JOBIMB, 2025, Vol 13, No 2, 116-124
https://doi.org/10.54987/jobimb.v13i2.1154

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

JOBIMB VOL 13 NO 2 2025
AI and Biochemistry

Chemical and Functional Characterization of *Syzygium cumini* Leaves with Antioxidant Potential

Aisha Abubakar Jinjiri[1], Zainab Bello Musa[1], Aminu Jibril Sufyan[2], Nafisa Isiyaka Rabiu[1], Amina Abdulmalik Juda[1], Husna Usman Turadu[1], Fatima AbdulGaffar Nasir[1], Amina Lawal Garba[1,3], Innocent Ojeba Musa[1], Miracle Uwa Livinus[1], Haruna Bala Tsoho[2], Abba Babandi[4] and Hafeez Muhammad Yakasai[1,2]*

[1]Department of Biochemistry, School of Science and Information Technology, Skyline University Nigeria, No. 2 Zaria Road, P.M.B 3076, Kano, Kano State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano, P.M.B 3011, Kano, Kano State, Nigeria.
[3]Integrated Science Department, School of Science Education, Federal College of Education (Technical) Bichi, P.M.B 3473, Bichi, Kano State, Nigeria.
[4]Medical Biochemistry Unit, Faculty of Basic Medical Sciences, Federal University Dutse, P.M.B 7156, Dutse, Jigawa State, Nigeria.

*Corresponding author:
Hafeez Muhammad Yakasai,
Biochemistry Department,
Faculty of Basic Medical Sciences, College of Health Science,
Bayero University Kano,
P.M.B 3011, Kano State,
Nigeria.
Email: hmyakasai.bch@buk.edu.ng

History

Received: 4th Oct 2025
Received in revised form: 28th Nov 2025
Accepted: 4th Dec 2025

Keywords

Syzygium cumini
Antioxidant activity
Phenolic compounds
GC-MS profiling
Oxidative stress

SDG Keywords

SDG 3 Good health and well-being
SDG 12 Responsible consumption and production
SDG 15 Life on land

Abstract

Natural antioxidants found in plants have thus proven to be important potential therapeutic agents against oxidative damage caused by free radicals. This study evaluated the antioxidant properties and functional characteristics of the bioactive compounds in *Syzygium cumini* leaves via *in vitro* and *in vivo* approach. The total phenolic content (TPC: 631.43 ± 163.90 µg/mL) was found to be higher than flavonoid content (TFC: 38.05 ± 3.02 µg/mL). Based on the IC_{50} values, ethanolic fraction was shown to be most effective in quenching electron pairs of DPPH radicals (IC_{50}: 0.02 ± 0.004 µg/mL), higher than methanolic extract (IC_{50} = 1.49 ± 0.90 µg/mL) and other fractions. The total antioxidant potential was also high in ethanolic fraction (1327 ± 5.89 µg/mL). Similarly, *in vivo* studies in type 2 diabetic rat model showed a reduction in the activity of catalase and a subsequent increase in malondialdehyde (MDA) content. However, *S. cumini* treatment abolished this antioxidant imbalance in a dose-dependent manner. The 1000 mg/kg dose resulted in a 27.28 ± 2.82 kU increase in catalase activity and a 0.67 ± 0.02 nmol/mg reduction in MDA content. The FTIR spectroscopy revealed strong O-H stretching peaks around 3260 cm^{-1} typical for phenolic compounds. Additionally, peaks were observed for C=O, C-O, and N-H bonds. Interestingly, GC-MS profiling further confirmed the presence of strong antioxidant phenolic compounds such as catechol, 1,2,3-benzenetriol, and 3,4,5-trihydroxybenzoic acid methyl ester. Besides this, moderate levels of antioxidants with lipid solubility were also found. Derivatives of linoleic acid, oleic acid, and octadecadienoic acid belonged to this group. This indicates a combination of hydrophilic radical scavenging capacity.

INTRODUCTION

Natural antioxidants have always remained in the limelight of biomedical research because of their versatile roles in protecting organisms from oxidative damage. A number of chronic illnesses, including diabetes, heart disease, and cancer, are significantly influenced by oxidative stress [1]. This occurs because of the uncontrolled generation of reactive oxygen species [2]. Natural antioxidants include a series of naturally occurring compounds such as flavonoids and other plant-derived molecules [3]. *Jamun,* or *Syzygium cumini* (L.) Skeels, is a medicinal herb that is widely used in traditional medicine. The leaves, seeds, and fruits of *S. cumini* have been reported to contain phenolic acids, flavonoids, and biolipids having antioxidant and antidiabetic qualities. Prior research has demonstrated that *S. cumini* extracts can raise enzymatic antioxidant levels and decrease oxidative biomarkers both in vitro and in vivo [4,5]. This has established *S. cumini* as a

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

potential novel therapeutic agent. Despite this data, a thorough chemical profiling of identified fractions and their roles in antioxidant activity has still not been fully ascertained. A comprehensive assessment regarding the presence and associated role of phytochemicals along with dedicated antioxidant assays gives a better insight about a particular molecule that significantly influences this observed phenomenon. Thus, this particular study intends to examine the level of antioxidant activity possessed by the fraction of *S. cumini* leaves along with its major identified bioactive.

MATERIALS AND METHODS

Reagents and chemicals

The extraction process involved the use of the following solvents: absolute methanol, chloroform, n-hexane, ethyl acetate, distilled water and ethanol. The reagents used in the antioxidant activity experiments include 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), iron (III) chloride, hydrochloric acid (HCl), distilled water, acetate buffer at pH 3.6, gallic acid.

Collection and identification of plant material

The plant samples were collected from a home garden at Hotoro, Tarauni Local Government Area of Kano state, Nigeria. The plant sample was authenticated by a plant scientist in the Department of Plant Biology, Bayero University Kano with a voucher number of BUKHAN 0406. The sample was washed under running tap water, air dried at room temperature for 7 days and milled with a mechanical blender into fine powder and stored under cool condition until use.

Extraction of crude plant material

The methanolic extract was prepared with some slight changes according to the method described by [6]. First, 200 grams of plant powder was placed in an ultrasonic bath and mixed with 1000 mL of methanol. The extraction took place for 40 minutes at a temperature of 50 °C. Various experimental factors were adjusted to improve the extraction process, including the liquid-to-solid ratio, ultrasonic power level (ranging from 100 to 200 W), methanol concentration (95%), and extraction time (between 20 and 40 minutes). After the extraction was complete, the crude extract was filtered using Whatman® filter paper (120 mm, China), and then the solvent was allowed to evaporate at room temperature. The resulting extracts were stored in a vacuum at optimal temperature until they were analyzed [6].

Solvent-based fractionation using column chromatography

Fractionation of the methanolic extract of *S. cumini* was carried out using column chromatography, which is based on the principle of separating compounds according to their polarity and differential affinity between a stationary phase (silica gel) and a mobile phase (solvents of varying polarity). A chromatographic column was carefully packed with silica gel and pre-conditioned with a non-polar solvent to ensure an even bed and eliminate air pockets. About 5–10 g of the crude extract was mixed with a small amount of silica gel, dried to form a free-flowing powder, and gently layered onto the column.

Elution was performed sequentially with solvents of increasing polarity: n-hexane to remove lipophilic substances such as fats, oils, waxes, and terpenes; chloroform to extract chlorophylls, alkaloids, and other medium-polar compounds; ethyl acetate to isolate flavonoids, tannins, and phenolics; ethanol for glycosides, phenolics, and certain sugars; and distilled water to collect highly polar constituents such as polysaccharides, amino acids, and organic acids [7]. Each solvent was added slowly in sufficient volume (50–150 mL) to ensure complete elution, and the fractions were collected separately, evaporated, and later subjected to FTIR and GC-MS characterization as well *in vitro* antioxidant testing [8,9].

Determination of antioxidant activity of *S. cumini*

Total flavonoid content

Using the method outlined by [10], the total flavonoid content was measured. The crude extract (0.5 mL) was mixed with 0.5 mL of 2% AlCl solution in ethanol. The mixture was allowed to stand for one hour at room temperature, after which, the absorbance was read at 420 nm with a spectrophotometer. A yellow color indicated that flavonoids were present. The concentration of the extract was calculated to be 1 mg/mL using the formula from the calibration curve: $y = 0.025x + C$; $R^2 = 0.9812$, where x represented the concentration and was equivalent to quercetin.

Total phenolic content

The total phenolic content of the extract was measured using a modified Folin-Ciocalteu method as described by [11]. For this determination, a crude extract solution at a concentration of 1 mg/mL was prepared and combined with 5 mL of Folin-Ciocalteu reagent followed by the addition of 4 mL of sodium carbonate solution. The resulting mixture was vortexed for about 15 seconds to ensure proper mixing and then incubated at 40 °C for 30 minutes to allow color formation. The absorbance of the developed solution was subsequently recorded at 765 nm using a spectrophotometer. The total phenolic content was expressed as mg/mL equivalent to gallic acid by using this equation based on the calibration curve: $y = 0.1216x$, where x represents the concentration as gallic acid equivalent (mg/mL).

DPPH assay

The DPPH radical scavenging activity of the various samples and fractions was assessed using the method described by [12] with some minor modification. A freshly prepared 0.1 mM DPPH solution in methanol was combined with about 2 mL of 4 mg/mL crude extract and fractions. After incubation in the dark for 30 minutes at room temperature, the absorbance of the different samples was measured at 517 nm, using gallic acid as a positive control.

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where;
Ac—Control reaction absorbance
As—Testing specimen absorbance.

Catalase (CAT) Assay

To determine catalase activity a sample was incubated with an H_2O_2 standard solution, and the decrease of H_2O_2 concentration in the sample was measured. The assay was run in two reactions. The serum samples were incubated in reaction A with 65 mM H_2O_2, and the reaction was stopped by sodium azide addition. This remaining H_2O_2 concentration was measured through an enzyme-linked colorimetric detection method in reaction B: that produces quinoneimnine dye which was read at 540 nm to determine the rate of decomposition of H_2O_2 as index of catalase activity [13].

Superoxide Dismutase (SOD) Assay

The serum samples were used to assay for superoxide dismutase (SOD) activity. The superoxide radicals reacted with a colorless substrate to form a yellow-colored product. This product was measured for absorbance at 450 nm. The higher the levels of SOD in the sample, the less the amount of the yellow product, and

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

hence the greater enzyme activity. These were added to the wells and xanthine oxidase was added. After 20 minutes at room temperature, the reaction was read in absorbance. The rate of inhibition calculation was done to determine the SOD activity and check for appropriate blanks for accurate measurement [14].

Malondialdehyde (MDA) Assay

A reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) formed an MDA-TBA_2 adduct absorbing at 532 nm and this was subjected to the MDA assay. Butylated hydroxytoluene (BHT) and EDTA were added to the sample to prevent artificial lipid oxidation. To minimize lipid hydroperoxide decomposition, the reaction mixture temperature was controlled. All wells were prepared as standard, blank and sample and the detection reagents were added. The plate was washed multiple times at 37 °C and incubated. A blue color change was observed in addition to a substrate solution followed by a yellow color change on the addition of stop solution. Immediately, MDA concentration was determined by measuring absorbance at 450 nm [15].

Characterization of *S. cumini* fractions

The bioactive in *S. cumini* solvent fractions were characterized based on FTIR and GCMS.

Fourier Transform Infrared (FTIR) Spectroscopy

The Fourier transform infrared (FTIR) spectroscopy method, based on [16] with some minor changes, was used to find the functional groups in the fractionated extracts of *S. cumini*. About 1 mg of each dried sample fraction was mixed with 100 mg of potassium bromide (KBr) in an agate mortar to create a smooth powder. Next, the mixture was pressed into a clear disc using a hydraulic press under vacuum. The prepared KBr pellets was scanned in the mid-infrared area using an FTIR spectrometer (Shimadzu FTIR-8400S or equivalent). The wavelength range was from 4000 to 400 cm^{-1}, and the resolution was set at 4 cm^{-1}, averaging 32 scans for each spectrum to enhance the signal-to-noise ratio. The spectra obtained were compared with standard functional group frequencies to find the key peaks and identify the types of compounds present [16].

Gas chromatography-mass spectrometry (GC-MS) analysis

The separation and identification of sample constituents were carried out through gas chromatography coupled with mass spectrometric detection. The analysis utilized a Thermo Scientific Trace GC Ultra system integrated with an ISQ quadrupole detector. A capillary column composed of fused silica (TG-5MS type, 30 m length, 0.25 mm internal diameter, and 0.1 µm film thickness) facilitated compound separation. High-purity helium functioned as the carrier medium at a uniform flow rate of 1 mL per minute. A 1 µL aliquot of each sample was introduced into the instrument.

The ionization of analytes occurred under an electron impact energy of 70 eV. Both the injector and the transfer line were thermostatically regulated at 280 °C. The oven temperature programming began at 40 °C, maintained for three minutes, then progressively increased at a rate of 5 °C per minute until reaching 280 °C, where it was held constant for an additional five minutes. To quantify all identified components, the percent relative peak area was calculated.

Compounds were tentatively identified by comparing their relative retention times and mass spectra with those found in the NIST and Wiley libraries associated with the GC-MS system [17].

RESULTS

Total phenolics and flavonoids contents of *S. cumini*

The mean total phenolics and flavonoids contents of the leaves of *S. cumini* were presented in **Table 1**. It was found that the leaves contain significantly high ($p<0.05$) total phenolic than flavonoid content.

Table 1. Total phenolics and flavonoid contents of *S. cumini* methanolic extract.

Antioxidants	Leaves (µg/mL)
Total Phenolic (TPC)	631.43 ± 16.39*
Total Flavonoid (TFC)	38.05 ± 3.02*

Results are mean ± S.D of triplicate, values in the same raw bearing same superscript are significantly different at $p<0.05$.

2,2-diphenyl-1-picrylhydrazyl (DPPH) antiradical property of *S. cumini*

The result of the DPPH radical scavenging activity of *S. cumini* expressed as µg/mL was presented in **Fig. 1**. It was shown that the crude extract and various solvent fractions of the leaves revealed diverse antioxidant activity. Ethanol fraction of the leaves showed highest antioxidant activity indicated by the least IC_{50} of 0.02 ± 0.004 µg/mL compared to all other solvent fractions. The significantly lower ($p<0.05$) lower IC_{50} of the gallic acid standard than all the solvent fractions of the leaves indicate higher antioxidant activity.

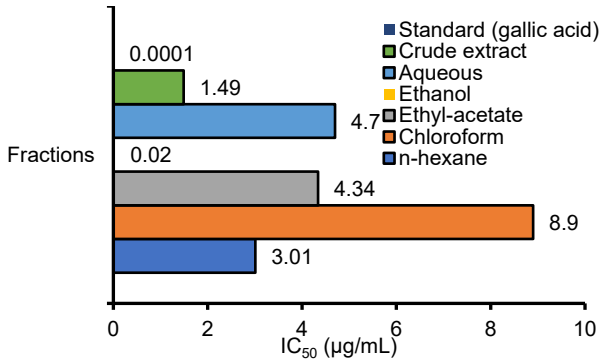

Fig. 1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *S. Cumini.* Data are presented as mean ± SD (n=3).

Total antioxidant capacity (TAC) of *S. cumini*

The total antioxidant capacity (TAC) of *S. cumini* extract and fractions expressed in µg/mL was presented in **Fig. 2**. It was found that different solvent fractions of the leaves revealed varied total antioxidant power. The ethanol fraction of the leaves showed the highest TAC with 1327 ± 5.89 µg/mL, while the chloroform fraction showed the least TAC (217.8 ± 3.80 µg/mL).

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

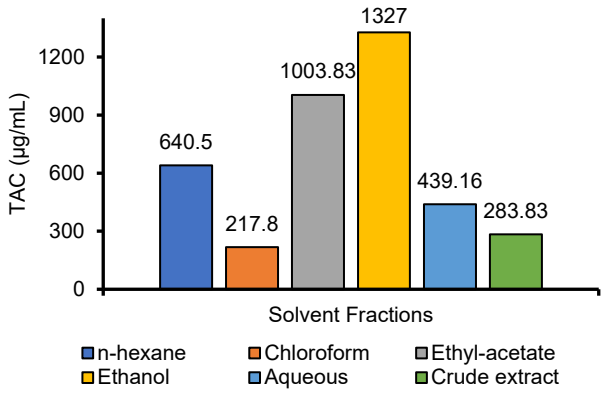

Fig. 2. Total antioxidant capacity (TAC) of *S. cumini.* Data are presented as mean ± SD (n=3).

Effect of *S. cumini* on serum MDA and antioxidant enzymes

The effect of *S. cumini* on the serum malondialdehyde and antioxidant enzymes was presented on **Table 2**. The result shows that serum catalase activity was highest in the drug control group (33.56 ± 5.46 kU), followed by the normal control (30.25 ± 4.08 kU), and lowest in the diabetic control (16.78 ± 3.68 kU). Among the extract-treated groups, the high dose (1000 mg/kg) recorded 27.28 ± 2.82 kU, the medium dose (500 mg/kg) had 24.87 ± 2.79 kU, and the low dose (250 mg/kg) showed 24.48 ± 4.25 kU. Superoxide dismutase (SOD) activity was 0.44 ± 0.03 U/ml in the normal control and 0.44 ± 0.01 U/ml in the drug control. The diabetic control showed 0.61 ± 0.03 U/ml. The extract-treated groups recorded 0.55 ± 0.02 U/ml at both low and medium doses, while the high dose showed 0.84 ± 0.02 U/ml.

Similarly, the serum malondialdehyde (MDA) levels were 0.64 ± 0.01 nmol/mg protein in the normal control and elevated to 0.92 ± 0.05 nmol/mg in the diabetic control. The drug control group had 0.65 ± 0.03 nmol/mg. The low, medium, and high extract doses recorded MDA levels of 0.72 ± 0.04, 0.68 ± 0.03, and 0.67 ± 0.02 nmol/mg protein, respectively.

Table 2. *In vivo* antioxidant effect of *S. cumini* in diabetic rat model.

Group	Catalase Activity (kU)	SOD Activity (U/mL)	MDA (nmol/mg protein)
I (Normal Control)	30.25 ± 4.08	0.44 ± 0.03	0.64 ± 0.01
II (Diabetic Control)	16.78 ± 3.68*	0.61 ± 0.03*	0.92 ± 0.05*
III (Drug Control)	33.56 ± 5.46	0.44 ± 0.01	0.65 ± 0.03
IV (250 mg/kg)	24.48 ± 4.25	0.55 ± 0.02*	0.72 ± 0.04*
V (500 mg/kg)	24.87 ± 2.79	0.55 ± 0.02*	0.68 ± 0.03
VI (1000 mg/kg)	27.28 ± 2.82	0.84 ± 0.02**	0.67 ± 0.02

Values are presented as mean ± standard deviation (n = 6). Within each column, means with different numbers of asterisks are significantly different ($p < 0.05$).

FTIR characterization of ethanol fraction of *S. cumini* leaves

Table 3 presented the FTIR spectral data that reveal the types of functional groups present in the ethanol fraction of *S. cumini* leaves. The presence of a strong, broad O-H stretches and H-bonded band at 3260 cm^{-1} typically indicates the presence of alcohols and phenols (**Fig. 3**). The medium intensity C-H stretching vibrations at 2925 cm^{-1} and 2854 cm^{-1}, along with the C-H rocking at 724 cm^{-1}, confirm the presence of alkane (aliphatic) structures. A weak absorption band at 2117 cm^{-1} indicates the possible presence of alkynes (–C≡C–). A strong absorption band at 1693 cm^{-1} is characteristic of a C=O stretch, specifically pointing towards alpha, beta-unsaturated aldehydes or ketones.

The medium intensity N-H bending vibration at 1611 cm^{-1} suggests the presence of primary amines, while the C-N stretch at 1197 cm^{-1} indicates aliphatic amines. A strong N-O asymmetric stretch at 1514 cm^{-1} suggests the presence of nitro groups. A strong C-O stretch at 1320 cm^{-1} signifies the presence of alcohols, carboxylic acids, esters and ethers. Medium intensity C-Cl stretch absorption bands at 821 cm^{-1} and 769 cm^{-1} suggest the presence of (alkyl halides).

GC-MS Analysis ethanol fraction of leaves

The GC-MS analysis of the ethanolic leaf fraction revealed several bioactive constituents with documented antioxidant relevance (**Table 4)**. Phenolic compounds dominated the profile, notably catechol and 1,2,3-benzenetriol, both of which are recognized for strong radical-scavenging activity. A methylated trihydroxybenzoic acid derivative, consistent with a gallic acid ester, was also detected and further supports the phenolic contribution to antioxidant potential.

In addition to these high-activity phenolics, the extract contained multiple unsaturated fatty acid derivatives, including linoleic acid ethyl ester, 9-octadecenoic acid methyl ester, and several peaks corresponding to 9,12-octadecadienoic acid (**Figs. 4** to **11)**. These compounds are known to provide lipid-phase antioxidant effects. Oleic acid and n-hexadecanoic acid were also present and may offer secondary protective activity. Together, the profile indicates that both phenolic constituents and unsaturated fatty acids contribute to the antioxidant properties of the *S. cumini* ethanolic leaf fraction.

Table 3. FTIR Identified functional groups in ethanol fraction of *S. cumini* leaves**.**

Absorbance (cm^{-1})	Class of compound	Functional group	Intensity
3260	O-H stretch, H-bonded	Alcohols, phenols	Strong, broad
2925	C-H stretch	Alkanes	Medium
2854	C-H stretch	Alkanes	Medium
2117	-C (triple bond) C-stretch	Alkynes	Weak
1693	C =O stretch	Alpha, beta-unsaturated aldehydes, ketones	Strong
1611	N-H bend	Primary amines	Medium
1514	N-O asymmetric stretch	Nitro compounds	Strong
1320	C-O stretch	Alcohols, carboxylic acids, esters, ethers	Strong
1197	C-N stretch	Aliphatic amine	Medium
821	C-Cl stretch	Alkyl halides	Medium
769	C-Cl stretch	Alkyl halides	Medium
724	C-H rock	alkanes	Medium

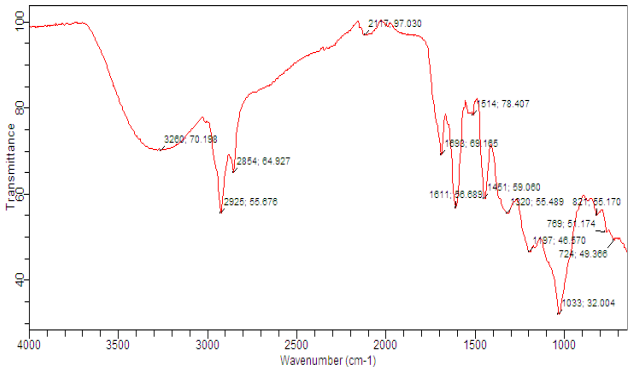

Fig. 3. FTIR spectrum of ethanolic fraction of *S. cumini* leaves.

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

Table 4. GC-MS identified compounds from ethanolic leaves fraction of *S. cumini.*

Peak	Compound	RT (min)	Area (%)	MW (g/mol)
1	Catechol	5.1	2230706.21	110.11
2	1,2,3-Benzenetriol	6.7	61963670.43	126.11
3	3,4,5-Trihydroxy benzoic acid methyl ester	11.5	1378446.53	184.15
4	Linoleic acid ethyl ester	12.6	7318041.63	308.51
5	9-Octadecenoic acid methyl ester	12.7	5899501.46	296.50
6	9,12-Octadecadienoic acid (Z,Z)	13.5	55188669.73	280.45
7	Oleic acid	13.8	7238524.82	282.47
8	n-Hexadecanoic acid	11.3	20460473.25	256.43

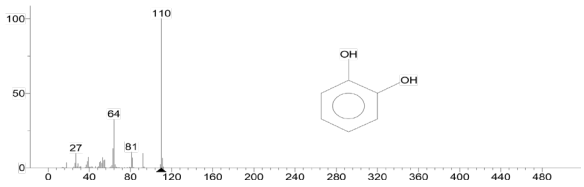

Fig. 4. Chromatogram of catechol with a retention time (RT) of 5.1 minutes.

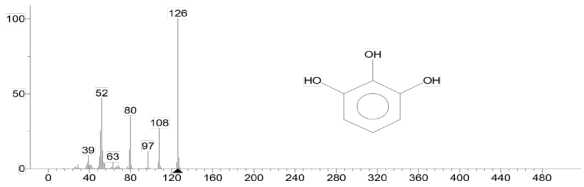

Fig. 5. Chromatogram of 1,2,3-benzenetriol with a retention time 6.7 minutes.

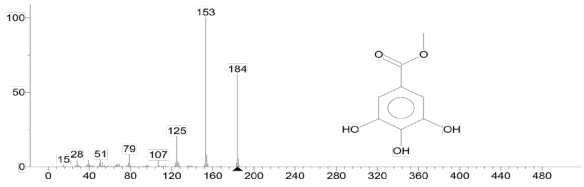

Fig. 6. Chromatogram of Benzoic acid, 3,4,5-trihydroxy-, methyl ester with a retention time (RT) of 11.5 minutes.

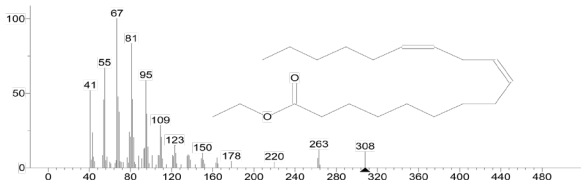

Fig. 7. Chromatogram of Linoleic acid ethyl ester with a retention time (RT) of 12.6 minutes.

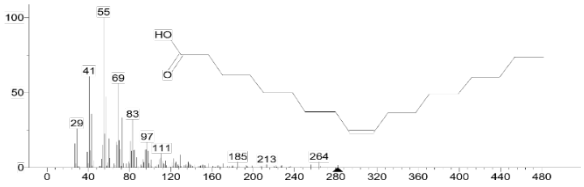

Fig. 8. Chromatogram of oleic acid with a retention time (RT) of 13.8 minutes.

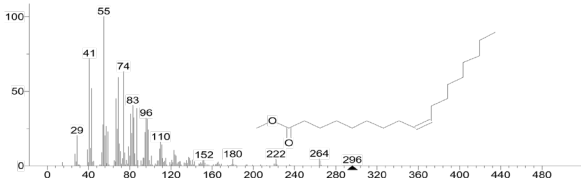

Fig. 9. Chromatogram of 9-octadecenoic acid (Z)-, methyl ester with a retention time (RT) of 12.7 minutes.

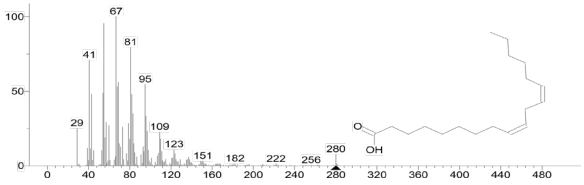

Fig. 10. Chromatogram of 9,12-Octadecadienoic acid (Z,Z) with a retention time (RT) of 13.5 minutes.

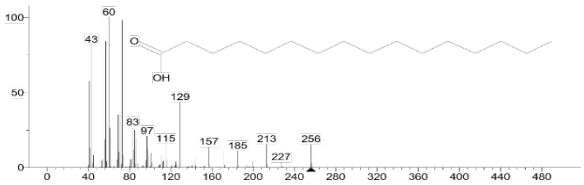

Fig. 11. Chromatogram of n-hexadecanoic with a retention time (RT) of 11.3 minutes.

DISCUSSION

Syzygium cumini has been reported to have high phenolic component and antioxidant capacity [18]. The higher phenolic content than flavonoid found in this study suggests that the leaves are a rich reservoir of phenolic compounds, which encompass a vast array of phytochemicals known for their free radical scavenging capabilities, metal chelation, and enzyme inhibition properties [19,20]. Studies consistently highlight that plant phenolics are key contributors to the overall antioxidant capacity of plant extracts [21]. Similarly, the leaves also show a slightly high mean total flavonoid. Flavonoids, a prominent subgroup of phenolic compounds, are well-established for their diverse pharmacological activities, including potent antioxidant, anti-inflammatory, and antidiabetic effects [22]. The slightly elevated flavonoid content in the leaves reinforces their potential as a source of these beneficial compounds. These findings shows that the higher TPC and TFC in the leaves suggest that this plant part possess a greater inherent antioxidant potential [23].

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

Assessment of the free radical (DPPH) scavenging capacities of various solvent fractions from the leaves of *S. cumini* revealed a lower IC_{50} value indicating stronger antioxidant activity. The IC_{50} value serves as an exceptionally potent positive control for comparison. The ethanol fraction of the leaves displayed exceptionally potent DPPH radical scavenging activity, a value outstandingly low, nearing the potency of the standard and suggesting that the ethanol-soluble compounds in the leaves are highly effective radical scavengers. The n-hexane, ethyl-acetate and crude leaves extract also show good activity. However, the chloroform and aqueous leaves fractions were less potent.

Recent investigations on the DPPH radical scavenging activity of *S. cumini* extracts consistently report strong antioxidant properties, particularly from leaf extracts. The IC_{50} of the leaf extracts of methanol, ethyl-acetate and hexane are almost inactive, compared to standard ascorbic acid, indicating methanol fractions are in the very powerful category [24]. The solvent-dependent nature of antioxidant activity is a widely recognized phenomenon, with polar solvents, ethanol and methanol for extracting phenolic and flavonoid compounds responsible for DPPH scavenging [25].

Specific IC_{50} values vary across studies due to differences in plant origin, processing, extraction methodologies, and assay conditions, the general trend of *S. cumini* exhibiting potent radical scavenging activity is well-established [23]. Antioxidant compounds in *S. cumini* are diverse and vary in polarity, requiring different solvents for optimal extraction. In this study, the ethanol fraction from the leaves stands out as having an exceptionally high total antioxidant capacity, with a remarkably mean TAC value. This suggests the presence of extremely potent antioxidant compounds soluble in ethanol within the leaves. The crude leaf extract also shows good antioxidant capacity.

Other leaf fractions, such as n-hexane, chloroform, ethyl-acetate, and aqueous, exhibit varying, generally lower, capacities compared to the ethanolic fraction. Overall, the leaves are valuable antioxidant sources but their active compounds differ significantly in solubility and extractability [26]. Recent works on the antioxidant capacity of *S. cumini* extracts generally support the concept of solvent-dependent activity and often confirm the potent antioxidant properties of leaf extracts [27]. Many studies reported the leaves as a rich source of antioxidants, consistent with the high activity of the ethanolic leaf extract observed here [28]. Research frequently correlates TAC values with the content of specific phytochemicals like phenolics and flavonoids, which are known to be extracted differentially by various solvents [29]. Variations in TAC values across published works are common due to differences in extraction protocols, plant material origin, maturation stage, and the specific TAC assay employed [30].

Additionally, findings of the *in vivo* antioxidant effect of *S. cumini* in this study showed that the catalase activity of the diabetic control group was markedly reduced (16.78 ± 3.68 kU), reflecting oxidative stress and compromised antioxidant defense in the absence of treatment. Conversely, catalase activity was restored significantly in the drug control group (33.56 ± 5.46 kU) and was also elevated in the *S. cumini*-treated groups in a dose-dependent manner, with the highest catalase activity recorded in the high-dose group (27.28 ± 2.82 kU). These results align closely with previous studies that reported an increase in catalase activity following the administration of *S. cumini*. For instance, [18] demonstrated that rats administered with *S. cumini* extract for 21 days showed a significant dose-dependent improvement in catalase activity, attributed to the presence of flavonoids and other phenolic compounds in the extract. Similarly, [31] suggested that the polyphenolic compounds in *S. cumini* may upregulate the expression of catalase and other antioxidant genes through modulation of cellular signaling pathways like the Nrf2-ARE (nuclear factor erythroid 2-related factor-2 antioxidant response element) pathway.

Research [32] also reported similar improvements in catalase levels in diabetic rats treated with *S. cumini* pulp extract, highlighting the plant's potential to stimulate endogenous antioxidant systems. In this study, while the catalase activity did not statistically differ across groups, the numerical trend suggests a meaningful biological impact, particularly at the medium and high doses of *S. cumini*. The pattern observed with SOD activity further reinforces the antioxidative role of *S. cumini*. In diabetic conditions, the overproduction of superoxide radicals overwhelms the antioxidant system, often leading to the inactivation of SOD. In this study, the diabetic control group recorded a moderately elevated SOD activity (0.61 ± 0.03 U/ml), possibly due to an adaptive response to increased oxidative load. However, treatment with *S. cumini* significantly improved SOD activity, particularly in the high-dose group, which showed a peak activity of 0.84 ± 0.02 U/ml, well above both the diabetic and normal controls.

These findings are corroborated by the work of [33], who observed enhanced SOD activity in diabetic rats administered with *S. cumini* seed extract, again citing the antioxidative phytoconstituents as the primary agents responsible for the improved enzymatic response. Similarly, [34] found that SOD activity was significantly restored in streptozotocin-induced diabetic mice after oral administration of *S. cumini* extract. Enhanced SOD activity, as seen in this study and others, suggests the extract's ability to improve mitochondrial function and reduce oxidative burden. In another relevant study by [25], rats fed with *S. cumini* leaf extract showed significant increase in both SOD and catalase activities, further supporting the notion that different parts of the plant, seeds, leaves, and pulp, possess bioactive compounds that target oxidative stress. Given that the high-dose extract in this current research produced an even greater SOD activity than drug control, it can be inferred that *S. cumini*'s antioxidant capacity may, in certain contexts, rival or complement that of standard pharmaceutical interventions.

MDA is a final product of polyunsaturated fatty acid peroxidation and serves as a robust marker of oxidative damage. As expected, MDA levels were significantly elevated in the diabetic control group (0.92 ± 0.05 nmol/mg protein), indicating increased lipid peroxidation under diabetic stress. Notably, *S. cumini* administration brought about a dose-dependent reduction in MDA levels, with the high-dose group reaching near-normal values (0.67 ± 0.02 nmol/mg protein), closely comparable to the normal control (0.64 ± 0.01 nmol/mg). These results agree with the observations of [35], who reported that diabetic rats treated with *S. cumini* seed oil showed significantly reduced MDA levels, demonstrating decreased lipid peroxidation and improved antioxidant defense. Similarly, [32] found that aqueous extract of *S. cumini* seeds effectively reduced MDA concentrations in the liver and kidneys of diabetic rats. The consistent decrease in MDA across extract doses in this study reflects the protective effect of *S. cumini* against membrane damage and oxidative injury. Fourier-transform infrared (FTIR) spectroscopy analysis is a qualitative technique provides valuable insights into the types of chemical bonds and functional groups present in the ethanol fraction of *S. cumini* leaves. This qualitative technique is fundamental for phytochemical profiling, as it identifies the

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

characteristic chemical bonds and functional groups present in the extract. **Table 3** lists specific absorbance wavenumbers (cm^{-1}), the corresponding class of compound, the identified functional group, and its intensity (e.g., strong, medium, weak, strong, broad). The most prominent feature is the strong and broad O-H stretch absorption band at 3260 cm^{-1}, which strongly indicates the abundant presence of alcohols and phenols. These classes of compounds are well-established for their potent antioxidant properties, acting as free radical scavengers, metal chelators, and reducing agents. The strong signals for these groups provide a robust chemical basis for the high antioxidant capacity observed in *S. cumini* leaves. This is a crucial finding, as phenolic compounds (including flavonoids and tannins) are well-known for their significant biological activities [18].

The C-H stretching vibrations at 2925 cm^{-1} and C-H rocking at 724 cm^{-1} confirm the presence of alkane (aliphatic) structures. These are common components of fatty acids, waxes, and the carbon backbone of various organic molecules, including the aliphatic chains within more complex secondary metabolites. A weak -C≡C- stretch absorption band at 2117 cm^{-1} suggests the possible presence of alkynes (compounds containing a carbon-carbon triple bond). While less common, some specialized plant metabolites can possess alkyne functionalities. A strong C=O stretch absorption band at 1693 cm^{-1}, specifically suggesting alpha, beta-unsaturated aldehydes or ketones. These functional groups are found in various secondary metabolites, including some pigments, terpenes, and volatile compounds, many of which are biologically active. The medium intensity N-H bending vibration at 1611 cm^{-1} indicates primary amines, while the C-N stretch at 1197 cm^{-1} suggests aliphatic amines. These groups are characteristic of amino acids, peptides, and alkaloids, which are important nitrogen-containing compounds often with pharmacological properties.

A strong N-O asymmetric stretch absorption band at 1514 cm^{-1} points to the presence of nitro groups. Their strong intensity indicates they are a significant component of this extract, and their biological roles can be diverse. A strong C-O stretch absorbance at 1320 cm^{-1} broad category includes alcohols (consistent with the O-H stretch), carboxylic acids, esters, and ethers. This peak likely represents the contribution of various oxygenated compounds prevalent in plant extracts, such as carbohydrates, phenolic compounds, and organic acids. Many phenolic acids, flavonoids, and other compounds containing the identified functional groups (e.g., carbonyls, amines) possess significant anti-inflammatory properties. They can modulate inflammatory pathways, inhibit pro-inflammatory enzymes, and reduce the production of inflammatory mediators. The presence of these compounds supports the plant's antioxidant and anti-inflammatory potential.

The medium intensity C-Cl stretching vibrations at 821 cm^{-1} suggest the presence of carbon-chlorine bonds (alkyl halides). Recent FTIR studies and broader phytochemical analyses of *S. cumini* leaf extracts, particularly those using ethanol or hydroalcoholic solvents, consistently report the dominant presence of O-H groups (from phenols and alcohols), C-H stretches (from alkanes), and various C-O stretches and aromatic signals. These findings align well with the known richness of *S. cumini* leaves in polyphenols (flavonoids, tannins) and carbohydrates [36]. While the specific appearance of alkynes, nitro compounds, or alkyl halides might be less universally reported, their detection here suggests unique constituents or the influence of specific environmental or genetic factors. Variations in peak intensities and the presence of minor peaks are common across studies due to differences in plant origin, maturity, extraction protocols, and analytical conditions [37].

The GC-MS profile of the ethanolic leaf fraction revealed a chemical composition consistent with strong antioxidant potential, driven primarily by phenolic constituents and supported by unsaturated fatty-acid derivatives. Catechol and 1,2,3-benzenetriol (pyrogallol) were among the major phenolics, both of which are well-established radical scavengers due to their hydroxyl-rich aromatic structures that readily donate hydrogen atoms and stabilize resulting phenoxyl radicals. Their antioxidant behavior is consistent with classical structure–activity studies showing that ortho- and tri-hydroxylated phenolics exhibit some of the highest electron-donating efficiency among simple phenolic acids [38,39]. The presence of a trihydroxy-benzoic acid methyl ester, structurally related to gallic acid, further supports this interpretation, as both gallic acid and its esters show potent reducing and metal-chelating activity across multiple antioxidant assays [40].

The extract also contained several unsaturated fatty-acid derivatives, including linoleic acid ethyl ester, 9,12-octadecadienoic acid, and oleic-acid analogues. Although these compounds are not as potent as phenolics on a per-molecule basis, unsaturated fatty acids can mitigate oxidative damage by slowing lipid peroxidation and interacting with lipid-phase radicals, contributing to broader antioxidant protection in biological systems [41,42]. The presence of palmitic and oleic acids may also provide mild membrane-stabilizing or cytoprotective effects under oxidative conditions, as reported in plant-based antioxidant studies.

Taken together, the chemical profile indicates that the antioxidant activity of the *S. cumini* ethanolic leaf fraction is likely driven by the synergistic interaction of high-activity phenolics with lipid-phase antioxidant contributions from unsaturated fatty acids. This pattern aligns with previous investigations demonstrating that *S. cumini* leaves are enriched in phenolic acids, flavonoids, and bioactive lipids that collectively enhance oxidative stability and biological antioxidant responses.

CONCLUSION

Syzygium cumini leaves demonstrate strong antioxidant potential driven by their high phenolic content, potent free-radical scavenging activity and a diverse profile of bioactive compounds confirmed by FTIR and GC-MS. The ethanolic fraction showed the highest activity, reflecting its enrichment in catechol, pyrogallol and other tri-hydroxylated phenolics. *In vivo*, the extract restored catalase activity and reduced lipid peroxidation in diabetic rats, indicating effective protection against oxidative stress. These findings support *S. cumini* leaves as a promising natural source of antioxidant agents with relevance for managing oxidative stress-related conditions.

REFERENCES

1. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058–70. https://doi.org/10.1161/circresaha.110.223545
2. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol cell Biol. 2020;21(7):363–83. http://dx.doi.org/10.1038/s41580-020-0230-3
3. Goyal S, Thirumal D, Singh S, Kumar D, Singh I, Kumar G, et al. Basics of antioxidants and their importance. Antioxidants Nature's Def Against Dis. 2025;1–20. http://dx.doi.org/10.1002/9781394270576.ch1
4. Hossain S, Rahaman A, Nahar T, Basunia MA, Mowsumi FR, Uddin B, et al. *Syzygium cumini* (L.) skeels seed extract ameliorates

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

in vitro and in vivo oxidative potentials of the brain cerebral cortex of alcohol-treated rats: *Syzygium cumini* (L.) skeels seed extract inhibits oxidative potentials of brain. Orient Pharm Exp Med. 2012;12(1):59–66. http://dx.doi.org/10.1007/s13596-011-0044-0

5. Borges RM, Bitencourt PER, Stein CS, Bochi G V, Boligon A, Moresco RN, et al. Leaves and seeds of *Syzygium cumini* extracts produce significant attenuation of 2, 2 azobis-2-amidinopropane dihydrochloride-induced toxicity via modulation of ectoenzymes and antioxidant activities. J Appl Pharm Sci. 2017;7(6):37–48. http://dx.doi.org/10.7324/japs.2017.70606
6. Hossain Z, Khanam M, Sarker AR. Out-of-pocket expenditure among patients with diabetes in Bangladesh: a nation-wide population-based study. Heal Policy Open. 2023;5:100102. http://dx.doi.org/10.1016/j.hpopen.2023.100102
7. Thapa CB, Paudel MR, Bhattarai HD, Pant KK, Devkota HP, Adhikari YP, et al. Bioactive secondary metabolites in Paris polyphylla Sm. and their biological activities: A review. Heliyon. 2022;8(2). http://dx.doi.org/10.1016/j.heliyon.2022.e08982
8. Srivastava N, Singh A, Kumari P, Nishad JH, Gautam VS, Yadav M, et al. Advances in extraction technologies: Isolation and purification of bioactive compounds from biological materials. In: Natural bioactive compounds. Elsevier; 2021. p. 409–33. http://dx.doi.org/10.1016/b978-0-12-820655-3.00021-5
9. Mukherjee S, Rizal S, Singh S, Hooi A, Ghosh PK, Hossain A, et al. Methodologies for identification, purification, and characterization of bacterial secondary metabolites. In: Bacterial secondary metabolites. Elsevier; 2024. p. 381–97. http://dx.doi.org/10.1016/b978-0-323-95251-4.00004-1
10. Ordonez AAL, Gomez JD, Vattuone MA. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. 2006;97(3):452–8. http://dx.doi.org/10.1016/j.foodchem.2005.05.024
11. Wolfe K, Wu X, Liu RH. Antioxidant Activity of Apple Peels. J Agric Food Chem. 2003;51(3):609–614. http://dx.doi.org/10.1021/jf020782a
12. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules. 2022;27(4):1326. http://dx.doi.org/10.3390/molecules27041326
13. Shangari N, O'Brien PJ. Catalase activity assays. Curr Protoc Toxicol. 2006;27(1):7. http://dx.doi.org/10.1002/0471140856.tx0707s27
14. Flohe L. [10] Superoxide dismutase assays. In: Methods in enzymology. Elsevier; 1984. p. 93–104. http://dx.doi.org/10.1016/s0076-6879(84)05013-8
15. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. 2017;524:13–30. http://dx.doi.org/10.1016/j.ab.2016.10.021
16. Mabasa XE, Mathomu LM, Madala NE, Musie EM, Sigidi MT. Molecular Spectroscopic (FTIR and UV-Vis) and Hyphenated Chromatographic (UHPLC-qTOF-MS) Analysis and *In Vitro* Bioactivities of the Momordica balsamina Leaf Extract. Biochem Res Int. 2021;2021(1):2854217. http://dx.doi.org/10.1155/2021/2854217
17. Pasternak Z, Avissar YY, Ehila F, Grafit A. Automatic detection and classification of ignitable liquids from GC–MS data of casework samples in forensic fire-debris analysis. Forensic Chem. 2022;29:100419. http://dx.doi.org/10.1016/j.forc.2022.100419
18. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol Reports. 2019;24:e00370. http://dx.doi.org/10.1016/j.btre.2019.e00370
19. Pinchuk I, Shoval H, Dotan Y, Lichtenberg D. Evaluation of antioxidants: scope, limitations and relevance of assays. Chem Phys Lipids. 2012;165(6):638–47. http://dx.doi.org/10.1016/j.chemphyslip.2012.05.003
20. Mensah MLK, Komlaga G, Forkuo AD, Firempong C, Anning AK, Dickson RA. Toxicity and Safety Implications of Herbal Medicines. Herb Med. 2019;63. http://dx.doi.org/10.5772/intechopen.72437
21. Tambe BD, Pedhekar P, Harshali P. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.)(Myrtaceae) leaves extracts. Asian J Pharm Res Dev. 2021;9(5):50–4. http://dx.doi.org/10.22270/ajprd.v9i5.1023
22. Dash JR, Kar B, Pattnaik G. In-silico, in-vitro and in-vivo Biological Activities of Flavonoids for the Management of Type 2 Diabetes. Curr Drug Discov Technol. 2024;21(5):80–91. http://dx.doi.org/10.2174/0115701638290819231228081120
23. Imran A, Ye S, Li JA, Ajaj R, Rauf A, Ahmad Z, et al. LC-ESI-QTOF-MS/MS Characterization of Phenolic Compounds in the Stem, Roots, and Leaves of *Syzygium cumini* and Their Antioxidant Potential. Food Sci Nutr. 2025;13(4):e70112. http://dx.doi.org/10.1002/fsn3.70112
24. Itam A, Wati MS, Agustin V, Sabri N, Jumanah RA, Efdi M. Comparative study of phytochemical, antioxidant, and cytotoxic activities and phenolic content of *Syzygium aqueum* (Burm. f. Alston f.) extracts growing in West Sumatera Indonesia. Sci World J. 2021;2021(1):5537597. http://dx.doi.org/10.1155/2021/5537597
25. Qamar M, Akhtar S, Ismail T, Wahid M, Abbas MW, Mubarak MS, et al. Phytochemical Profile, Biological Properties, and Food Applications of the Medicinal Plant *Syzygium cumini*. Food. 2022;11(378):1–21. http://dx.doi.org/10.3390/foods11030378
26. Asanaliyar M, Nadig P. Syzygium cumini (jamun) therapeutic potential: a comprehensive review on phytochemical constituents and emphasis on its pharmacological actions related to diabetic intervention. Int J Basic Clin Pharmacol. 2020;9(2):363. http://dx.doi.org/10.18203/2319-2003.ijbcp20200192
27. Eshwarappa RSB, Iyer RS, Subbaramaiah SR, Richard SA, Dhananjaya BL. Antioxidant activity of *Syzygium cumini* leaf gall extracts. BioImpacts BI. 2014;4(2):101. http://dx.doi.org/10.4103/0974-8490.147225
28. de Jesus Soares J, da Silva Rosa A, Motta PR, Cibin FWS, Roehrs R, Denardin ELG. Protective role of *Syzygium cumini* leaf extracts against paraquat-induced oxidative stress in superoxide-dismutase-deficient Saccharomyces cerevisiae strains. Acta Sci Biol Sci. 2019;41:47139. http://dx.doi.org/10.4025/actascibiolsci.v41i1.47139
29. Akullo JO, Kiage-Mokua BN, Nakimbugwe D, Kinyuru J. Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic. Heliyon. 2023;9(8). http://dx.doi.org/10.1016/j.heliyon.2023.e18806
30. Sadowska-Bartosz I, Bartosz G. Evaluation of The Antioxidant Capacity of Food Products: Methods, Applications and Limitations. Processes 2022, 10, 2031. http://dx.doi.org/10.3390/pr10102031
31. Amir Rawa MS, Mazlan MKN, Ahmad R, Nogawa T, Wahab HA. Roles of Syzygium in Anti-Cholinesterase, Anti-Diabetic, Anti-Inflammatory, and Antioxidant: From Alzheimer's Perspective. Plants. 2022;11(11). http://dx.doi.org/10.3390/plants11111476
32. Patel H, Mandle N. Pharmacodynamic Evaluation of *Syzygium cumini* Extract in Experimental Diabetic Models. Int J Pharmacogn Herb Drug Technol. 2025;02(04):1–13. https://aktpublication.com/index.php/ijphdt/article/view/63
33. Kaur D, Yousuf B, Qadri OS. Syzygium cumini anthocyanins: recent advances in biological activities, extraction, stability, characterisation and utilisation in food systems. Food Prod Process Nutr. 2024;6(1). http://dx.doi.org/10.1186/s43014-023-00177-6
34. Nahid S, Mazumder K, Rahman Z, Islam S, Rashid MH, Kerr PG. Cardio- and hepato-protective potential of methanolic extract of *Syzygium cumini* (L.) Skeels seeds: A diabetic rat model study. Asian Pac J Trop Biomed. 2017;7(2):126–33. http://dx.doi.org/10.1016/j.apjtb.2016.11.025
35. Seker U, Kaya S, Kandemir SI, Sener D, Demirel OU, Nergiz Y. Effects of black cumin seed oil on oxidative stress and expression of membrane-cytoskeleton linker proteins, radixin, and moesin in streptozotocin-induced diabetic rat liver. Hepatol Forum. 2022;3(1):21–6. http://dx.doi.org/10.14744/hf.2021.2021.0035
36. Feng Y, Chen W, Jia Y, Tian Y, Zhao Y, Long F, et al. N-Heterocyclic molecule-capped gold nanoparticles as effective antibiotics against multi-drug resistant bacteria. Nanoscale. 2016;8(27):13223–7. http://dx.doi.org/10.1039/c6nr03317b
37. Taylor CJ, Pomberger A, Felton KC, Grainger R, Barecka M, Chamberlain TW, et al. A brief introduction to chemical reaction optimization. Chem Rev. 2023;123(6):3089–126. http://dx.doi.org/10.1021/acs.chemrev.2c00798
38. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933–56. http://dx.doi.org/10.1016/0891-5849(95)02227-9
39. Kiokias S, Proestos C, Oreopoulou V. Phenolic Acids of Plant

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

Origin-A Review on Their Antioxidant Activity *In Vitro* (O/W Emulsion Systems) Along with Their in Vivo Health Biochemical Properties. Foods (Basel, Switzerland). 2020 Apr;9(4). http://dx.doi.org/10.3390/foods9040534

40. Marinova E, Yanishlieva N. Antioxidant activity and mechanism of action of some phenolic acids at ambient and high temperature. Food Chem - FOOD CHEM. 2003 May 1;81:189–97. http://dx.doi.org/10.1016/s0308-8146(02)00411-9
41. Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacol Res. 2008 Jun;57(6):451–5. http://dx.doi.org/10.1016/j.phrs.2008.05.002
42. Tsaluchidu S, Puri BK. Fatty acids and oxidative stress. Ann Gen Psychiatry [Internet]. 2008;7(1):S86. https://doi.org/10.1186/1744-859X-7-S1-S86

This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).