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Research Article

Comparative evaluation of the leaves, fruit pericarp, seeds, and bark of *Syzygium cumini* (L.) Skeels for their phenolic contents, *in vitro* antioxidant, and *α*-glucosidase inhibitory activities.

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ABSTRACT

The different parts of *Syzygium cumini* (L.) Skeels have been widely studied for their medicinal values, most notably the antidiabetic properties. However, for *S. cumini* growing in Egypt, no previous study was undertaken to determine the most valuable part regarding the biological activity. This study aims to compare the leaves (**L**), fruit pericarp (**FP**), seeds (**S**), and bark (**B**) of *S. cumini* growing in Mansoura, Egypt, based on their total phenolic contents, antioxidant and *α*-glucosidase inhibition activities. Among the extracts, **S** demonstrated the highest phenolic content with a value of 337.36 mg gallic acid equivalent (GAE)/g dried extract, followed by **B** and **L** showing values of 126.22 and 108.23 mg GAE/g, respectively, while **FP** showed considerably low phenolic content 26.98 mg GAE/g as compared to the other parts. For ABTS assay, **L** and **S** showed high anti-oxidant activity with IC_{50} values of 3.77 and 5.75 µg/ml, respectively. Meanwhile, **B** showed comparable anti-oxidant activity (IC_{50}=13.52 µg/ml) to ascorbic acid (IC_{50}=10.67 µg/ml), while **FP** was the least active (IC_{50}=27.92 µg/ml). Finally, **FP** was the most active against *α*-glucosidase with IC_{50} value of 25.82 µg/ml, followed by **S** (32.66 µg/ml), while **L** (60.95 µg/ml) and **B** (108.49 µg/ml) were much less active, all compared to acarbose (8.5 µg/ml). This study revealed the anti-oxidant and antidiabetic potential of the seeds and fruit pericarp of *S. cumini*, respectively. Accordingly, a further comprehensive *in vivo* study is recommended to elucidate the possibility of their use as a functional food or natural supplement.

Keywords: *Syzygium cumini*; anti-oxidant; phenolic contents; ABTS; *α*-glucosidase.

1. INTRODUCTION

Syzygium cumini (L.) Skeels (*Eugenia jambolana* Lam.), Myrtaceae, is an evergreen tree mainly cultivated for its ornamental value and edible fruits. It is commonly known as Black Plum, Indian Blackberry, Jambolan, and Purple Plum.[1] The leaves of *S. cumini* are coriaceous, opposite, oval, or elliptic-oblong with acute apex. Its fragrant flowers are white, arranged in cross panicled cymes. The fruits are purple-colored berries with a central large seed. The bark is exfoliating and has a gray color.[2] The seeds are pale green in color and have an astringent taste.[3] The main habitat for *S. cumini* is East Indies. It is also found in Australia, Malaysia, and Sri Lanka. The tree has been introduced from India to many tropical regions such as East and West Africa, West Indies, and other sub-tropical regions such as California, Florida, and Algeria.[4]

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Previous phytochemical studies showed that the leaves of *S. cumini* are rich in flavonoids including quercetin, myricitrin, and myricetin; triterpenes such as maslinic acid and betulinic acid; and sterols such as *β*-sitosterol. Its fruits contain anthocyanins, cyanidin diglycosides, malic acid, oxalic acid, gallic acid, and sugars. The seeds contain ellagitannins, gallic acid, corilagin, jamboline, terpenoids, fats, proteins, and resins. While, betulinic acid, friedelin, 3*β*-friedelanol, gallic acid, ellagic acid, kaempferol, and *β*-sitosterol were previously isolated from the bark.[5-7]

Traditionally, the leaves, seeds, and bark of *S. cumini* have been used for diabetes, skin diseases, and diarrhea, respectively. In Brazil, both the leaves and fruits have been used for diabetes and stomachache. In Unani medicine, the fruits have been used for sore throat and diarrhea, while the seeds have been used as a liver tonic and as a mouthwash to strengthen the teeth and gums in India.[8-9]

Several biological activities have been reported for the different parts of *S. cumini*. Its leaves, fruits, seeds, and bark have been reported to exhibit anti-oxidant,[10-13] anti-micrbial[10, 14-16] and *in vitro* anti-cancer[13, 17-19] activities. Also, the fruits showed acaricidal activity against *Tetranychus urticae* and the bark showed anti-plasmodial[20] and anthelmintic activities.[21] The different parts of *S. cumini* have been mainly used for diabetes and several studies were conducted to study their mechanism. *In vitro* studies showed that the leaves and seeds exhibited inhibition of *α*-amylase,[22, 23] while, the leaves, fruits, seeds, and cortex showed inhibition against *α*-glucosidase.[24-26] *In vivo* studies showed that the leaves improved the peripheral insulin sensitivity and pancreatic islet function[27] while the seeds and bark exhibited hypoglycemic effect.[5, 28]

Previously the different parts of *S. cumini* growing in Indonesia were compared for their *α*-glucosidase inhibitory activity,[26] however no such study was performed for *S. cumini* growing in Egypt. In addition, no comparative studies regarding the anti-oxidant activity and total phenolic contents were performed. Accordingly, this study aims to compare the leaves (**L**), fruit pericarp (**FP**), seeds (**S**), and bark (**B**) of *S. cumini* growing in Mansoura, Egypt, based on their phenolic contents, anti-oxidant and antidiabetic properties.

2. METHODS

2.1. Plant Material

The leaves, ripen fruits, and bark of *Syzygium cumini* (L.) Skeels, family Myrtaceae, were collected in November 2019 from the gardens of the Faculty of Agriculture, Mansoura University, Egypt. The plant was authenticated by Professor Mohanad Mohamed Abd Elbaset, Department of Ornamental Plants, Faculty of Agriculture, Mansoura University, Egypt. A voucher sample Sc-11-2020 was placed at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Egypt. The mature seeds were separated from the fruit pericarps. All parts were dried under shade at room temperature and then grounded. The different parts of *S. cumini* are shown in **Figure 1**.

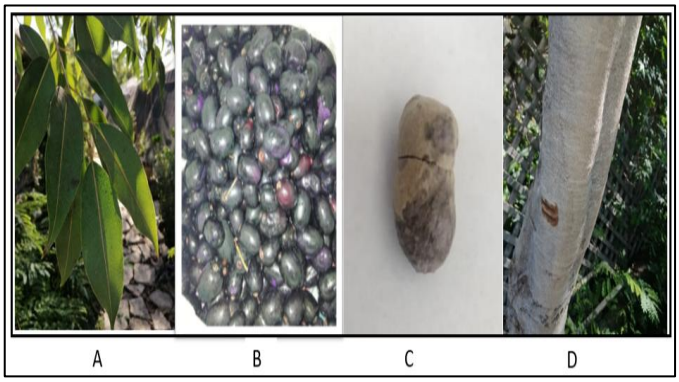

Figure 1: The different parts of *Syzygium cumini* (L.) Skeels, A) leaves, B) fruits, C) seeds, D) bar

2.2. Reagents:

Folin-Ciocalteau reagent, gallic acid (Sigma, Missouri, USA), and sodium carbonate (Hi-Media, Mumbai, India) were used for the determination of total phenolic content. Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) [(ABTS) Sigma, Missouri, USA], potassium persulfate (Hi-Media, Mumbai, India), ascorbic acid tablets (Cevarol®) (Memphis Pharmaceutical, Cairo, Egypt) were used for ABTS assay. Acarbose and *α*-glucosidase enzyme (*Saccharomyces cerevisiae*) (Sigma, Missouri, USA), *p*-nitrophenyl-glucopyranoside (p-NPG), sodium carbonate (Na_2CO_3), disodium hydrogen phosphate, sodium dihydrogen phosphate (Hi-Media, Mumbai, India) were used for *α*-glucosidase assay. Methanol and anhydrous calcium chloride ($CaCl_2$) were obtained from (EL-Nasr company for pharmaceutical chemicals, Mansoura, Egypt).

2.3. Extraction:

The powdered plant material for each part, 250 g each, was extracted separately at room temperature by maceration with 70% methanol (3 × 400 ml) till exhaustion. Each extract was evaporated to dryness under a vacuum. Then, the residue was kept in a desiccator containing anhydrous $CaCl_2$ till complete dryness. The yields of **L**, **FP**, **S** and **B** were 25 g (10%), 37 g (14.8%), 18 g (7.2%) and 65 g (26%), respectively.

2.4. Estimation of the total phenolic content:

The colorimetric Folin-Ciocalteu method[29, 30] was used to estimate the total phenolic content (TPC) of each plant part using gallic acid as a standard. One mg of each methanolic extract was dissolved in 1 ml methanol, then mixed with 5 ml of (10% v/v) Folin-Ciocalteau and 4 ml (7.5% w/v) Na_2CO_3. The reaction solution was incubated for 30 minutes at room temperature. The absorbance was measured at λ_{max} 765 nm using a UV/Vis spectrophotometer (Milton Roy Spectronic 1201, Ontario, Canada) against blank [1ml methanol was mixed with 5 ml (10% v/v) Folin-Ciocalteau and 4 ml (7.5% w/v) Na_2CO_3]. The results of TPC were expressed as mg gallic acid equivalents (GAE)/g of the dried extract. The calibration curve **(Figure 2)** was plotted using the absorbance values estimated at different gallic acid concentrations (0-0.5) mg/ml. All determinations were performed in triplicates.

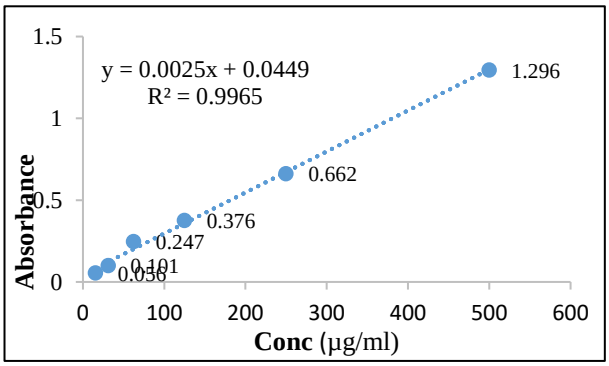

Figure 2: Calibration curve of gallic acid

2.5. ABTS assay:

The antioxidant activity of the different plant parts was estimated by the ABTS radical scavenging method according to the procedure previously described.[31, 32] It is a colorimetric assay where the ABTS is oxidized to the radical cation $ABTS^{\bullet+}$ in a persulfate system. Then, the generated blue-green colored radical $ABTS^{\bullet+}$ directly reacts with antioxidants to decolorize ABTS solution.[33]

The radical cation $ABTS^{\bullet+}$ solution was generated by reacting equal volumes of colorless (1.8 mM) ABTS stock solution and (0.63 mM) potassium persulfate, then the mixture was left to stand for 12-16 h at room temperature in the dark. The prepared $ABTS^{\bullet+}$ solution was diluted using ethanol to adjust the absorbance at 0.700 at λ_{max}734 nm. Then, 10 µl of each extract at different concentrations (0.5-1000) µg/ml in ethanol were mixed with 190 µl of the radical solution in a microtiter plate. The absorbance was measured every 1 minute for 13 minutes using a microplate reader. Ascorbic acid and ethanol were used as positive and negative controls, respectively. Measurements were performed three times. The percentage of radical scavenging was calculated according to:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where ($A_{control}$) is the absorbance of the negative control and (A_{sample}) is the absorbance of the sample.

2.6. *α*-Glucosidase inhibition assay:

The assay was performed according to the methods previously reported,[34] with minor adjustments. Both *α*-glucosidase (1 U/ml) and *p*-NPG (10 mM) were prepared in phosphate buffer (0.1 M), adjusted at pH 6.9. The tested samples and acarbose (positive control) were prepared at different concentrations (4000-2 µg/ml). In a test tube, 50 µl *α*-glucosidase was added to 250 µl phosphate buffer (0.1 M, pH 6.9), and 100 µl of the tested sample/ acarbose, the mixture was pre-incubated for 20 minutes at 37 °C. Then, 10 µl of *p*-NPG (10 mM) was added as a substrate and further incubated for 30 minutes at 37 °C. The reactions were stopped by the addition of 650 µl of sodium carbonate (1 M), and the absorbance was measured in a spectrophotometer (Amersham Biosciences, USA) at λ_{max} 405 nm. Measurements were done three times. The percentage of inhibition was calculated according to:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where ($A_{control}$) is the absorbance of the negative control and (A_{sample}) is the absorbance of the sample.

2.7. Statistical Analysis

All experiments were conducted in triplicates. Data were presented as the mean ± SD. Statistical analysis (mean ± SD) and figures were created using Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1. Estimation of the total phenolic content:

The methanolic extract of **S** showed considerably high phenolic content (337.36 ± 4.08 mg GAE/g) as compared to the other parts, estimated approximately three times as **L** (108.23 ± 1.22 mg GAE/g) and twelve times as **FP** (26.98 ± 1.39 mg GAE/g), meanwhile, **B** and **L** showed comparable content (١٢٦،٢٢ ± 1.68 and 108.23 ± 1.22 mg GAE/g, respectively) and the lowest phenolic content was demonstrated by **FP (Table 1)**. It is worth noting that a previous study reported that the total phenolic content of the ripen fruit pericarp of *S. cumini* was less than the seeds[35].

Table 1: Total phenolic contents (TPC) of the different parts of *Syzygium cumini* (L.) Skeels.

Sample	TPC (mg GAE/g)	Average absorbance at 765 nm
Leaves **(L)**	108.23 ± 1.22	0.316 ± 0.0032
Fruit pericarp **(FP)**	26.98 ± 1.39	0.112 ± 0.0028
Seeds **(S)**	337.36 ± 4.08	0.888 ± 0.0103
Bark **(B)**	**126.22** ± 1.68	0.360 ± 0.0042

Each experiment was done in triplicate, and results are expressed as mean ± S.D., n=3

3.2. Antioxidant activity:

ABTS assay showed high antioxidant activity for **L** and **S** with IC_{50} values of 3.77 ± 0.13 and 5.75 ± 0.38 µg/ml, respectively, as compared to ascorbic acid (IC_{50}=10.67 ± 0.84 µg/ml). **B** showed comparable anti-oxidant activity to ascorbic acid (IC_{50}=13.52 ± 0.62 µg/ml), while **FP** was the least active (IC_{50}=27.92 ± 0.41 µg/ml). The results were nearly matched to their phenolic contents, where **FP** demonstrated the lowest phenolic content and the lowest antioxidant activity **(Table 2).**

Even though the phenolic content of **L** and **B** was nearly comparable, the antioxidant activity of **L** was about three times that of **B**. This may suggest that the antioxidant principles in **L** are different in nature i.e. chlorophyll,[37] carotenoids, or others. It is worth noting that all parts were previously reported to have antioxidant activity.[10-13] The antioxidant activity is usually correlated to phenolic constituents, due to their ability to scavenge free radicals, chelate transition metals, and inhibit lipoxygenase.[10]

Table 2: ABTS-scavenging activity of the different parts of *Syzygium cumini* (L.) Skeels.

Sample	ABTS assay (IC_{50} µg/ml)
Leaves **(L)**	3.77 ± 0.13
Fruit pericarp **(FP)**	27.92 ± 0.41
Seeds **(S)**	5.75 ± 0.38
Bark **(B)**	13.52 ± 0.62
Ascorbic acid*	10.67 ± 0.84

*Positive control, each experiment was done in triplicate, and results are expressed as mean ± S.D., n=3

3.3 *α*-Glucosidase inhibition activity:

α-Glucosidase enzyme is responsible for converting disaccharides and polysaccharides into *α*-glucose, consequently, its absorption.[38, 39] Inhibitors of *α*-glucosidase are among the most effective approaches for the management of type 2 diabetes mellitus, including acarbose and voglibose.[40] They regulate postprandial hyperglycemia in type 2 diabetes mellitus induced by *α*-glucosidase enzyme.

The methanolic extract of **FP** and **S** were the most active among the tested parts, displaying nearly three times and four times the IC_{50} values of acarbose, demonstrated as 25.82 ± 1.27, 32.66 ± 2.13 and 8.5 ± 0.21 µg/ml respectively. Meanwhile **L** and **B** were less active showing IC_{50} values of 60.95 ± 1.86 and 108.49 ± 3.05 µg/ml, respectively.

The inhibition of *α*-glucosidase enzyme was demonstrated in the following order, **FP** > **S** > **L** > **B (Table 3)**. Traditionally, all the parts of the plant were used for treating diabetes and its complications.[41] Previous comparative studies were performed on *S. cumini* growing in the different regions of Indonesia. For *S. cumini* growing in Mojokerto, Saraswaty *et al* reported the *α*-glucosidase inhibition activity of the ethanolic extracts of the different parts in the following order, cortex > young seed > young fruit > leaves.[26] Meanwhile for *S. cumini* growing in Pasuruan, Ishartati *et al*. reported that the fruits had higher *α*-glucosidase inhibitory activity than the seeds in the case of the *n*-hexane and ethyl acetate extracts, but for the ethanol extracts, the seeds showed higher activity.[42] It is worth noting that our study was conducted on the fully ripe fruit pulp and mature seeds growing in Mansoura, Egypt.

FP and **S** were the most active among the tested parts. Their activity may be attributed to their chemical components. For instance, cyanidin, delphinidin, petunidin, malvidin, and their glucosides, mainly present in **FP**,[9, 36] are reported to

possess α-glucosidase inhibitory activity.[43, 44] Meanwhile, quercetin and rutin from **S**,[34] are reported as α-glucosidase inhibitors.[45] It is worth noting that **S** and **FP** showed considerable antioxidant effects which can support their use as antidiabetic agents. Antioxidants can play a significant role in managing and preventing the progression of diabetes-related complications. In diabetes, elevated blood glucose levels can lead to increase in the production of reactive oxygen species (ROS), which damage cells, tissues, and organs over time, contributing to the development of complications such as cardiovascular disease, neuropathy, and nephropathy. Antioxidants help to mitigate this damage by neutralizing ROS, thereby reducing inflammation, cellular injury, and the risk of long-term diabetic complications.[46-47]

Table 3: α-Glucosidase inhibitory activity of the different parts of *Syzygium cumini* (L.) Skeels.

Sample	α-Glucosidase inhibition assay (IC_{50} µg/ml)
Leaves **(L)**	60.95 ± 1.86
Fruit pericarp **(FP)**	25.82 ± 1.27
Seeds **(S)**	32.66 ± 2.13
Bark **(B)**	108.49 ± 3.05
Acarbose*	8.5 ± 0.21

*Positive control, each experiment was done in triplicate, and results are expressed as mean ± S.D., n=3

4. CONCLUSION

This study aimed to compare the different parts of *S. cumini* regarding their phenolic content, antioxidant, and α-glucosidase inhibitory activities. The seeds demonstrated considerably high phenolic content followed by the leaves and bark which showed comparable results, while the fruit pericarp showed considerably lower phenolic content as compared to the other parts. The highest antioxidant effect was demonstrated by the leaves and the seeds, followed by the bark and finally the fruit pericarp was the least active. For the -glucosidase inhibitory activity, the fruit pericarp demonstrated the highest activity, followed by the seeds, their αIC_{50} values estimated nearly three and four times that of acarbose, followed by the leaves while the bark was the least active. In conclusion, the seeds and fruit pericarp of *S. cumini* can be candidates a functional food or natural supplement for their anti-oxidant and antidiabetic properties, respectively. According to these findings, a comprehensive *in vivo* study and standardization of the extracts regarding the active principles, are recommended.

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CONFLICT OF INTEREST

The authors claim that there is no conflict of interest.

AUTHOR CONTRIBUTION

Conceptualization, M. E, and M. A.; sampling and extraction, M. A. and M. E; biological assay, M. A.; original draft preparation, M. A. and M. E; writing, review, and editing. M. A., M. E, K. F, and M. Z. All authors have read and agreed to publish this version of the manuscript

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