition activity of test-disc. (d) cytotoxicity assay. In addition, we propose which compound are present in the poly phenol riched fraction analysed with UHPLC-QTOF- MS and GC-MS.

Figure 1. *Syzygium cumini* (Jamun) fruit

CHAPTER 2

LITERATURE REVIEW

Syzygium cumini has various pharmacological activities such as antidiarrhoeal, astringent, digestive, antibacterial, antioxidant, antiviral but most important activity is antidiabetic [7].

In a study conducted by Basky and colleagues, the phytochemical components of the *Syzygium cumini* seed extracts were positive in terms of alkaloids, flavonoids, glycosides, steroids, cardiac glycosides, saponins, resins, phenols, tannins, and terpenoids. Seed extracts contain large quantities of flavonoids, steroid, and moderate quantities of alkaloids, glycosides, and phenols. Alkaloids are natural chemical compounds with basic nitrogen atoms used as pharmaceuticals and recreational substances. Animal studies also indicate that terpenoids reduce blood sugar levels. Flavonoid improves the benefits of vitamin C and acts as an antioxidant. Bioflavonoids stimulate glucose absorption in peripheral tissues and regulate the activity or expression of enzymes that limit the rate of carbohydrate metabolism. Polyphenols and flavonoids are natural anti-inflammatory drugs that inhibit the inhibition of hydrolysis of carbohydrates by a protein bond. In the present study, seed extracts contain a significant amount of flavonoids, which explain the antidiabetic properties [1].

The antioxidant activity of *Syzygium cumini* extracts and their active ingredients, specifically focusing on anthocyanidins and ellagic acid. The most often used antioxidant assay for plant samples is the 1,1-diphenyl 1-2-picrylhydrazine (DPPH) radical scavenging assay, antioxidant capacity. The *Syzygium cumini* pulp and seeds have the ability to scavenge while there are other assays available to determine a

compound's free radicals and act as antioxidants. Jamun pulp contains large concentrations of the pigments Cy- and Dp, which have groups called catechol (ortho-dihydroxyl) and pyrogallol (vicinal trihydroxyl), respectively [8].

Indian traditional medicine practitioners use *Syzygium cumini* seeds as an antidiabetic medication. Diabetes is emerging as the third “killer” disease in human health after cancer, cardiovascular, and cerebrovascular disorders due to its high frequency, morbidity, and mortality (Li et al., 2003). According to the Gajera et al. (2017) studied about pulp of *Syzygium cumini* was shown to be negatively linked with phenols, anti-diabetic and antioxidant properties. Additionally, it was established that the kernel percentage of seeds gave them greater antioxidant and anti-diabetic properties[2]. According to Raza et al. (2017) studied, the effects of extracts from the pulp and seeds of the *Syzygium cumini* were investigated. The results showed that the extract could both lower blood glucose levels in rats and control insulin levels[9]. In an investigation conducted by Shankar et al. (2007), the anti-diabetic effect of two novel androgen derivatives-JB1 and JB2 isolated from ethanolic extracts of *Syzygium cumini* seeds was assessed using alloxan as a diabetogenic agent. It was discovered that JB1 out performed over JB2 in terms of effectiveness[10]. It was confirmed by Sharma et al.(2003) that an ethanolic concentrate of jamun seeds is an effective treatment for diabetes using alloxan [11].

Chemotherapy controls a complex parasitic disease. A similar impact was observed in a new research area where *Syzygium cumini* extracts had more active components such as sesquiterpenes, monoterpenes, and other essential oil constituents, which can be

improved for novel medicine formulations. *Syzygium cumini* extract's immunomodulatory activity has the ability to influence the Leishmaniasis impact [12].

Another study done by Katiyar et al., examined Jamun is abundant in iron, therefore it can work as a blood purifier, allowing for sufficient haemoglobin production. As a result, it serves as a protective diet for menstruating women [13].

CHAPTER-3

MATERIALS AND METHODS

3.1 Plant material collection and authentication

Syzgium cumini fruits were collected from the tree growing at Delhi technological university in New Delhi, India. The fruits botanical samples were verified by the CSIR-NIScPR, Raw materials Herbarium and Museum, Delhi India, and the voucher sample (NIScPR/RHMD/Consult/2023/4618-19) was placed at the RHMD, Delhi.

3.2 Preparation of extract

The fruits had been obtained were cleaned and the seeds were gently removed from the fruits. Then, 50 g of crushed seeds and pulp extracted with methanol individually. After that, it was kept on stirrer for 48 hours at room temperature. The sample was filtered with muslin cloth after complete extraction. The extract was stored at 4 ^{o}C until further use [14].

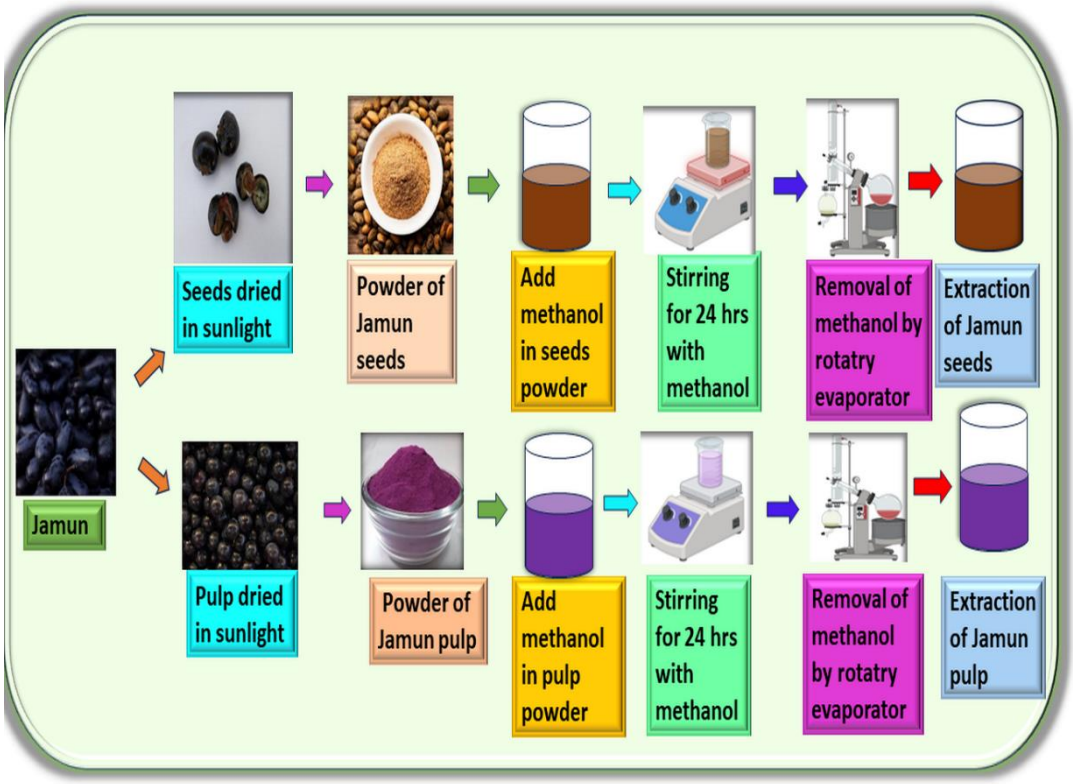

Figure 2. Preparation of Extraction of *Syzygium cumini* seeds and pulp

3.3 Phytochemical analysis

The methanolic extract of *Syzgium cumini* seeds and pulp underwent a qualitative phytochemical analysis from the following method. Identification tests for various kinds of phytoconstituents including alkaloids, flavonoids, total phenolic content, glycosides, proteins and amino acid, terpenoids were carried out [1].

For the phytochemical analysis, following tests were performed [15].

Tannins:

Braymer's test: 1mL of extract of pulp and seed was taken in a test tube. Then added 2 mL of distilled water. 2-3 drops of 5 % $FeCl_3$ were added to the extracts.

Saponins:

Foam test: 0.5 g of the extract of the pulp and seed was shaken with 2 mL of water. Persistence of foam for 10 minutes indicates the presence of saponins.

Phalobatannins:

HCl test: 2mL of the aqueous extracts were added into dilute HCl. Red colour precipitate formed indicates the presence of phalobatannins.

Carbohydrate:

Molisch's test: A few drops of molisch's solution was added to the 2mL of the extracts. Then, a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface of purple coloured observed indicated the presence of carbohydrate.

Flavonoids:

Lead acetate test: A few drops of lead acetate solution treated with extracts. The yellow precipitate indicated the presence of flavonoids.

Glycosides:

Keller killani's test: 10 mg of extracts dissolved in 1mL glacial acetic acid containing 1 drop of $FeCl_3$ solution and 1 mL of concentrated sulphuric acid was added. A brown ring interface indicated the presence of glycosides.

Terpenoids:

Salkowski test: 2 mL of chloroform and 1 mL of concentrated sulphuric acid was added to 1 mg of extract. Reddish brown colour indicated the presence of terpenoids.

Proteins and amino acids:

Xanthoproteic test: The extracts were treated with few drops of concentrated nitric acid. The yellow colour formed indicated the presence of proteins and amino acid.

Coumarins test:

NaOH test: 2 mL of extracts was treated with 3 mL of 10 % NaOH in a test tube. The solution turns to yellow colour, then it contains coumarins.

Phenolic compound:

Ferric chloride test: 2 mL of 5 % $FeCl_3$ solution were added to 1 mL of extracts. The dark blue colour indicated the presence of phenolic compound.

3.4 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS was used to evaluate the volatile components in methanol extract of *Syzgium cumini* seeds and pulp. At 260 ºC, helium was used as a carrier gas in split mode when both samples were injected into the GC-MS. When electron ionization (EI) was used as the ionization method, the column flow rate was maintained at 1.21 mL/minute. The ion source and temperatures at the contact were 270 ºC and 220 ºC respectively. The temperature of the oven was set to 80 °C for 3 minutes to 300 °C for 20 minutes with a solvent delay time of 3.50 minutes. To locate the separate peaks National Institute of Standards and Technology (NIST) used mass spectrum data [16].

Figure 3. GC-MS instrument

3.5 Ultra High Performance Liquid Chromatography-Quadrupole time of Flight- Mass Spectrometry (UHPLC-QTOF-MS) analysis

The methanolic extract of *Syzygium cumini* seeds and pulp was analyzed using UHPLC and mass spectrometry, HPLC (Waters, SYNAPT-XS HDMS, UK) fitted with AD pump, degasser, AD auto sampler controller, AD column, and quadrupole time-of-flight mass spectrometer (QTOF-MS). It was used to determination of secondary metabolites in the methanol extracts of the seeds and pulp. Both extracts were combined with formic acid (1%, 10 mL) in water and put it away for 10 minutes. 10 mL of acetonitrile and 10 mL of methanol were combined and vortexed for 1 minute then 5 minutes centrifuged at 5000 rpm. The sample was diluted with acidified water and then it was injected (5 µL) into the instrument. The samples were separated by chromatography using column C18 (Waters, Acquity BEH 2.1), which has a size of 100 mm x 2.1 mm. The mass spectroscopic analysis was performed in positive mode (ESI+) with the following conditions: desolvation gas flow of 950 L/h, source

temperature of 120 °C, cone gas flow of 50 L/hour and capillary voltage of 3.22 keV. The secondary metabolites were eluted via a binary mobile phase at a flow rate of 0.2 mL/minute where solvent A was LC-MS grade water with 1% formic acid and solvent B was 1% formic acid with acetonitrile. The Chem Spider program was used to gather and process the data [17].

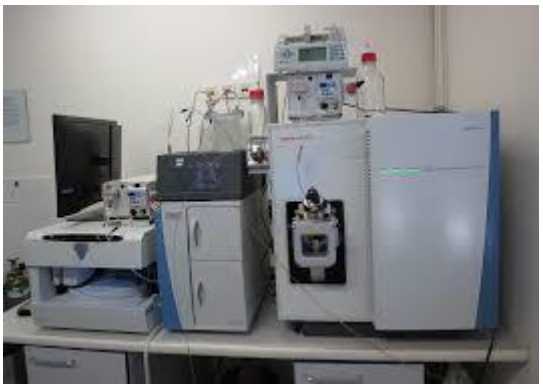

Figure 4. UHPLC-QTOF-MS (Waters, SYNAPT-XS HDMS, UK)

3.6 *In-vitro* biological activity

3.6.1 Anti-oxidant activity

3.6.1.1 $DPPH^+$ radical scavenging activity

The activity of antioxidants was evaluated using the method 1,1-diphenyl-2-picrylhydrazyl (DPPH). The different concentrations of 5 µL of methanol extracts (0.5 µg/mL, 0.08 µg/mL, 0.16 µg/mL, 0.31 µg/mL, 0.63 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5 µg/mL) were mixed with 0.0.1 ml of DPPH methanol solution. Samples were incubated for 30 minutes in the darkness and each sample's absorption was measured at 495 nm with the help of microplate readers. 20 µL deionized water was used as a control in a reaction mixture. Using the following formula, determine % of the radical scavenging by using the following formula [18].

$$\%\ DPPH\ Scavenging\ activity$$

$$= \frac{\text{Absorbace of control} - \text{Absorbance of sample}b}{\text{Absorbance of control}} \times 100$$

3.6.2Antifungal activity

3.6.2.1 Agar well diffusion method

The antifungal activity of *Syzygium cumini* methanol extracts has been evaluated using a Zone Inhibition Method (Kirby-Bauer). To inoculate SDA plates, spread 100 µL of *C. albicans* fungal culture (adjusted to 0.5 McFarland units - approximate cell density 1.5 ×108 CFU/mL) and place a disk containing 10 µL of different concentrations (0-100 percent). Each plate contains only one disc loaded with solvents, which is used to control the vehicle, and the Amphotericin B disc (50 g) is used as a positive control. *C. albicans* plates were incubated for 24 hours at 37°C. Clear zones formed around the disc were measured and reported [19].

3.6.3Anti-diabetic activity

3.6.3.1 α-amylase inhibition assay

α- amylase inhibition was determined by Syama et al., with some modifications. The α- amylase activity was used 96 well plate. Sodium phosphate buffer with 0.6 mM NaCl formed at pH of 6.9. In each well containing 20 µL of soluble starch (1% W/V, 20 mg/mL), 10 µL of plant extract (1 mg/mL), and 20 µL of acarbose. 10 µL of α-amylase (12 mg/1mL) was added and then incubated at 37 ºC for 15 minutes. 100 µL of iodine reagent and 10 µL of HCl (1M) were added. Two different wells were utilized for positive and negative controls: 50 µg/mL and 20 µL/mL acarbose. The absorbance was determined at 620 nm using a microplate reader [10].

$$\%\ inhibition = \frac{\text{Absorbance of control} - A\textit{bsorbanceof sample}}{\textit{Absorbance of control}} \times 100$$

3.6.3.2 α- glucosidase inhibitory activity

With some modifications, the inhibition activity of α- glucosides was determined by Rauf et al., and the pH was maintained at 6.8 by the formation of a 100 mM sodium phosphate buffer. A reaction mixture contains 50 µL of phosphate buffer (100 mM, pH 6.8), 20 µL of extracts (0 to 1000 g/mL) and 10 mL of α-glucosidase (1 U/mL). Then, 15 minutes pre incubation at 37 °C on 96 well plates. Subsequently, add 20 µL of P-NPG (5mM) to the substrate and incubate the reaction mixture for 20 minutes at 7°C. Add 50 µL Na_2CO_3 to stop the reaction. Measurement of 405 nm p-nitrophenol absorption using ELISA microplate reader. Acarbose was utilized as positive control at 1mg/mL [20].

$$Inhibitory\ activity\ (\%) = 1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ conrol} \times 100$$

3.6.3.3 DPP-IV (Dipeptidyl peptidase) inhibitor activity

The DPP-IV inhibitory activity was carried out using Gly-pro-pNA as a substrate described by Robbani et al., A total of 100µL of Gly-Pro-pNA (0.5 mM Gly-Pro-pNA in 50 mM tris buffer, pH 8.3, with 0.1% Tween 20) was combined with 10 µL of both samples on a 96-well plate. The DPP-IV activity kinetics was evaluated by monitoring the velocities of pNA release from the chromogenic substrate over 10 minutes incubated at 37 ^{o}C. The absorbance was analyzed at 405 nm [21].

$$DPP - IV\ Activity\ (\ U\ /\ mL) = \frac{\mathrm{OD} \times \mathrm{V}}{\varepsilon \times v \times df}$$

ΔOD = Change in absorbance per minute at 405 nm.

V = Volume of the reaction mixture

v = Volume of Sample

ε = micromolar Extinction Coefficient at 405 nm (0.0102 $\mu M^{-1}cm^{-1}$)

df = sample dilution factor

3.6.3.4 Reactive oxygen species (ROS) activity measurement

The probe 2'-7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) was used to produce ROS. L6 cells were cultured at 37 ºC for 24 hours after being plated 6 well plates at density of 5000–8000 cells/well in 1 mL Dulbecco's Modified Eagle Medium (DMEM) with 1% Antibiotic solution and 10% FBS (Fetal Bovine Serum). The cells were incubated at different concentrations for further 24 hours. After incubation, the medium was withdrawn and cells were collected using trypsin-EDTA in a 1.5 mL tube and then again washed with 500 µL cold PBS. 100 µL of PBS (Phosphate Buffered Saline) containing 2 µM DCFDA added in cell plate and then samples were collected using a BD FACS Calibur flow cytometry in an hour. The acquired data were examined using the 2.5.1 version of the flowing [22].

3.6.3.5 Insulin secretion assay

Insulin secretion in cells was determined by Z. Sameermahmood et al., with some modifications [23]. For this purpose, the cells were lysed in 50 µL buffer in each well of a 96 well plate, 200-500 µL for 1 well of a well plate or 50 mM dish plate. After that, it was centrifuged for 5 minutes at 10,000 rpm and the supernatant was collected. Then, 100 µL metformin standard sample, 100 µL samples (both pulp and seeds) and 100 µL detection antibody (conjugated detection antibody) solution were added to each well and then plate was sealed and incubated for 2 hours at room temperature. After that, the plate was washed with wash buffer four times and the buffer was blotted by

firmly tapping plate upside down on absorbent paper. 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) solution was added and incubated for 15 minutes in the dark. To stop the reaction, 100 µL of stop solution was added to each well and the absorbance was measured at 450 nm within 5 minutes [24].

3.6.4 Cytotoxicity assay

Cytotoxicity of extracts on L6 cell line was previously described by S. kim et al., with some modification. 10,000 cells/well were cultured in DMEM (Dulbecco Modified Eagle Medium) in 96 well plates containing 10% FBS (Female Bovine Serum), 1% antibiotic solution and 1 day at 37 °C in an incubator with 5% CO_2. Then, the cells added various samples (0 µg/mL, 1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL). After 24 hours, a 10 µL MTT solution was placed in each well, and incubated for two hours [25].

CHAPTER- 4

RESULTS AND DISCUSSION

4.1 Phytochemical analysis of *Syzygium cumini* pulp and seeds

The data obtained from phytochemical study determined that saponins, flavonoids, carbohydrate, coumarins and glycoside were present in methanol extract of *Syzyium cumini* pulp while absence of phlobatannin, tannins, proteins and amino acids terpenoids and phenolic compound. The methanolic extract of *Syzyium cumini* seeds shows the presence of saponins, tannins, flavonoids, carbohydrate, coumarins, glycoside and phenolic compound while absence of terpenoids, phalobatannin, proteins and amino acids. Saponins, carbohydrate, flavonoids, glycoside and coumarins were identified in both pulp and seeds as shown in table 1. These phytochemicals were identified as biologically active components in both *Syzygium cumini* seeds and pulp. These phytoconstituents shows various activities such as flavonoids shows anti-diabetic, anti-cancer, anti-inflammatory [26]. Tannins also shows anti-diabetic, anti-inflammatory, anti-oxidant, anti-microbial properties [27].

Table 1. Phytochemical analysis of *Syzygium cumini* pulp and seeds

Phytochemicals	**Result**		
	Test name	**Pulp extract**	**Seed extract**
Tannins	Braymer's test	-	+
Saponins	Foam test	+	+
Phalobatannins	HCl test	-	-
Carbohydrate	Molisch's test	+	+
Flavonoids	Lead acetate test	+	+
Glycoside	Keller -Killani test	+	+
Terpenoids	Salkowski test	-	-
Proteins and Amino acids	Xanthoproteic test	-	-

Coumarins	NaOH test	+	+
Phenolic compound	Ferric chloride test	-	+
+ = Presence of Phytochemicals - = Absence of Phytochemicals			

4.2 Gas Chromatography- Mass Spectrometry (GC-MS) analysis of *Syzygium cumini* seeds and pulp extract

21 components were determined in the methanolic extract of *Syzgium cumini* pulp and 33 components in seeds by GC-MS studies. The major components were found to be 5- [hydroxy methyl] -2-furaldehyde (60.61%), Pyranone (12.76%), 2-furoic acid (5.08%) ,5-oxy-dimethylene-bis(2-furaldehyde) (4.33%) in pulp and major components were found to be 5-Oxymethylfurfurole (45.78%), Pyranone (9.84%), Pyrogallol (4.24%), Cycloisolongifolene (3.12%) in seeds as shown in table 2. These bioactive compounds have various biological activities. Pyranone shows anti-fungal, cytotoxic, antileishmanial properties [28].

Table 2. Bioactive compounds identified by GC-MS analysis of methanol extract of *Syzgium cumini*

Compound Name	**RT (min)**	**Area%**	**MW (g mol^{-1})**	**MF**
Pulp extract				
2-Butenolide	3.377	0.27	84	$C_4H_4O_2$
Heptanol	3.490	0.11	114	$C_7H_{14}O$
Furan-2-carboxylic acid	3.713	0.48	112	$C_5H_4O_3$
5-Methylfuran-2-al	3.957	1.33	110	$C_6H_6O_4$
2,4-Dihydroxy-2,5dimethyl-3(2H) furnone	4.200	1.81	144	$C_6H_8O_4$
Levulinic acid	5.130	1.97	116	$C_5H_8O_3$
5-Formylfurfural	5.597	1.20	124	$C_6H_4O_3$
2-Furoic acid	5.680	5.08	126	$C_6H_6O_3$
n-Nonyl acetate	6.127	0.23	186	$C_{11}H_{22}O_2$
Pyranone	6.707	12.76	144	$C_6H_8O_4$

2-Methyl-2H-pyran-3,4,5(6H)-trione	7.237	0.61	142	$C_6H_6O_4$
1-Methylbutyl propionate	7.470	0.48	144	$C_8H_{16}O_2$
5-[hydroxy methyl] -2-furaldehyde	8.120	60.61	126	$C_6H_6O_3$
Butane,1,I'-[methylene bis(oxy)]bis [3-methyl]	10.323	0.70	188	$C_{11}H_{24}O_2$
Hexose	11.873	2.76	180	$C_6H_{12}O_6$
Machilol	13.223	0.12	222	$C_{15}H_{26}O$
(+)-β- eudesmol	13.520	0.13	222	$C_{15}H_{26}O$
Octadec-9-enoic acid	16.603	0.19	282	$C_{18}H_{34}O_2$
5-5'-oxy-dimethylene-bis(2-furaldehyde)	16.957	4.33	234	$C_{12}H_{10}O_5$
3,4-furandimethanol	18.313	0.40	128	$C_6H_8O_3$
4,6-Dihydrofuro[3,4-b] furan	24.743	0.17	110	$C_6H_6O_2$
Seeds extract				
2-Oxepanone	3.363	0.44	114	$C_6H_{10}O_2$
5-Methyl furfural	3.960	0.59	110	$C_6H_6O_2$
2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	4.197	1.51	144	$C_6H_8O_4$
4-Hydroxy-3-hexanone	4.677	0.19	116	$C_6H_{12}O_2$
2,5-Dimethyl-3(2H) furanone	5.277	0.09	128	C_6H_8O3
2-Methyl-3-hydroxy-4-pyrone	5.943	0.14	126	$C_6H_6O_3$
Nonyl acetate	6.123	0.17	186	$C_{11}H_{22}O_2$
Triethylaluminum	6.337	0.19	114	$C_6H_{15}Al$
5-(hydroxymethyl) furan-2-carbaldehyde	6.420	0.14	126	$C_6H_6O_3$
Pyranone	6.663	9.84	144	$C_6H_8O_4$
5-hydroxy-2-(hydroxymethyl) pyran-4-one	7.217	0.47	142	$C_6H_6O_4$
Tetrahydro-5-methyl-2-furanmethanol	7.443	0.61	116	$C_6H_{12}O_2$
5-Oxymethylfurfurole	8.007	45.78	126	$C_6H_6O_3$
(4S)-1-methyl-4-(6-methylhepta-1,5-dien-2-yl) cyclohexene	10.123	0.60	204	$C_{15}H_{24}$

Benzene-1,2,3-triol	10.250	4.24	126	$C_6H_6O_3$
1,1,4,7-tetramethyl-1a,2,3,4,5,6,7,7b-octahydrocyclopropa[e]azulene	10.887	0.53	204	$C_{15}H_{24}$
1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene	11.117	0.50	202	$C_{15}H_{22}$
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	11.253	0.23	204	$C_{15}H_{24}$
α- amorphene	11.317	0.23	204	$C_{15}H_{24}$
α- cubebin	11.373	0.28	204	$C_{15}H_{24}$
β-selinene	11.490	0.24	204	$C_{15}H_{24}$
(-)-α-gurjunene	11.637	3.00	204	$C_{15}H_{24}$
Hexose	11.773	1.47	180	$C_6H_{12}O_6$
CycloisolongiIfolene	12.520	3.12	202	$C_{15}H_{22}$
β-Vetivenene	12.563	2.20	202	$C_{15}H_{22}$
1H-Cycloprop[E]azulen-4-ol, decahydr	12.720	1.07	222	$C_{15}H_{26}O$
1,1,7,7a-Tetramethyl-1a,2,6,7,7a,7b-hexahydro-1H-cyclopropa[a]naphthalene	13.083	1.01	202	$C_{15}H_{22}$
Zizanal	14.663	0.13	218	$C_{15}H_{22}O$
1-[2-(2,2,6-Trimethylbicyclo [4.1.0]hept-1-yl)ethyl]vinyl acetate	15.267	2.22	250	$C_{16}H_{26}O_2$
Palmitinic acid	16.590	0.24	256	$C_{16}H_{32}O_2$
Cis-linoleic acid methyl ester	17.847	0.09	294	$C_{19}H_{34}O_2$
(E)-phytol	18.007	0.17	296	$C_{20}H_{40}O$
β-dihydrofucosterol	28.597	0.56	414	$C_{29}H_{50}O$
RT = Retention Time MF = Molecular Formula MW = Molecular Weight				

4.3 UHPLC-QTOF- MS analysis of methanol extract of *Syzygium cumini* seeds

UHPLC-QTOF-MS analysis was done to detect secondary metabolites present in methanol extract of *Syzygium cumini* seeds given in Table 3., and it validated the existence of theoplus, allolactose, and melibiose. 9-{2-deoxy-5-O-[(phosphonatooxy) phosphinato]pentofuranosyl}-9H-purin-amine,5Methylphenazine-1-carboxylate, gypsogenic acid, Juvenile hormone, 2-Oxo-10-methylthiodecanoate. Theophylline can be found in many of the "foods and drinks" that we consume on a regular basis. Caffeine metabolites, such as Theophylline, are widely utilized to treat adult asthma and bronchospasm. Caffeine is frequently contained in "nonprescription" medications such cold remedies, diet pills, diuretics, and stimulants[29]. Cellobiose dehydrogenase limits bacterial growth by generating hydrogen peroxide. Hydrogen peroxide acts on bacterial cells by generating radicals that target vital cell components such as proteins, lipids, and DNA [30].

Table 3**.** UHPLC- QTOF- MS Of Methanol Extract of *Syzygium cumini* Seeds

Peak no.	**Metabolites Name**	**RT (min)**	**MF**	**MM ($gmol^{-1}$)**	**$[M-H]^+$**	**Error (ppm)**	**Compound ID**
1.	1,3-Dimethyl-3,7-dihydro-1H-purin-2,6-dion	2.34145	$C_7H_8N_4O_2$	157.075	158.0828	3.894262	CSID2068
2.	4-O-β-D-Glucopyranosyl-β-D-glucopyranose	2.34145	$C_{12}H_{22}O_{11}$	319.1265	320.1343	-1.264841	CSID10261
3.	6-O-α-D-Galactopyranosyl-β-D-glucopyranose	2.34145	$C_{12}H_{22}O_{11}$	303.0179	304.0257	-1.667501	CSID10974
4.	9-{2-deoxy-5-O-[(phosphonatooxy) phosphinato]pentofuranosyl}-9H-purin-6-amine	3.34425	$C_{10}H_{12}N_5O_9P_2$	407.0048	408.0126	3.966657	CSID24785085

5.	Dihydroferulic acid 4-sulfate	4.381333	$C_{10}H_{12}O_7S$	274.9952	276.0303	4.2992	CSID162994
6.	5-Methylphenazine-1-carboxylate	4.381333	$C_{14}H_{10}N_2O_2$	237.0664	238.0742	3.9372	CSID26332581
7.	2-Isopropyl-3-oxosuccinat	5.1582	$C_7H_8O_5$	132.9399	133.9477	3.9863	CSID24785380
8.	5'-O-[({[2(Diméthylamino)ethoxy](hydroxy) phosphoryl} oxy)(hydroxy)phosphoryl]cytidine	12.15871	$C_{13}H_{24}N_4O_{11}P_2$	434.9933	436.0011	3.4730	CSID28184664
9.	4'-O-demethylrebeccamycin	12.15872	$C_{26}H_{19}Cl_2N_3O_7$	554.0521	555.0599	0.9139	CSID25052047
10.	Guanosine-5'-diphospho-D-mannose	12.52141	$C_{16}H_{25}N_5O_{16}P_2$	604.0693	605.0771	4.1306	CSID17372
11.	Gibberellin A44 diacid	13.97243	$C_{20}H_{26}O_6$	361.1662	362.174	-2.5501	CSID24784737
12.	Medicagenic acid	21.71473	$C_{30}H_{46}O_6$	439.2336	440.2414	2.0661	CSID385658
13.	Gypsogenic acid	21.71473	$C_{30}H_{44}O_5$	460.3224	461.3302	-0.21858	CSID24784682
14.	8',10-diapocarotene-8',10-dial	22.12	$C_{17}H_{20}O_2$	255.1385	256.1463	-1.599186	CSID58829813
15.	(1S,5R,8R)-1,5-dimethyl-8-[(1E)-3-oxo-1-buten-1-yl]-6-oxabicyclo [3.2.1] octan-3-on	23.73697	C13H18O3	183.1817	184.1895	2.632873	CSID59700305
16.	(-)-Pipecolic acid	23.73697	C6H11NO2	106.089279	107.097079	1.114818	CSID388365
17.	4α-carboxy-4β-methyl-zymosterol	24.5139333	$C_{29}H_{46}O_3$	441.3368	442.3446	-2.81901	CSID20059528
18.	Gypsogenin	24.5139333	C30H46O4	469.3318	470.3396	-1.321445	CSID 83794
19.	Juvenile hormone	24.5139333	C16H26O3	265.1804	266.1882	2.255609	CSID 4444856
20.	Xanthoxic acid	24.51393	$C_{15}H_{22}O_4$	265.1439	266.1517	-4.112643	CSID 4445404

21.	(-)-Cholesterol	24.51393	$C_{27}H_{46}O$	363.3651	364.3729	-0.6437	CSID 5775
22.	(1S,2R,4aS,6aR,6aS,6bR,8aR,9R,10R,11R,12aR,14bS)-10,11-Dihydroxy-9-(hydroxymethyl)-1,2,6a,6b,9,12a-hexamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid	24.51393	$C_{30}H_{48}O_5$	487.3423	488.3501	-1.61515	CSID 24785685
23.	4'-apo-β-carotenal	24.51393	$C_{35}H_{46}O$	443.2565	444.2643	1.871908	CSID21378036
24.	6-Deoxo-3-dehydro Teasterone	24.51393	$C_{28}H_{46}O_3$	407.3549	408.3627	3.7891	CSID58837421
25.	Lecithin	24.51393	$C_{44}H_{78}NO_8P$	778.5387	789.5465	-2.1406	CSID24766802
26.	2-Oxo-10-methylthio Decanoate	25.17118	$C_{11}H_{19}O_3S$	230.0982	231.1060	2.018333	CSID 24785262
27.	(+)-Costunolide	25.41257	$C_{15}H_{20}O_2$	231.1385	232.1463	4.175053	CSID 4444782
28.	Costus lactone	25.41257	$C_{15}H_{20}O_2$	231.1385	231.1463	4.175053	CSID4519051
29.	D-4'Phosphopantothenic acid	25.9308	$C_9H_{18}NO_8P$	298.0691	299.0769	1.042549	CSID128
30.	(-)-Narcotine	25.9308	$C_{22}H_{23}NO_7$	412.1396	413.1474	-0.20229	CSID242139
31.	L-γ-Glutamyl-N-(2-carboxypropyl)-D-cystein	25.9308	$C_{12}H_{20}N_2O_7S$	335.0913	336.0991	1.719594	CSID81407923
32.	4,4'-Tetrahydro-1H,3H-furo[3,4-c]furan-1,4-	27.65915	$C_{20}H_{22}O_6$	357.1338	358.1416	-0.31675	CSID204822

	diylbis(2-methoxyphenol)						
33.	Phaseolin fungicide	27.6591 5	$C_{20}H_{18}O_4$	321.1127	322.1205	-0.387478	CSID3279224
34.	(S)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one	27.6591 5	$C_{17}H_{26}O_4$	255.0848	256.0926	3.714655	CSID3354
35.	Uniconazole	27.6591 5	$C_{15}H_{18}ClN_3O$	290.1060	291.1138	-1.032795	CSID 4941263
36.	1-linoleoyl-GPC	28.6441 5	$C_{26}H_{50}NO_7P$	495.3349	496.3427	-1.997256	CSID 9181014
37.	Rosafluine	29.6125	$C_{14}H_{20}O_2$	219.1385	220.1463	1.440591	CSID 23107138
38.	6-O-β-D-glucopyranosyl-β-D-glucopyranoside de (2R)-4-[(1R)-2,6,6-triméthyl-4-oxo-2-cyclohexén-1-yl]-2-butanyle	29.6125	$C_{25}H_{42}O_{12}$	533.2598	534.2676	1.121297	CSID 59700315
39.	2-Hydroxy-4-isopropyl-3-methoxy-10a-methyl-7-(4-methylpent-3-en-1-yl)-9,10-dioxo-8,8a-dihydro-5H-anthracene-1-carbaldehyde	29.9926 6	$C_{26}H_{32}O_5$	401.2352	402.2430	0.531847	CSID 35015011
40.	Heliocide H3	30.5626 3	$C_{25}H_{30}O_5$	371.1110	372.1182	1.637917	CSID 158697
41.	(1R,4R)-4-[(1E,3R)-3-Hydroxy-1-buten-1-yl]-3,5,5-trimethyl-2-cyclohexen-1-ol	30.8224 5	C13H22O2	209.1541	210.1619	3.094544	CSID 10364083
42.	1,5-Diaminopentane	30.8224 5	$C_5H_{14}N_2$	79.126	80.1338	-2.89467	CSID 13866593
43.	(-)-Deoxypodophyllotoxin	31.8938 2	$C_{22}H_{22}O_7$	397.1287	398.1365	4.435146	CSID 2118

44.	1,2-dioctanoyl-1,2,6-hexanetriol	33.70798	$C_{22}H_{42}O_5$	385.2954	386.3032	0.517021	CSID 58829687
RT = Retention Time MF = Molecular Formula MM = Molecular Mass							

4.4 UHPLC-QTOF-MS study of methanol extract from *Syzygium cumini* pulp

UHPLC-QTOF-MS analysis was done to detect secondary metabolites contained in methanol extract of Syzygium cumini pulp given in Table 4, which demonstrates the presence of 1-Oxo-1-[(2-oxo-8-heptadecanyl)oxy-9-octadecanyl (9E)-9-octadecenoate, Curcumin, Gypsogeninic acid, Geroquinol, 2-(2-Methylencyclopropyl)-3-oxosuccinate, Leinolic acid, Geraniol acetate, and Dioxindole-3-acetate. Geraniol demonstrates a variety of biochemical and pharmacological activities. It is a key molecule in the taste and scent industries. It has both insecticidal and repellant effects. It contains antimicrobial and anti-tumor effects[31]. Curcumin scavenges superoxide radicals, hydrogen peroxide, and nitric oxide while inhibiting lipid peroxidation. These effects could explain several of its pharmacological and therapeutic benefits. It has potent anti-carcinogenic, anti-inflammatory, antioxidant, antimicrobial, and anti-parasitic properties [32].

Table 4. UHPLC- QTOF- MS of Methanol Extract *of Syzgium cumini* Pulp

Peak No.	**Metabolites identified**	**RT (min)**	**MF**	**MM ($gmol^{-1}$)**	**$[M-H]^+$**	**Error (ppm)**	**Compound ID**
1.	1-Oxo-1-[(2-oxo-8-heptadecanyl) oxy]-9-octadecanyl (9E)-9-octadecenoate	2.39	$C_{53}H_{100}O_5$	793.7673	794.7751	3.8	CSID24785451
2.	(2R,3S,4S)-leucocyanidin	12.53	$C_{15}H_{14}O_7$	266.9756	267.9834	1.6	CSID389677

3.	2,4-diamino-6-ethyl-5,3'-(2-trifluoromethyl-4-sulphonamidophenoxy) prop-1'-yloxypyrimidine	13.83	$C_{16}H_{20}F_3N_5O_4S$	434.1110	435.1178	1.7	CSID58829803
4.	Eupatolitin 3-glucoside	14.59	$C_{23}H_{24}O_{13}$	485.1320	486.139	-1.4	CSID8544432
5.	Rosafluin	21.74	$C_{14}H_{20}O_2$	219.1385	220.1463	2.4	CSID23107138
6.	Gypsogeninic acid	22.75	$C_{30}H_{46}O_5$	485.3267	486.3345	-3.2	CSID10217372
7.	1-Hexadecanoyl-sn-beta-D-galactopyranosylglycerol	23.54	$C_{25}H_{48}O_9$	491.3220	492.3298	-4.0	CSID58829698
8.	2-(2-Methylencyclopropyl)-3-oxosuccinat	1.99	$C_8H_6O_5$	181.0148	182.0226	1.2	CSID24785550
9.	Lambertine	2.20	$C_{20}H_{19}NO_4$	314.1417	315.1495	-2.8	CSID9800
10.	Geraniol acetate	2.34	$C_{12}H_{20}O_2$	195.1385	196.1463	-0.3	CSID1266019
11.	Desethylsimazine	3.34	$C_5H_8ClN_5$	133.9485	134.9563	4.9	CSID13278
12.	4-(2-Hydroxyethyl) phenylhydrogen sulphate	4.38	$C_8H_{10}O_5S$	195.0351	196.0429	-4.9	CSID30777652
13.	1-Glycerophosphate	10.70	$C_3H_7O_6P$	147.0094	148.0172	-1.4	CSID2939444
14.	Dioxindole-3-acetate	12.15	$C_{10}H_8NO_4$	205.0380	206.0458	1.7	CSID24784782
15.	(1S,5R,8R)-1,5-Dimethyl-8-[(1E)-3-oxo-1-buten-1-yl]-6-oxabicyclo [3.2.1] octan-3-one	18.72	$C_{13}H_{18}O_3$	221.1177	222.1255	3.6	CSID59700305
16.	Gypsogenate	21.71	$C_{30}H_{44}O_5$	483.3121	484.3199	-4.4	CSID24784682
17.	(+) Gomisin-M2	22.12	$C_{22}H_{26}O_6$	385.1651	386.172943	-2.0	CSID10278518
18.	Geroquinol	23.73	$C_{16}H_{22}O_2$	245.1541	246.1619	-1.6	CSID4445151
19.	Juvenile hormone 3	24.51	$C_{16}H_{26}O_3$	265.1804	266.1882	-0.2	CSID4444856

20.	Costundide	25.41	$C_{15}H_{20}O_2$	231.1385	232.1463	4.1	CSID4444782
21.	11-Hydroxylaurate	25.93	$C_{12}H_{23}O_3$	214.1574	215.1652	-4.8	CSID24785390
22.	Leinolic acid	27.65	$C_{18}H_{32}O_2$	279.2324	280.2402	-1.2	CSID4444105
23.	Harmidol	28.64	$C_{12}H_{12}N_2O$	177.1052	178.1130	3.4	CSID11262879
24.	Curcumin	29.61	$C_{21}H_{20}O_6$	367.1181	368.1259	1.4	CSID4445080
25.	2-Hydroxy-4-isopropyl-3-methoxy-10a-methyl-7-(4-methylpent-3-en-1-yl)-9,10-dioxo-8,8a-dihydro-5H-anthracene-1-carbaldehyde	29.99	C26H32O5	401.2352	402.2430	0.5	CSID35015011
RT = Retention Time MF = Molecular Formula MM = Molecular Mass							

4.5. *In vitro* biological assays

4.5.1. Antioxidant activity

4.5.1.1. DPPH radical scavenging activity

The methanolic extract of S*yzygium cumini* seeds and pulp showed significant variation in oxidant activity. DPPH (2, 2diphenyl-1-picrylhydrazyl) is a dark-colored crystalline powder constituted of stable free radical molecules, the scavenging of which is the basis of an antioxidant assay. DPPH was used to determine the possible antioxidant principles present in the extracts by its radical scavenging capacity measurement. The assay is based on the interaction of DPPH with antioxidants, which involves either the transfer of hydrogen atoms or electrons from the antioxidant to DPPH, thus neutralising its free radical character [2]. Liang and Yi (2009) observed hydrolysable tannins obtained from *Syzygium cumini* pulp shown excellent DPPH radical scavenging activity as well as ferric reducing/antioxidant power. The results are satisfactory, showing that the pulp can be used as a significant source of natural

oxidants. The ability of *Syzygium cumini* to scavenge free radicals and reactive oxygen species formed as a result of ionising radiation exposure demonstrates its antioxidant effectiveness. The potential for radical scavenging was discovered to be proportional to the amounts of phenolic compounds present in the fruit. Our result of Antioxidant property (DPPH scavenging) was observed in seeds (IC_{50} = 0.1592 ± 0.068µL/mL) and pulp (IC_{50}= 0.2469 ± 0.047 µL/mL) which was found equivalent to (IC_{50} = 1.12 ± 0.32µg/mL) of Ascorbic acid.

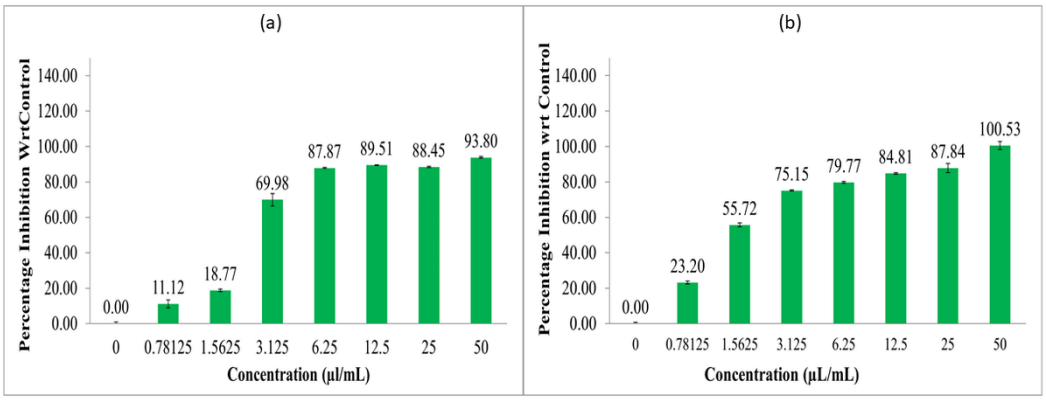

Figure 5. DPPH Scavenging activity of methanolic extract of *Syzygium cumini* (a) pulp (b) seeds

4.5.2. Anti-fungal activity

Methanol extract of the seeds and pulp of *Syzygium cumini* showed significant antibacterial activity against all the test microorganism[11]. Based on the results obtained from the study, when test organism was treated with different amount of sample on agar plate, it was found that *Syzygium cumini* seeds (Max zone of Inhibition 8.6 mm at 6.25 % dose) and *Syzygium cumini* pulp (Max zone of Inhibition 7 mm at 25% dose) having antifungal activity the test organism C. albicans as compared to positive control (Max zone of Inhibition of seeds 23.66 mm and for pulp 22.66 mm at 50 µg dose). The zone of inhibition is an area around a disk on an agar plate where no

bacterial growth is observed due to the presence of an antimicrobial agent. We can conclude from the above results that the methanol extract *Syzygium cumini* may be a source of antimicrobial agent in food, cosmetic, homeopathic and traditional ayurvedic treatments [11].

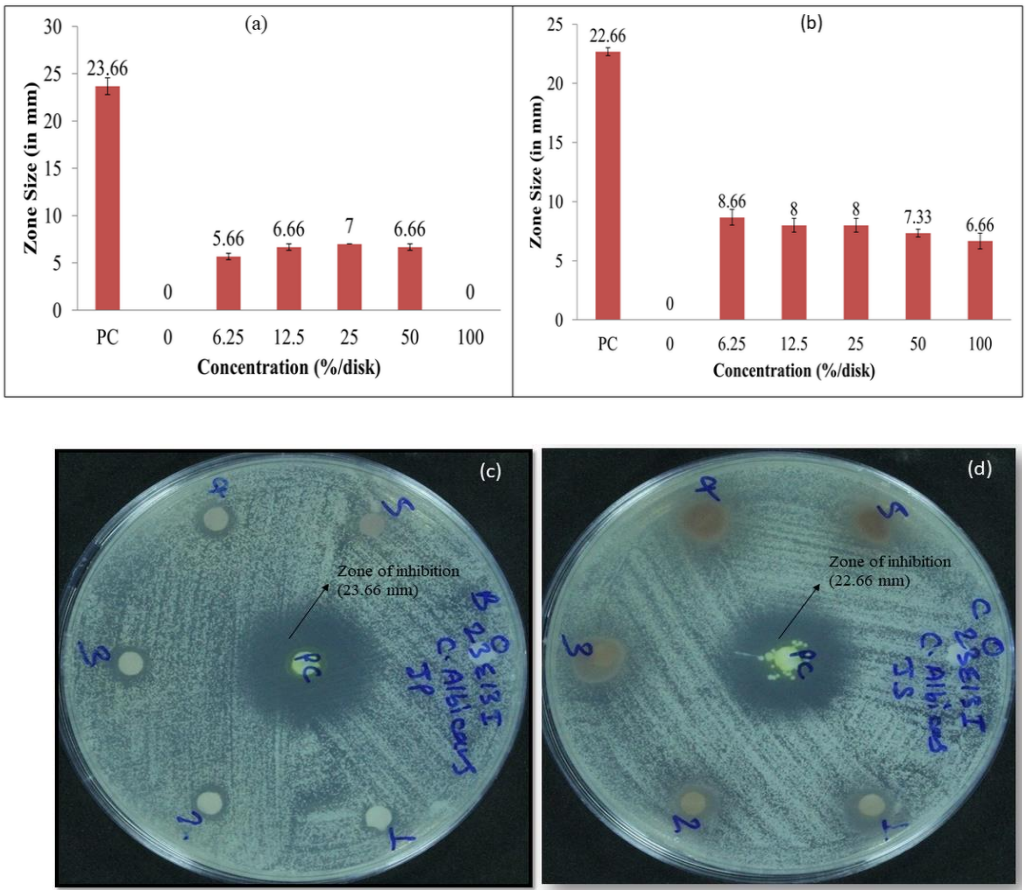

Figure 6. Antifungal activity of methanolic extract of *Syzygium cumini* (a) and (c) for pulp, (b) and (d) for seeds

4.5.3. Antidiabetic activity

4.5.3.1. α-Glucosidase inhibitory assay

The α-glucosidase enzyme is crucial for digestion and can be found in the small intestine's mucosal brush boundary. Its job is to convert and degrade complicated carbs into smaller, simpler, and more absorbable ones. Its blockage is an efficient way to postpone glucose absorption while also preventing excessive postprandial blood

glucose levels, which may slow diabetes progression. Figure 7. illustrates the inhibition of α-glucosidase enzyme by methanolic extracts of *Syzygium cumini* pulp and seeds. The inhibition of α-glucosidase enzyme is dose-dependent, as the concentration of both extracts significantly impacts the quantity of enzyme. The IC_{50} values for pulp and seed extracts are 4.80 µL/mL and 1.65 µL/mL, respectively, indicating that the seeds' methanolic extract has a higher inhibitory capacity than the pulp extract. Acarbose was utilized as a positive control and had an IC_{50} value of 2.50 µL/mL inhibited [33].

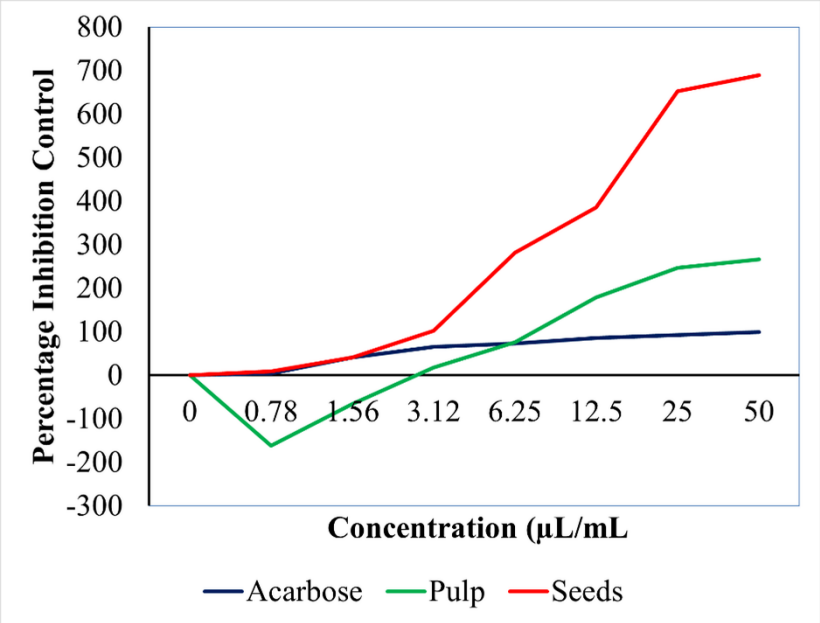

Figure 7. α-glucosidase inhibitory assay of methanolic extract of *Syzygium cumini* for pulp and seeds

4.5.3.2. DPP-IV inhibitor activity assay

Figure 8. shows the results of DPP-IV inhibitory activity in both methanolic extracts of *Syzygium cumini*. Pulp extract had the strongest inhibitory activity, with a percentage inhibition value of 1.14%, compared to seeds, which had a percentage inhibition value of 1.48%, and the control, which was a positive control, showed 2.23%

inhibition. The results show that methanolic extracts of seeds are more potent against the DPP-IV enzyme than pulp [21].

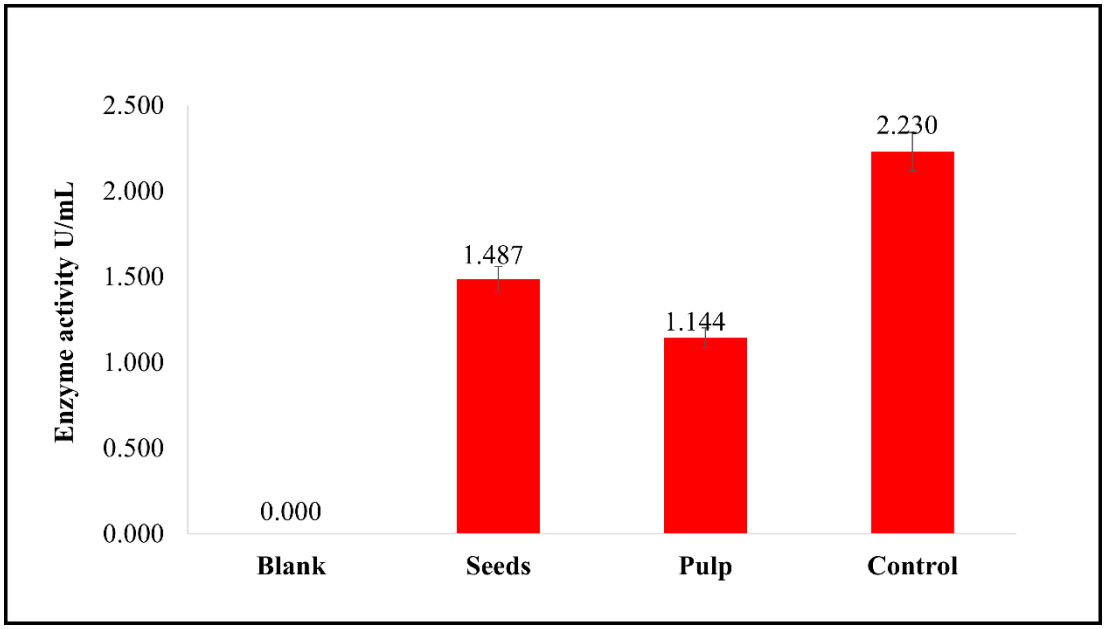

Figure 8. DPP-IV inhibitor activity assay of methanolic extracts of *Syzygium cumini* for pulp and seeds

4.5.3.3 ROS measurement

The results of ROS measurement in L6 cell line exposed to both pulp and seeds of methanolic extract of *Syzygium cumini* are shown in Figure 9. The ROS measurement was found to increase in a dose-dependent manner. Highest ROS production was observed in methanol extract of seeds (9.12%) as compared to pulp (6.56%) [22].

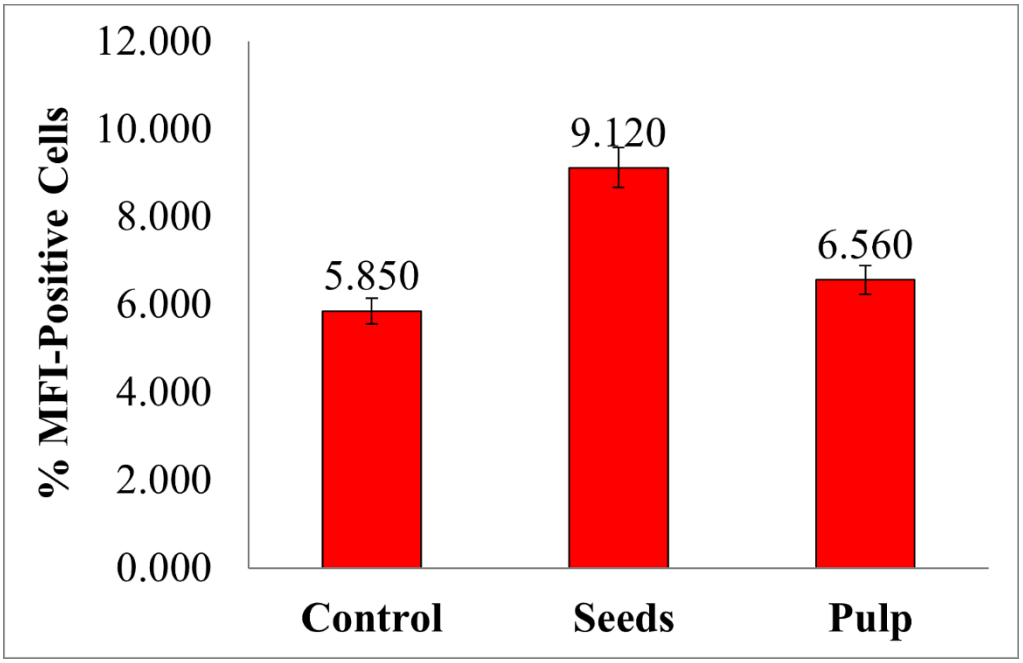

Figure 9. ROS measurement of methanolic extract of *Syzygium cumini* pulp and seeds

4.5.3.4. α-Amylase inhibitory assay

The antidiabetic efficacy of methanol extracts of *Syzygium cumini* pulp and seeds was tested to corroborate its traditional use in the treatment of diabetes. α-Amylase, found mostly in saliva and pancreatic fluid, plays a crucial role in digestion. Targeting and blocking this enzyme is a potential method for preventing high postprandial blood glucose levels. Our findings also support its use by demonstrating substantial antidiabetic activity against various diabetes enzymes in figure 10. When compared to pulp extract, seed extract shows a promising effect. The pulp methanol extract had the highest inhibition rate (IC_{50} = 0.238 µL/mL), whereas the seed extract had an IC_{50} of 0.05 µL/mL. Acarbose was employed as a positive control and had an IC_{50} value of 0.60 µL/mL. It shows that the methanolic extract of pulp and seeds significantly inhibited α-amylase activity[34].

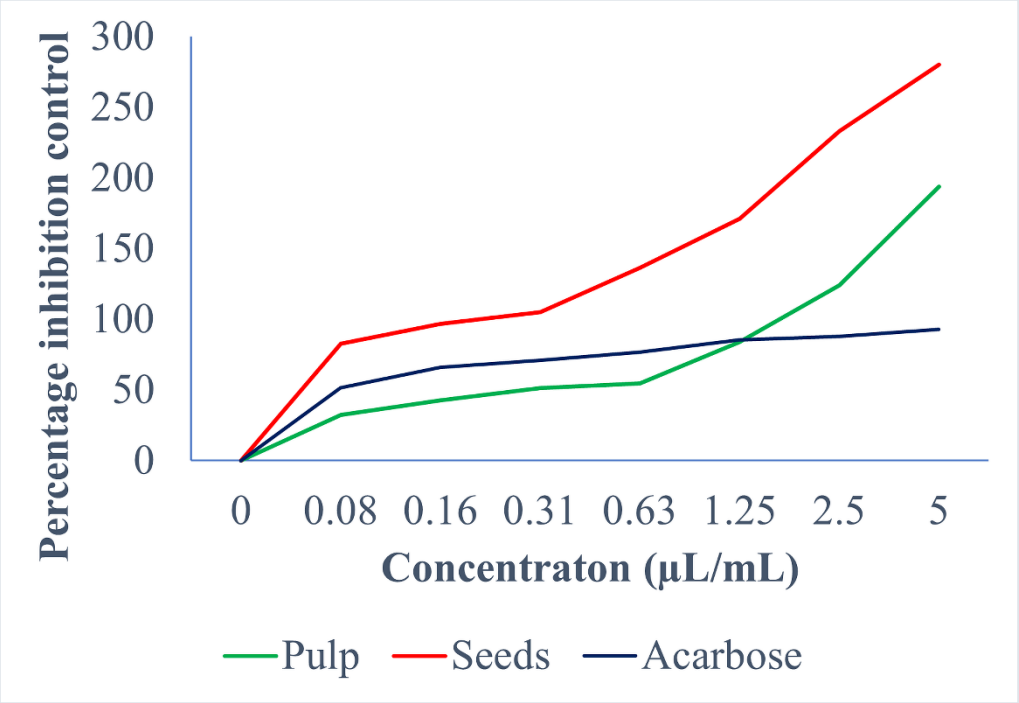

Figure 10. α-Amylase Inhibitory assay of pulp and seeds extracts of *Syzygium cumini*

4.5.3.5. Insulin secretion assay

According to the results obtained after analysis, different insulin concentrations were observed in the sample of *Syzygium cumini* seeds and pulp shown in Figure 11. The highest concentration was observed in sample *Syzygium cumini* pulp (297.73 U/mL Lysate) and the lowest in sample *Syzygium cumini* seeds (244.45 U/mL Lysate) with respect to control [35].

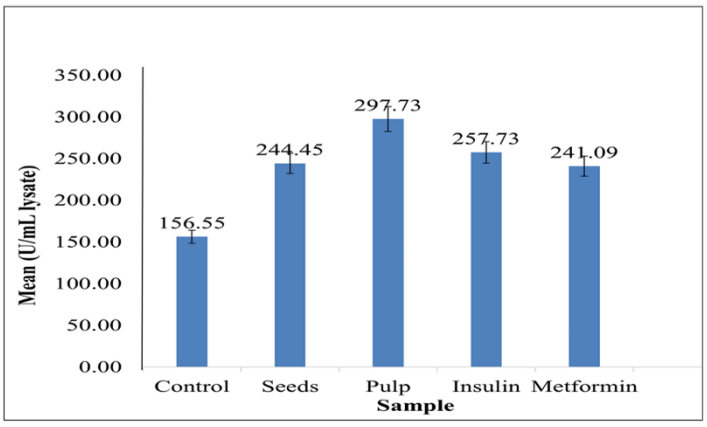

Figure 11. Quantitative estimation of Insulin

4.5.4. Cytotoxicity assay

The anti-cytotoxic effect of methanol extracts of both pulp and seeds of *Syzygium cumini* against the L6 cell line (myoblast cell line) is represented in Figure 12. At varying concentrations, there were notable differences in the proportion of viable cells in both the pulp and seed extracts. On the L6 cell line, pulp extract showed 34.48 % viability at 1000 µg/mL, but seed extract shown in figure 8(b) demonstrated 22.14 % viability. However, the pulp extract shown in figure 8 (a) demonstrated 94.82 % vitality at a 1 µg/mL concentration, while the seed extract showed 80.82% viability. The decline in cell viability was observed in a concentration dependent manner. The inhibitory concentration (IC_{50}) values obtained by the MTT assay for pulp and seeds were 314 ± 0.09 µg/mL and 83.9 ± 0.1 µg/mL respectively. Interestingly, both pulp and seed extracts did not show any toxic effects at lower concentrations and less cytotoxicity was found at the increased concentration of pulp extract above 250 µg/mL, while for seed extract it was above 50 µg/mL. Hence, it can be inferred from the results that at a limited dosage, both the pulp are biocompatible and not harmful the human beings as well as other living organisms [25].

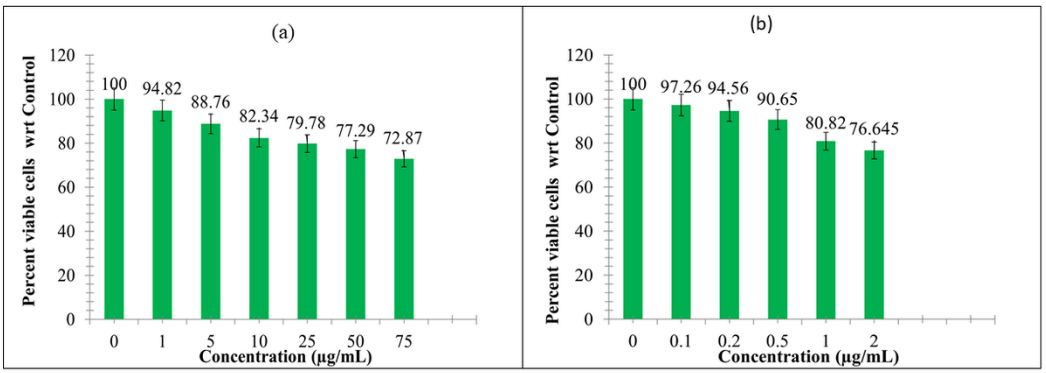

Figure 12. MTT assay – L6 of both extract of *Syzygium cumini* (a) pulp and (b) seeds

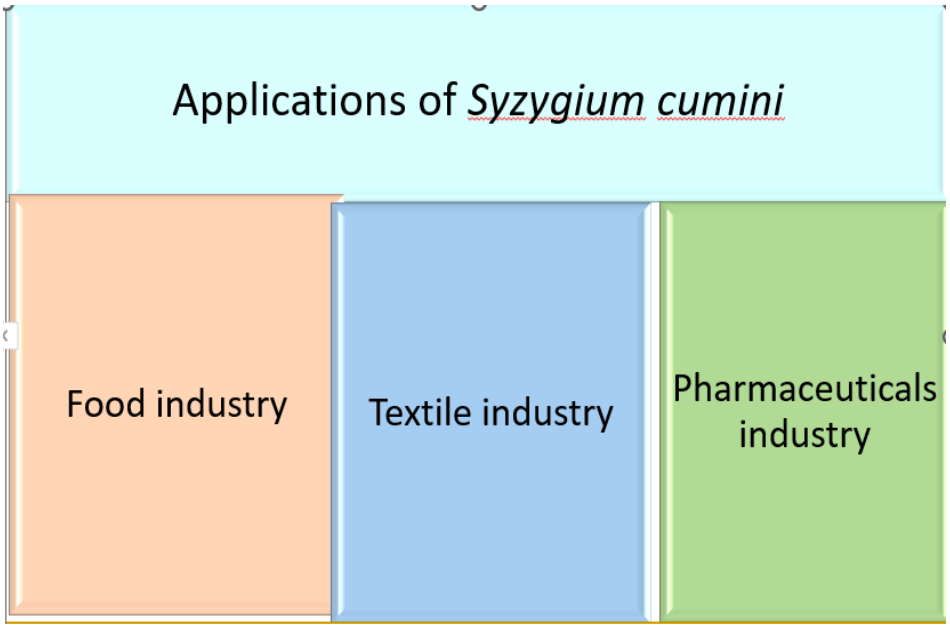

Figure 13. Applications of *Syzygium cumini*

CHAPTER 5

CONCLUSION

Jamun is rich in source of nutritional and medicinal values. Its various value-added products need to catch national and international focus so that their medicinal and nutritional characteristics can be utilized in ideal manner. Jamun has many phytochemicals and bioactive compounds which are used in pharmaceuticals industries but its cultivation is very limited. Therefore, it needs more exploration. Jamun has various biological properties such as antioxidant, antifungal, anti-inflammatory. Among all of them, jamun seeds are popular for its antidiabetic property. They are used in various applications such as in food industries, pharmaceuticals industries, cosmetics and traditionally used to treatment of various diseases and concluded that seeds and pulp may be beneficial for new researchers on the application of investigated phytocompounds.

APPENDICES

Conference Participations

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