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RESEARCH ARTICLE

Antidiabetic and Antibacterial Activities of *Syzygium cumini* Seeds

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Abstract

Bioactive compounds of plants have been used in treating various diseases from ancient times. In this study, *Syzygium cumini* seed extract was studied for its antioxidant, antibacterial, and anti-diabetic potentials. The antioxidant potential was evaluated by DPPH assay. Whereas, the antibacterial feature was tested against *Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Klebsiella pneumonia* by agar well diffusion method. The *in-vitro* anti-diabetic potential was performed by the α-amylase enzyme inhibition method using CNPG3 (2-chloro-4-nitrophenyl-α-D-maltotriose) as a substrate. The methanolic extract showed a total phenolic content of 128.8 ± 1.44 mg GAE/g and a total flavonoid content of 115.6 ± 8.01 mg QE/g. The extract scavenged free radicals where strong antioxidant activity was observed with IC_{50} values of 91.63 ± 6.54 μg/mL in the concentration range of 50-6.25 μg/mL. The DCM and EA fractions exhibited moderate antidiabetic potentials with IC_{50} values of 92.1 ± 0.5 μg/mL, 150.8 ± 0.8 μg/mL, and 42.2 ± 4.6 μg/mL in the concentration range of 400-25 μg/mL against acarbose standard having IC_{50} values of 6.02 ± 0.1 μg/mL in the concentration range 50-1.6 μg/mL. The methanolic extract having ZOI (19mm) showed antibacterial activity however standard neomycin demonstrated ZOI (22.5 mm) against *S. aureus* at the dosage of 50 μg/mL. Methanolic extract having ZOI (20 mm) demonstrated effective antibacterial activity when compared to standard neomycin with ZOI (15mm) against *K. pneumonia* at the dosage of 50 μg/mL.

Keywords: *Diabetes, Syzygium cumini, Antioxidant, phytochemical constituents, antibacterial activity*

Introduction

Phytochemicals are plant-derived compounds, produced by plants as a result of primary or secondary metabolism. They typically have biological activity in the plant host and help the plant grow, reproduce, or protect itself against parasites, or predators [1]. The compounds that quench the oxidants are called antioxidants, and having a wide range of biological effects, including anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic properties. These activities may be linked to their antioxidant activity [2], which stops the generation of free radicals, scavenge them, or promote their breakdown to prevent tissue damage caused by free radicals [3]. Diabetes mellitus (DM) is a metabolic disorder characterized by persistently high blood glucose levels and abnormalities in the metabolism of carbohydrates, fats, and proteins caused by a total or relative deficiency of insulin secretion. Diabetes causes long-term disruption, dysfunction, and loss of multiple organs, as well as the eyes, kidneys, nerves, heart, and blood vessels [4].

The *Syzygium cumini* belongs to the family Myrtaceae and is locally known as "Jamun". *S. cumini* is a tropical evergreen tree native to Bangladesh, India, Nepal, Pakistan, and Indonesia, and belongs to the Myrtaceae flowering plant family [5]. Jamun is a fast-growing tree that can reach 100 feet in height and produces clusters of fruits in the autumn. Fruits grow in clusters, with each cluster consisting of a few to ten to forty fruits [6]. Jambolan is high in anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol, and myrecetin. The seeds are said to contain the alkaloid, jambosine, as well as the glycoside jambon that inhibits the diastatic transformation of starch to sugar, as well as seed extract has been shown to reduce blood pressure by 34.6%, due to ellagic acid content [7]. *S. cumini* contains bioactive phytochemicals such as 1,8-cineole, Acetyl oleanolic acid, Anthocyanins, Bergenins, Betulinic acid, Caffeic acids, Cinnamaldehyde, (cis/ trans), Citronellol, Ellagic acid, Eugenol, Ferulic acids, Gallic acid, Isoquercetin, Kaempferol, Lauric acid, Linalool, Malic acid, Myrtenol, N-hentriacontane, α-terpinene, ß-pinene, ß-phellandrene, ß-sitosterol and so on [8].

GC-MS research showed that the major oil constituents in *S. cumini* were α-pinene, α-terpineol, and alloocimene. *S. cumini* had a total antioxidant activity (TAA %) of 11.13 [9]. The highest total phenolic and flavonoid content was contained in the methanol extract of *S. cumini* seed having 474 2.2 mg of GAE/g dry weight and 668 1.4 mg of QUE/g dry weight, respectively [10].

The kernel had the highest antioxidant activity (84.7%) of the fruit components, followed by the seed (80.3%) [11]. The essential oil isolated from the leaves of Egyptian *S. cumini* were found to contain α-pinene, β-pinene, and trans-caryophyllene as the major components by gas

chromatography-mass spectrometry. The essential oil, methanolic and methylene chlorides extracts of the plants revealed significant antibacterial activity against both of gram negative and gram positive bacteria [12].

The primary objective of this research is to analyze the phytochemicals found in the methanolic extract of *S. Cumini* and investigate their effectiveness in combating microbes and diabetes. This particular plant is readily available and has been employed as a traditional remedy by the local population. The local residents consume ripe fruits and seeds to alleviate gastric issues, while they use the bark to address dysentery, renal problems, and headaches in the Gulmi district of Nepal. In this investigation, we examined the methanol extract and its various fractions from the plant to estimate total phenolic and flavonoid content. Additionally, we assessed their antioxidant, antibacterial, and α-amylase inhibitory properties using established scientific methods. This research aims to validate the traditional medicinal applications of this plant within the local community for addressing various health concerns.

Materials and Methods

The fresh seeds of *S. cumini* were collected from Gulmi, district, Nepal. The plant samples were collected based on ethnobotanical knowledge of local people and a literature review. The taxonomy identification of plants was confirmed by Dr. Suresh Kumar Ghimire, of the Central Department of Botany, Tribhuvan University.

Preparation of methanolic extract

Using a cold percolation process, the crude fraction was extracted in stages with (80%) methanol. Weighed dried and powdered leaves were put in conical flasks and soaked in methanol for 72 hours at room temperature, with occasional stirring. The solvent was filtered through the Whatman filter paper 41 and the filtrate was stored in beakers after 72 hours.

Phytochemical screening

Using several specialized reagents, color reactions were used to identify the various phytochemicals present in various extracts. This result demonstrates that the extract contains the majority of the phytochemicals. The method employed for phytochemical analysis was based on the standard protocol [13].

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

Folin-Ciocalteu (FC) is a method that relies on chemically reducing the reagent, which is a combination of tungsten and molybdenum oxides. The results of the reduction of metal oxides are blue in color and have a broad light absorption spectrum with a maximum at 765 nm. Light absorption at that wavelength is proportional to phenol concentration. The extract's total flavonoid content was determined using a 96-well plate system modified from the colorimetric method [14], [15].

Antioxidant activity

The antioxidant activity of methanolic extract of *S. cumini* was observed by using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [16], [17]. 3.94 mg of DPPH was dissolved in methanol to make the 0.1 mM DPPH solution, with the final amount held at 100 mL. A quercetin stock solution was created by dissolving 1 mg of quercetin in 1 mL of methanol. The final concentrations of 20, 10, 5, 2.5, 1.25, and 0.625 μg/mL were achieved by diluting the stock solution of 1 mg/mL. First, a 50 mg/mL stock solution was prepared by implementing a vortex machine to dissolve 50 mg of plant extracts in 1 mL of DMSO. In a 50% DMSO solution, plant extract concentrations of 1000, 500, 250, 125, and 62.5 μg/mL were prepared.

The antioxidant activity of the plant extract was determined by using the 96 –well plate method which was modified from the colorimetric method (Chang *et al.,* 2002). For the DPPH test, positive control of 20 μg/mL quercetin was used, and negative control of 50% DMSO was used. 100 μL of plant samples, positive control quercetin, and negative control DMSO were placed in 96-well plates in triplicate. Then, 100 μL of DPPH reagent was added to each well. After that, it was incubated in the dark for 30 minutes. Afterward, a micro-plate reader was used to measure the absorbance at 517 nm. (Chang *et al.,* 2002). The following equation was used to calculate the percentage of DPPH free radical

$$\% \text{ Inhibition} = \left(\frac{A_{control} - A_{Sample}}{A_{Control}}\right) \times 100$$

Where, $A_{control}$ = Absorbance for the DPPH

A_{sample} = Absorbance of the DPPH + Sample

The IC_{50} (50% inhibitory concentration) meaning is the effective concentration of the sample needed to scavenge 50% of the DPPH free radicals. The extract concentration versus scavenging effect was plotted on an inhibition curve to get the IC_{50} values.

α -Amylase assay

The plant extract was prepared at a concentration of 500 μg/mL in a 50% dimethyl sulphoxide by the stock solution (50 mg/mL). The solutions of the substrate and the enzyme were prepared in 50 mM of phosphate buffer solution supplemented with 0.9% NaCl. The test was performed as: 20 µL of plant extract was added to 80 μL of porcine pancreatic α-amylase (PPA) up to a final concentration of 1.5 U/mL and incubated for 10 minutes at 37°C. After incubation, 100 μL of 0.8 mM 2-chloro-p- α nitrophenyl -D-maltotrioside ($CNPG_3$) taken as substrate was added and incubated for next 15 minutes. Then, the absorbance was recorded at 405 nm by using the microplate reader. The percentage inhibition of the plant extract and the standard were calculated by using the formula:

$$\% \text{ Inhibition capacity} = \left(\frac{A_{control} - A_{Sample}}{A_{Control}}\right) \times 100$$

Where, $A_{control}$ = Absorbance for the acarbose

A_{sample}= Absorbance of the Sample

Antibacterial analysis

The antibacterial activity was performed using Agar Well Diffusion Method in Muller Hilton Agar plates [18]. Muller Hilton Agar plates were prepared by dissolving 6.46 g MHA in 100 mL of distilled water in a conical flask by warming on the hot plate. The conical flask was cotton-plugged, wrapped in aluminum foil, and autoclaved at 121 °C for 15 minutes. The prepared media was then cooled at room temperature to 30-40°C before being poured into autoclaved Petri dishes (20 mL per plate). The Petri dishes remained at room temperature and incubated for 24 hrs. Contaminated plates were discarded after incubation and the remaining were kept in the refrigerator until further investigation.

In a conical flask, Nutrient Broth (1.12 g) was dissolved in 40 mL distilled water by warming on a hot plate. After that, the solution was poured into test tubes (5 mL each) and autoclaved for 15 minutes at 121 °C. The prepared nutrient broth was sterilized and placed in the refrigerator before further study.

With the aid of an inoculating loop from the primary culture plate, the bacteria (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia*) were examined aseptically touched and moved to a test tube containing 5 mL of Nutrient Broth solution. The inoculums were then incubated at 37 °C for 24 hours. Petri plates were analyzed for Zones of Inhibition (ZOI) caused by plant extract antibacterial action. A scale was used to calculate the diameter of the inhibition zone. The antimicrobial analysis was carried out at Central Department of Chemistry, Tribhuvan University, Nepal.

Results and Discussion

Phytochemical screening

Phytochemical analysis of a methanolic extract of seeds of *S. cumini* showed the presence of various secondary metabolites including polyphenols, alkaloids, flavonoids, terpenoids, quinones, and glycosides but the absence of reducing sugar, quinones, and saponins as illustrated in **Table 1**. The presence of alkaloids is responsible for antimicrobial properties whereas polyphenols act as antioxidant activity. Glucoside, terpenoids, and flavonoids are responsible for the production of glucose, to decrease blood sugar levels and diabetic complications, respectively. Due to different phytochemical components, methanolic extract of seeds of *S. cumini* has great potential in the biomedical field [19].

Table 1: *Preliminary phytochemical analysis of different extract of S. cumini*

S.N.	Phytochemicals	Methanolic extract
1.	Reducing Sugar	- -
2.	Polyphenols	++
3.	Alkaloids	++

4.	Glycosides	++
5.	Quinones	- -
6.	Flavonoids	++
7.	Saponins	- -
8.	Cumarins	++
9.	Terpenoids	++

Note: "++"presence, "- -" absence

Total phenolic content

Figure 1 shows the calibration curve of gallic acid taken as a standard. A linear plot of different concentrations of Gallic acid versus absorbance gives linear regression equation y = 0.035 and R^2 = 0.95. Total phenolic content was calculated using the concentration of gallic acid determined from the calibration curve in mg/mL which was 128.28 ± 1.4 mg GAE/g.

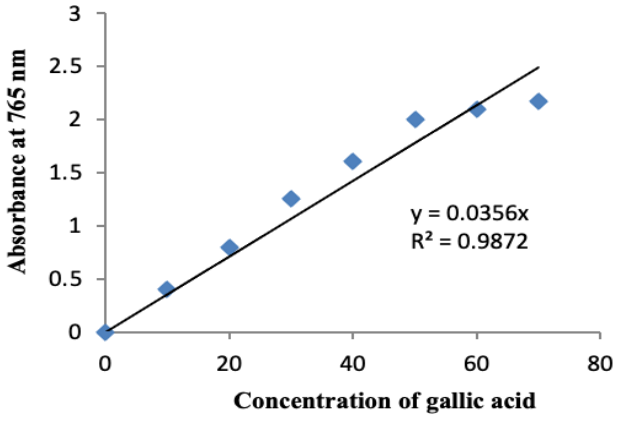

Figure 1: *Calibration curve of gallic acid*

Sonawane and Arya reported the phenolic content of fruits of Jambul has 83.9 mg GAE/g [20].

The methanol extract of the leaves of *Syzygium cumini* collected from the Terai and lesser Himalayas of Nepal was found to contain total phenolics of 12.8 ± 0.03 mg GAE/g and 28.28 ± 1.85 mg GAE/g that were quite lower than in the seeds of the plant in this study [21].

This difference in phenol content is due to various factors such as growth state, temperature, handling, degree of ripeness, harvesting period, environmental factors, storage, and analytical methods used [22]. Owing to the phenolic group content in plant extracts, it has great potential for antioxidants properties [23].

Total flavonoid content

Figure 2 showed the calibration curve of quercetin taken as a standard. The linear plot of different concentrations of quercetin versus absorbance gives linear regression equation y = 0.016 and R^2 = 0.965. The concentration of quercetin (C_1) noted from the calibration curve in mg/mL estimated total flavonoid content 115.6 ± 8.01 mg/mL in QE. The flavonoid content in the leaf extract of *S. cumini* was 51.5 ± 0.01 mg QE/g that was lower than observed in this study [21]. In another similar study, the methanol leaf extract of the plant was reported a total flavonoid content of 5.72 ± 0.52 mg QE/g when determined by the same procedure indicating the difference of flavonoid content in the same plant when collected at different place or method or state of maturity [24].

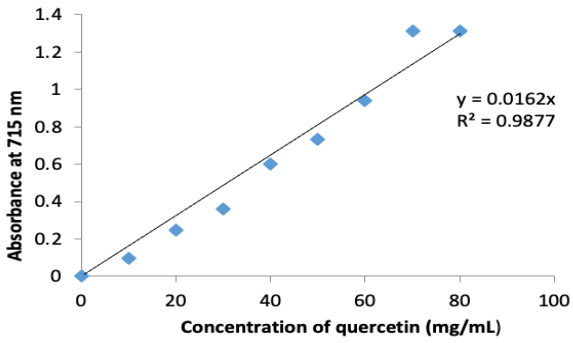

Figure 2: *Calibration curve of quercetin*

The flavonoids in a methanolic extract from the seeds of *S. cumini* help to prevent disease as well as display increased insulin secretion and controls glucose to have an anti-diabetic effect, minimize oxidative stress in muscle and fat cells and metabolism in liver cells. These results had been supported by previous findings [6].

Antioxidant activity

The IC_{50} value of ascorbic acid and *S. cumini* is presented in Figure 3. The IC_{50} of standard ascorbic acid was obtained to be 25.75 ± 0.90 μg/mL and different concentration of the crude methanolic extract of *S. cumini* was recorded as 91.63 ± 6.54 μg/mL.

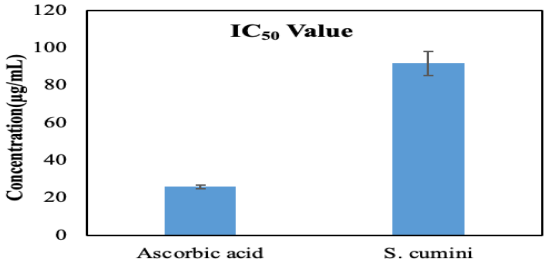

Figure 3: ***Antioxidant activity***

The methanol extract was determined to have the lowest IC_{50} value, which is compared to the value of ascorbic acid, according to the results shown in Fig. 3. This outcome displays its effectiveness as a potent antioxidant. In a previous study, different fractions ethanol extracts of the leaves of the plant from Brazil exhibited significant antioxidant capacity in DPPH and FRAP methods. The ethyl acetate and n-butyl alcohol fractions showed the highest capacity of 1154 ± 67.37 and 1178.27 ± 21.26 µmol trolox equivalent/g respectively. In the DPPH method, the same fractions showed the best results with IC_{50} values of 15.7 ± 2.4 and 23.5 ± 2.7 μg/mL respectively [25].

***In-vitro* antidiabetic activity**

The *in-vitro* anti-diabetic activity was performed by α-amylase inhibition using $CNPG_3$ as substrate. The result revealed that IC_{50} value of the α-amylase inhibitory action of standard acarbose was found to be 6.02 μg/mL at the concentration range of 50-1.56 μg/mL where methanolic extract, DCM fraction, and EA fraction gave IC_{50} values of 42.155, 92.11 and 150.77 μg/mL at the concentration range of 400-25 μg/mL, respectively as presented in Table 2 as well as in Fig. 4. Among the extracts, EA fraction inhibits more effectively which makes it a potential source of anti-diabetic activity. In a previous study, the dose of 1.25 g/Kg of body weight of *S. cumini* seed powder and ethanolic extract to the Type-2 diabetic rats was reported to significantly reduce the fasting glucose level in 21 days [25]. Different fraction of ethanolic

extract of leaves of *S. cumini* from Brazil were evaluated for antiglycant (Bovine serum albumin /methylglyoxal, (Bovine serum albumin /fructose), and arginine/methylglyoxal), and inhibitory capacity against α-amylase, α-glucosidase and lipase. Exception to the hexane, all of the fractions exhibited substantial activities indicating the possibility of the plant for the management of diabetics [26]. In Sri Lanka, two potent antidiabetic compounds gallic acid and umbelliferone were isolated from the decoction of stem bark of *S. cumini* by the activity guided fractionation followed by chromatographic techniques indicating the possibility of the plant material for the management of diabetics [27]. The methanol, 70% methanol and ethyl acetate fractions of the seeds of *S. cumini* collected from Southern India were reported to contain antidiabetic polyphenolic compounds like cinnamic acid, quercetin, ferulic acid, ellagic acid, syringic acid, and gallic acid by HPLC and LC-Q-ToF(MS/MS) techniques. The 70% methanol fraction showed a good α-amylase and α-glucosidase inhibition activities (IC_{50} = 1.7 and 7.62 μg/mL respectively. The methanol fraction demonstrated a good dipeptidyl peptidase-IV inhibition activity by 88.1% [6]. Several studies have revealed that the increased formation of free radicals and oxidative stress cause the damage of important cellular components leading to diabetics [28]. There are many medicinal plants that can be used for the treatment of diabetics. They are easy to use, cost effective, have no side effects and assessable to the people of low-income countries like Nepal. The commonly used plants including *Allium sativum, Momordica charantia, Zingiber officinale, Hibiscus sabdariffa*, etc. have been approved to exhibit good hypoglycemic property, enhance immune system and manage blood sugar level [29].

Table 2: *IC_{50} values of standard acarbose, EA fraction, Crude extract, and DCM fraction*

Sample	IC_{50} (μg/mL)
Acarbose	6.02±0.1
EA fraction	42.2±4.6
Crude extract	92.1±0.5
DCM fraction	150.7±0.8

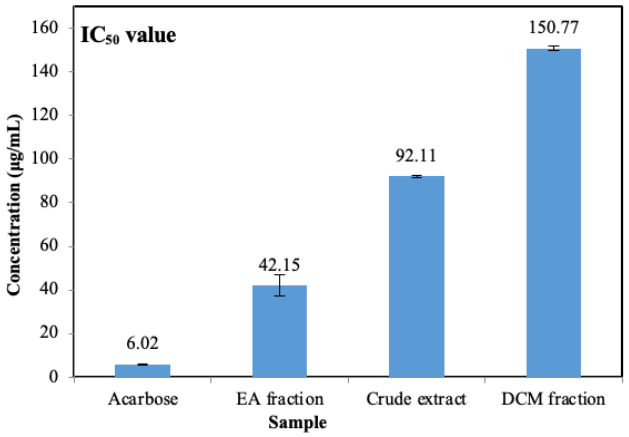

Figure 4: *α-Amylase inhibitory activities of different fractions of plant*

Antibacterial activity

An antimicrobial susceptibility test was done by agar well diffusion technique at a diameter of the well 6 mm. The negative inhibition was 50%

DMSO. The distance of the inhibition zone of the methanolic extract is presented in Table 3.

Table 3: *Antibacterial analysis showing Diameter of Zone of inhibition (nm)*

Bacteria	Neomycin*	Sample (methanolic extract)
E. coli	16	-
S. aureus	22.5	19
K. pneumoniae	15	20
S. typhi	12	-

Note: "-" represents No zone of inhibition, concentration: 50 μg/mL in each well, "*" represents positive control.

The above result indicates that the extract can inhibit *S. aureus* and *K. pneumonia.* There is no inhibition in *E. coli* and *S. typhi.* Due to the antibacterial activity of methanolic extract of *S. cumini, it has* great potential in the biomedical field. In a previous study, the leaf extracts of the plant was evaluated for its antibacterial activity by the agar well diffusion method. The plant exhibited significant susceptibility against *S. aureus and S. typhimurium* on zones of inhibition measurements. It was also found to exhibit wide range of effect against different multi drug resistant (MDR) bacteria. In this study, we evaluated the activity of the seed extracts which are found less active than that of leaf on the basis of zones of inhibition [24].

Conclusions

The phytochemical screening of methanol extracts showed the presence of various secondary metabolites. The total phenolic and flavonoid contents were observed higher in the methanolic extract of *S. cumini.* Results obtained show that methanolic extract of *S. cumini* was found to inhibit DPPH free radical when compared with standard ascorbic acid. Due to less IC_{50} value, the crude extract is more capable to inhibit free radicals. Carbohydrate-digesting enzymes α-amylase, which impel carbohydrates digestion by hydrolyzing α-1-4-glycosidic bonds and generates maltose and glucose from starch. Methanol extract, DCM fraction, and EA fraction showed potent antidiabetic activity. There are variations in the results of different fractions. The Methanol extract, DCM fraction, and EA fraction showed maximum inhibition of α-amylase. The EA fraction showed the lowest IC_{50} values. Thus, further studies are required in EA fraction for isolation of active compound on pure form. The crude extract has a broad range of antibacterial action against *S. aureus* as well as *K. pnuemoniae* but absent in *E. coli* and *S. typhi*

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