

Phytochemical Profile, Antioxidant Potential, and Synergistic Antibacterial Effects of *Syzygium Cumini* (L.) Skeels Leaf Extract in Combination with Antibiotics

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ABSTRACT

Background: *Syzygium cumini* (L.) Skeels, a member of the Myrtaceae family, is widely recognized for its diverse pharmacological properties, including anti-inflammatory, anti-cancer, anti-diabetic, and cardioprotective effects. Its bioactive compounds have demonstrated potential in antimicrobial and antioxidant applications. This study aimed to evaluate the chemical composition, antioxidant capacity, and synergistic antibacterial effects of *S. cumini* leaf extract in combination with antibiotics. **Methods:** Fresh *S. cumini* leaves were collected and extracted using a 50% aqueous-methanolic solvent. High-performance liquid chromatography (HPLC) was used to identify and quantify key phenolic compounds in the extract, including chlorogenic acid, catechin, kaempferol, myricetin, quercetin, ferulic acid, and gallic acid at concentrations of 74.1, 98.0, 165.8, 198.7, 201.4, 221.4, and 273.9 ppm, respectively. The antioxidant activity of the extract was assessed using the DPPH free radical scavenging assay, and the inhibitory concentration (IC₅₀) was determined. The antibacterial activity and synergistic effects of the extract were evaluated against multiple pathogenic bacterial strains in combination with six conventional antibiotics using the disk diffusion method. **Results:** The *S. cumini* leaf extract exhibited potent antioxidant activity, with a DPPH scavenging rate of 91.25% at a concentration of 200 µg/mL and an IC₅₀ value of 20.28 µg/mL. Synergistic antibacterial effects were observed when the extract was combined with six antibiotics, demonstrating an enhanced inhibitory effect against tested bacterial strains. The results suggest that the bioactive compounds present in *S. cumini* may potentiate the antimicrobial activity of conventional antibiotics, making them a promising adjunct in antimicrobial therapy. **Conclusion:** The findings of this study highlight the potential of *S. cumini* leaf extract as a natural source of antioxidants and antimicrobial agents. Its ability to enhance the efficacy of antibiotics suggests a possible role in combatting antibiotic resistance. Further studies are recommended to elucidate the underlying mechanisms and explore its clinical applications in laboratory medicine and pharmaceutical formulations.

Keywords: *Syzygium Cumini*, Myrtaceae, Antioxidant Activity, HPLC

INTRODUCTION

The Myrtaceae family is the eighth largest family of dicotyledonous flowering plants and the third largest family of trees. All species of the family are characterized by being woody, including climbers, small shrubs, and trees, which are spread in tropical and subtropical regions, and their distribution center is in America and Australia [1]. The species *Syzygium cumini* is one of the species of the myrtaceae family. It is a large evergreen tropical tree, reaching a height of 25-30 meters with dense, gray-brown bark. It is a fast-growing tree and can live for 100 years. The leaves of the plant are simple, opposite each other. The leaf blade is broadly elliptic and may be oblong-ovate, or elliptical, with a length of 6-12 cm. The leaf is somewhat smooth. Sometimes it is somewhat leathery [2]. Several studies have shown the effectiveness of essential oils extracted from the leaves of the species, as well as the hydro alcoholic extract, as an antidote to strains of bacteria and fungi. The essential oils extracted from the leaves have antibacterial properties against *Basillus sphaericus*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Samonella typhimurium* [3]. Antioxidants are a group of elements and compounds that are radical scavengers that work to stop or slow down the oxidation process in order to protect other compounds [4]. Several studies have indicated the biological effectiveness of *S.cumini* as an antioxidant. The study of [5, 6] confirmed that the plant *S.cumini* is rich in many chemical compounds, including anthrocyinins, glucoside, isoquercetin, kaemferol, ellagic acid and myrecetin. His study also revealed that

extracts of *S. cumini* contain high levels of flavonoids, tannis, carbohydrates and phenols. Chromatography, a word derived from the Greek word chroma for color and graphy for writing, is the comprehensive term for a group of techniques for separating mixtures. Chromatography is a technique for separating sample components based on differences in the speed of diffusion of compounds in a given medium. HPLC is a chromatographic technique that uses a solvent under pressure to separate, characterize and quantify substances in a sample. It is a technology that relies on the principle of adsorption, partitioning, ion exchange, and gel permeation. In this type of chromatography, compounds are separated based on their distinct polarities, as polar substances do not encounter any obstruction by the separation column, unlike non-polar substances that will encounter obstruction or retention by the separation column, and thus the materials will separate from each other [7]. Antimicrobial synergy, in which two drugs used in combination exert a greater effect than the additives, offers the possibility of rescuing existing antibiotics for use in the treatment of drug-resistant bacteria, even when these bacteria are resistant to one or both antibiotics separately.

MATERIAL AND METHODS

Collect Plant Samples

Samples were collected for the period from 7/12/2023 to 8/1/2024 and were diagnosed by Assis.Pro. Shaemaa Muhi Hassoun. After

washing with distilled water, it was placed on paper to dry for (3-5) days in the shade at room temperature. The leaves were ground using an electric grinder to obtain a dry plant powder, then packed in paper bags until use.

Preparation of Plant Extraction

The plant extract was prepared based on the method of Akowuah *et.al.*, 2004, with some modification using a solvent (methanol - distilled water) 50%. Place 70 gm of dried, ground leaves in volumetric flasks (1L) containing 700 ml of solvent. With the opening of the flask closed with aluminum foil to avoid evaporation of the mixture and to prevent air oxidation. After that, the flasks were placed in a shaking water bath at 37 °C for two hours at high speed. Then the extracts were filtered using medical gauze and then several layers of Whatman type filter paper (1). Then it was placed in a centrifuge at 2500 rpm for (10) minutes. Finally, place the filtrate in a glass dish and leave it in an oven at 45 degrees for 2-5 days to dry. Then scrape the extract and store it in clean containers until use.

High Performance Liquid Chromatography (HPLC)

Phenolic compounds were extracted from raw plant leaves by adding 100 ml of chloroform to 20 gm from the plant after grinding it well and placing it on an electric vibrator for 3 hours to remove the fat from the sample, then the chloroform layer was removed. Then, the sample was dried at a temperature of 50 °C to ensure that there were no traces of chloroform. A take 10 gm from the dried sample for extraction using ethanol/water solvent (70/30). Use 5 ml of liquid extract. The solvent was removed using a rotary evaporator under vacuum and dried at 40 °C to obtain a constant mass of the extract. Then, it was stored in glass bottles at a temperature of 4 °C to prevent oxidation until the test was conducted. The extraction process was performed for one hour, at room temperature, using an Ultrasonic Bath (USA). Reverse-phase HPLC analysis was used, using a SYKAM HPLC chromatographic system equipped with a UV detector and a C18-OSD (25cm, 4.6mm) column, the column temperature is 30 °C. The gradient elution method was conducted using eluent A (methanol) and eluent B (1% formic acid in water (v/v)). (For the purpose of quantitative measurement of individual phenolic compounds). initial 0-5 min, 40 % B; 6-20 min, 50 % B; and flow-rate of 0.7 mL/min. The spectra were acquired in the 280 nm

Estimation of Antioxidant Activity

The antioxidant activity of the leaves extract was estimated using a solvent (50% Aqueous-methanolic) using the DPPH method according to the method described by [8]. With some modification. Briefly, the DPPH solution (concentration 0.1 mM) was prepared by

dissolving 4 mg in 100 ml methanol. After that, 1 ml of this solution was added to 3 ml of extract at different concentrations (3.12, 6.25, 12.5, 50, 100 and 200) µg/ml. The test was conducted with three replicates. Shake the mixture vigorously, then leave it in a dark place for 30 minutes. Then, the radical scavenging activity was determined using a UV spectrophotometer, where the absorbance was measured at 517 nm. The percentage effect of the extract in scavenging DPPH was calculated using the following equation:

$$\text{Percent inhibition (\%)} = A_0 - A_1 / A_0 \times 100$$

A₀ = The Absorbance of control reaction.

A₁ = the absorbance in presence standard sample)

Synergistic Effect

The synergistic effect was determined by saturating the antibiotic tablets with plant extract at a concentration of 104 µg/ml to measure their effectiveness against G⁺ (*Streptococcus pneumonia*, *Staphylococcus aureus*). bacteria and at a concentration of 208 µg/ml against (*Escherichia coli*, *Pseudomonas aeruginosa*) and after growing them on MHA medium. MHA plates were kept at 4 °C for 1 h to allow proper diffusion, after which they were kept at 37 °C for 24 h. The inhibition zone was measured using a ruler.

Results and Discussion

Detection of Some Phytochemical Compounds

During the research, some preliminary (qualitative) chemical tests were conducted on the Aqueous -alcoholic extract of *S.cumini* leaves, which confirmed the presence of phenols, glycosides, flavonoids, saponins, terpenes, steroids, and tannins. Table (1, 2), Figure (1). These results are consistent with the study [9-12].

High-Performance Liquid Chromatography (HPLC)

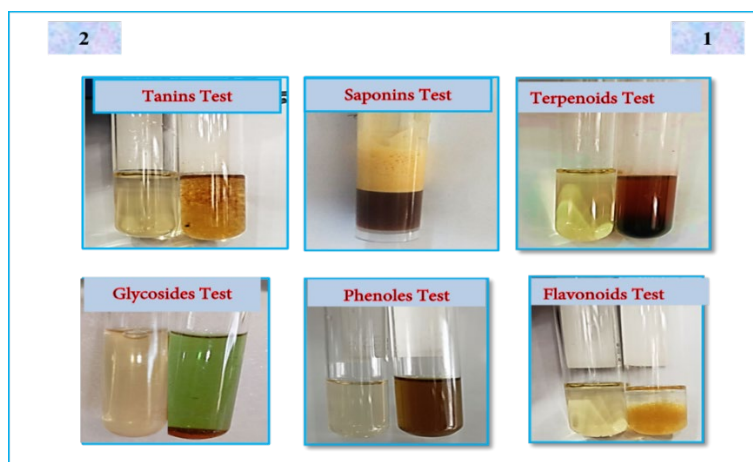
During the current research, some phenolic compounds found in *S.cumini* leaves were analyzed using HPLC technology, as it is a sensitive and accurate technology that is widely used to evaluate the quality of plant extracts. Quantitative and qualitative diagnosis (identification) of each of the compounds (chlorogenic acid, Catechin, Kaempferol, Myricetin, Quercetin, Ferulic acid and Gallic acid) was carried out, by applying appropriate chromatographic conditions from the stationary phase and a graduated polarity system for the mobile phase Schedule [13]. Several studies have confirmed the importance of these compounds as antioxidants, anti-inflammatory and anti-cancer. Through the results of chemical analysis, it was found that Gallic acid has a higher concentration than the rest of the compounds that were detected, and these results are consistent with the study of [14, 15] (Table 3,4).

Table 1: Detection of Some Chemical Compounds in *S.Cumini* Leaves Extract Based on Chemical Tests.

S.Cumini Leaves	Positive Results	Reagent	Phytochemical Compound
Extract	Yellow Color	Picric Acid + H ₂ O	Alkaloid
+	Green-bluish	Ferric chloride 1%	Phenols compound
+	Red Pellet	Benedict	Glycosides
+	yellow color	KOH + distilled water	Flavonoids
+	Formation of foam	Shaken	Saponin
+	Brown color	Chloroform + Acetic Acid + H ₂ SO ₄	Terpenes
+	Blue Color	Chloroform + Acetic Acid + H ₂ SO ₄	Steroids
+	White Gelatinous	Lead Acetate 1%	Tannins

Table 2: Concentrations of Phenolic Compounds in Leaves Extract Of *S.Cumini*.

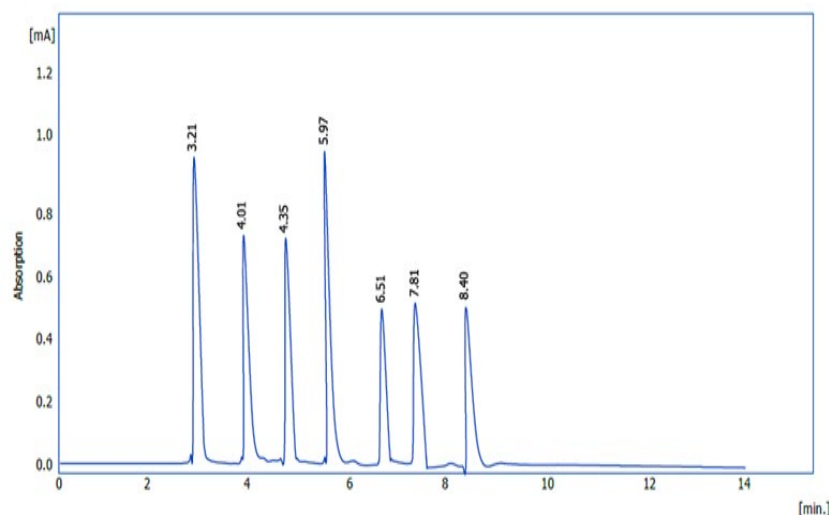
No	Name	Con (ppm)
1	Chlorogenic acid	74.1
2	Catechine	98.0
3	Kaempferol	165.8
4	Myricetin	198.7
5	Quercetine	201.4
6	Ferulic acid	221.4
7	Gallic acid	273.9

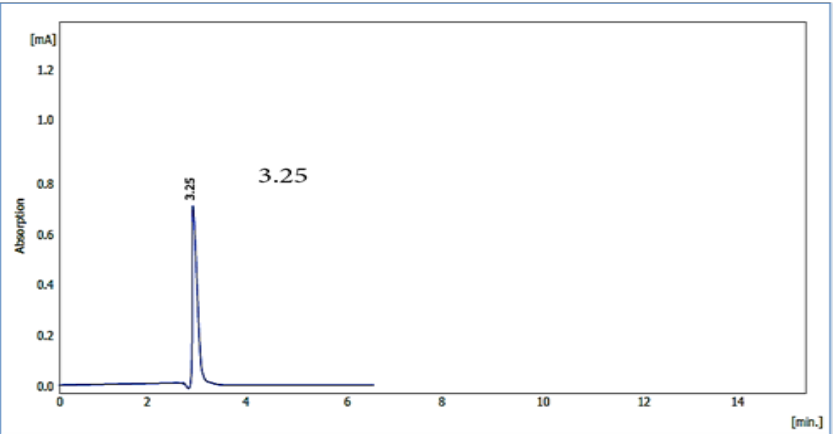
**Figure 1:** Some Qualitative Examinations of the of *S.Cumini* Leaves Extract. 1: Metanolic-Water Extraction, 2: Control Sample**Table 3:** Results of HPLC Analysis of Some Phenolic Compounds in *Syzygium Cumini* Leaves Extract

No.	Reten.time [Min.]	Area [MAU.s]	Height [MAU]	Area [%]	Height [%]	W05[min]	Compound Name
1	3.21	1255210.58	960.14	20.00	20.00	0.20	Chlorogenic acid
2	4.01	1124598.01	790.11	15.00	15.00	0.10	Ferulic acid
3	4.35	1395421.78	786.98	15.00	15.00	0.10	Quercetin
4	5.97	2047833.64	966.25	20.00	20.00	0.20	Gallic acid
5	6.51	521346.25	560.14	10.00	10.00	0.08	Myricetin
6	7.81	952467.11	564.80	10.00	10.00	0.08	Catechin
7	8.40	369805.14	562.48	10.00	10.00	0.08	Kaempferol
Total		7666682.14	5190.20	100.00	100.00		

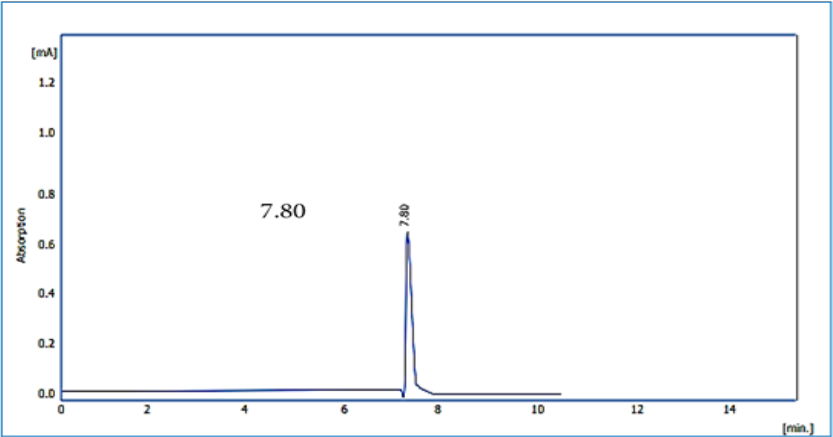
Table 4: Results of HPLC Analysis of Standard Compounds

No.	Reten. Time [Min]	Area [Mau.S]	Height [Mau]	Area [%]	Height [%]	W 05	Compound Name
1	3.25	985.60	711.80	100.00	100.00	]Min[	Chlorogenic acid
2	4.01	1985.44	640.15	100.00	100.00	0.25	Ferulic acid
3	4.36	2105.98	611.90	100.00	100.00	0.25	Quercetin
4	5.90	3201.49	800.74	100.00	100.00	0.25	Gallic acid
5	6.50	1950.14	784.11	100.00	100.00	0.25	Myricetin
6	7.80	1697.84	65.25	100.00	100.00	0.25	Catechin
7	8.45	1320.41	611.90	100.00	100.00	0.25	Kaempferol

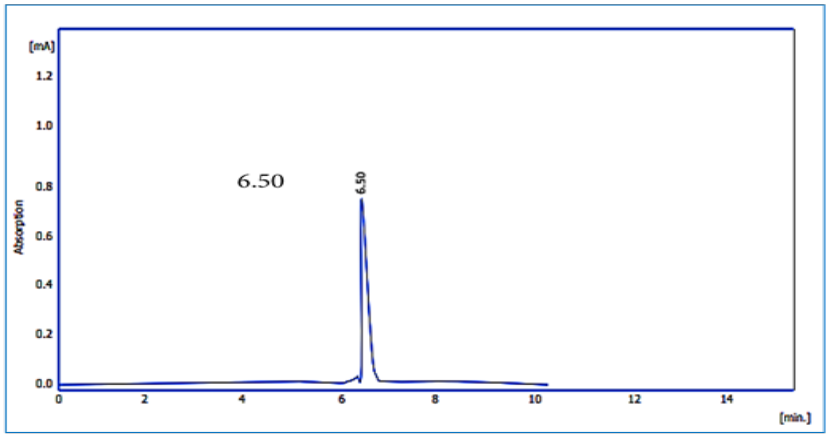
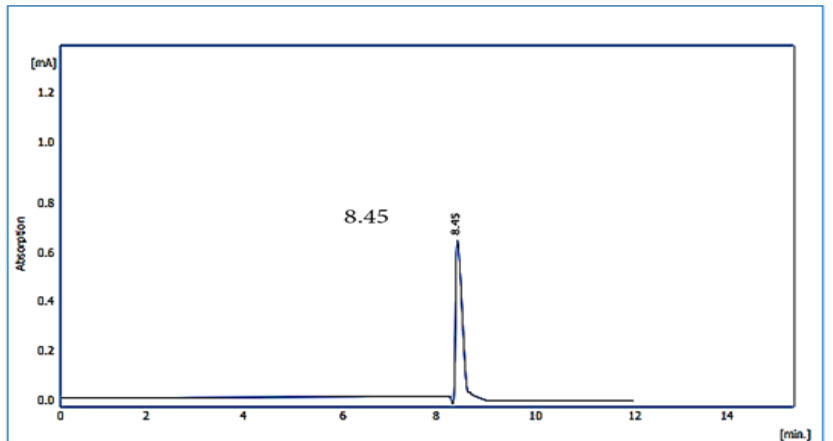




Result chromatography (Uncal - F:\ chlorogenic acid 10 ppm)



Result chromatography (Uncal - F:\ catechine 10 ppm)



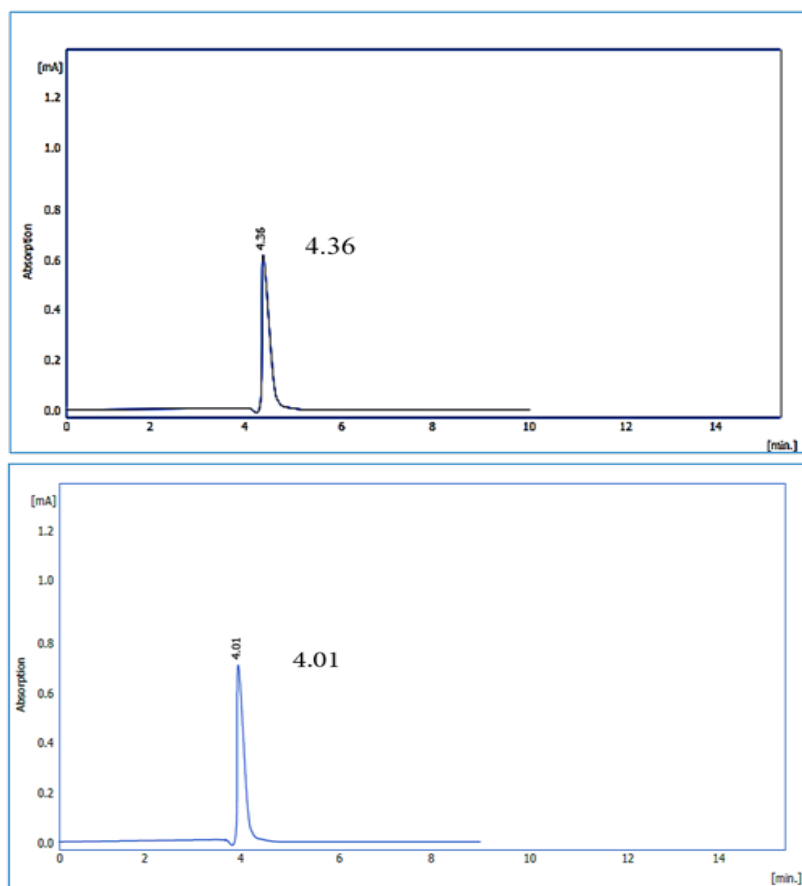


FIGURE 2: Chromatography of Syzygium Cumini Extract

Antioxidant Activity of Syzygium Cumini (L.) Skeels Extract

Antioxidants are substances capable of inhibiting oxidative processes and thus blocking the chemical reaction that transfers electrons or hydrogen to an oxidizing agent [16]. Scientific evidence has indicated that antioxidants reduce the risk of chronic diseases such as heart disease and cancer [17]. During the current study, the solvent Aqueous-Methanolic extract 50% was used to prepare an extract of *S. cumini* leaves. Different concentrations were taken: 3.12, 6.25, 12.5, 25, 100 and 200 µg/ml and using the DPPH method. The basis of this method is that the DPPH radical acts as an electron acceptor from a donor molecule as an antioxidant, as a result of which DPPH is converted to DPPH₂. DPPH radical scavenging is the basis for evaluating antioxidant capacity. The results of the current study found that *S. cumini* leaves extract has a high scavenging activity rate (Figure

2). The highest rate reached 91.25 at a concentration of 200 µg/ml. (Table 5, 6). The IC₅₀ value is 20.28 µg/ml, and this confirms that the alcoholic extract of *S. cumini* leaves has high antioxidant activity. These results are consistent with the results of the study [18-20].

These results are due to the presence of many active compounds in the leaves of *S. cumini*, especially phenolic compounds, flavonoids, and terpenes. Ferulic acid and catechin are phenolic compounds that play a role in the effectiveness of the extract as an antioxidant. These results are consistent with the study [21], as well as other studies that confirmed the high content of phenolic and flavonoids compounds in *S. cumini* leaves. Thus, the results of the current study confirm the basis for the use of *S. cumini* leaves extract in medical health applications, functional foods, and nutrients as a result of its antioxidant properties (Figure 3, 4).



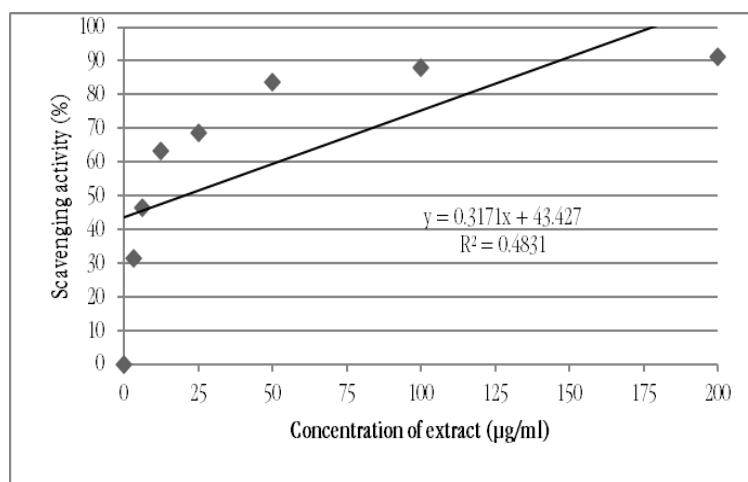
FIGURE 3: Antioxidant Activity by DPPH Method.

Table 5: Percentage of Scavenging Activity.

Concentration. (µg/ml)	Extract
0.0	0
200	91.25
100	88.06
50	83.75
25	68.81
12.5	63.25
6.25	46.63
3.12	31.50

Table 6: Antioxidant Activity of Plant Extracts Against Free-Radicals. [8]

Plant	IC ₅₀ (µg/ml)	Mark	Solvent
Syzygium.cumini	20.28	Strong	Methanol

**Figure 4:** Antioxidant Activity of the Aqueous - Methanolic Extract of *S. Cumini* Leaves.

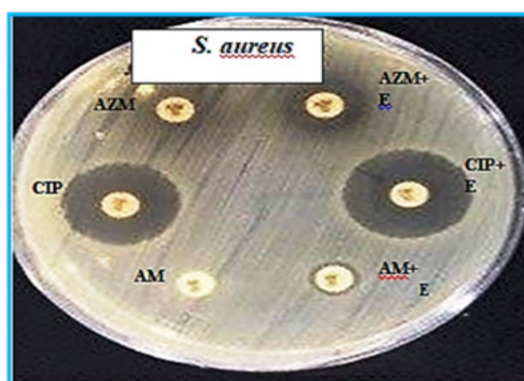
Synergistic Effect of *S. Cumini* Leaves Extract with Antibiotic

The synergistic effect of *S. cumini* leaves extract with 6 antibiotics against highly resistant isolates was examined. The results showed an increase in the effect of the antibiotic. This was done by saturating antibiotic tablets (Azithromycin, Nitrofurantoin, Imipenem, Amikacin, Nalidixic acid, ciprofloxacin) with the extract at a concentration of 104 µg/ml against *S. pneumoniae* and *S. aureus* and 208 µg/ml against *P. aeruginosa* and *E. coli* with an antibiotic tablet not saturated with the extract as a positive control (Figure 5).

The findings demonstrated that the antibiotic tablets saturated with the *S. cumini* leaf extract showed a significant increase in the

zones of inhibition compared to the control tablets. This indicated that the leaf extract enhanced the antibacterial activity of the antibiotics. For instance, tablets saturated with the extract exhibited larger zones of inhibition against *S. pneumoniae* and *S. aureus* at the lower concentration of 104 µg/ml, as well as against *P. aeruginosa* and *E. coli* at the higher concentration of 208 µg/ml.

This synergistic effect suggests that the compounds in *S. cumini* leaf extract may interact with the antibiotics to improve their efficacy, potentially by different mechanisms such as enhancing antibiotic uptake, disrupting bacterial cell walls, or interfering with bacterial resistance mechanisms.

**Figure 5:** Synergistic Effect of *S. cumini*

REFERENCES

- [1] P. Shafi, M. Rosamma, K. Jamil, and P. Reddy, "Antibacterial activity of Syzygium cumini and Syzygium travancoricum leaf essential oils," *Fitoterapia*, vol. 73, no. 5, pp. 414-416, 2002.

- [2] L. Leaves and L. Leaves, "Antioxidant activity by DPPH radical scavenging method of *ageratum conyzoides*," *American Journal of Ethnomedicine*, vol. 1, no. 4, pp. 244-249, 2014.
- [3] S. Phongpaichit *et al.*, "Biological activities of extracts from endophytic fungi isolated from *Garcinia* plants," *FEMS Immunology & Medical Microbiology*, vol. 51, no. 3, pp. 517-525, 2007.
- [4] N. Sarma, T. Begum, S. K. Pandey, R. Gogoi, S. Munda, and M. Lal, "Chemical composition of *Syzygium cumini* (L.) Skeels leaf essential oil with respect to its uses from North East region of India," *Journal of Essential Oil Bearing Plants*, vol. 23, no. 3, pp. 601-607, 2020.
- [5] R. M. R. Dulay and M. E. G. Castro, "Chemical constituents, antioxidant and antibacterial activity of *Syzygium cumini* (L.) Skeels (Myrtaceae)," *Der Pharma Chemica*, vol. 8, no. 20, pp. 317-321, 2019. [Online]. Available: <http://derpharmachemica.com/archive.html>.
- [6] B. B. Tewari and S. Gomathinayagam, "A critical review on *Ocimum tenuiflorum*, *Carica papaya* and *Syzygium cumini*: the medicinal flora of Guyana," *Revista Boliviana de Química*, vol. 31, pp. 28-41, 2014.
- [7] I. Jo *et al.*, "Ecological importance of the Myrtaceae in New Zealand's natural forests," *Journal of Vegetation Science*, vol. 33, no. 1, p. e13106, 2022.
- [8] S. Beiranvand, A. Williams, S. Long, P. R. Brooks, and F. D. Russell, "Use of kinetic data to model potential antioxidant activity: Radical scavenging capacity of Australian *Eucalyptus* honeys," *Food Chemistry*, vol. 342, p. 128332, 2021.
- [9] Z. P. Ruan, L. L. Zhang, and Y. M. Lin, "Evaluation of the antioxidant activity of *Syzygium cumini* leaves," *Molecules*, vol. 13, no. 10, pp. 2545-2556, 2008.
- [10] T. Devasagayam, J. Tilak, K. Boloor, K. S. Sane, S. S. Ghaskadbi, and R. Lele, "Free radicals and antioxidants in human health: current status and future prospects," *Japi*, vol. 52, no. 794804, p. 4, 2004.
- [11] M. Bajpai, A. Pande, S. Tewari, and D. Prakash, "Phenolic contents and antioxidant activity of some food and medicinal plants," *International journal of food sciences and nutrition*, vol. 56, no. 4, pp. 287-291, 2005.
- [12] A. Kumar and M. Kalakoti, "Phytochemical and antioxidant screening of leaf extract of *Syzygium cumini*," *Int J Adv Res*, vol. 3, no. 1, pp. 371-8, 2015.
- [13] M. Qamar *et al.*, "Phytochemical profile, biological properties, and food applications of the medicinal plant *Syzygium cumini*," *Foods*, vol. 11, no. 3, p. 378, 2022.
- [14] A. A. Shad *et al.*, "Phytochemical and biological activities of four wild medicinal plants," *The Scientific World Journal*, vol. 2014, no. 1, p. 857363, 2014.
- [15] G. C. Jagetia, "Phytochemical Composition and pleotropic pharmacological properties of jamun, *Syzygium cumini* skeels," *Journal of exploratory research in pharmacology*, vol. 2, no. 2, pp. 54-66, 2017.
- [16] M. Carochio and I. C. Ferreira, "A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives," *Food and chemical toxicology*, vol. 51, pp. 15-25, 2013.
- [17] E. E. Aung, A. N. Kristanti, N. S. Aminah, Y. Takaya, and R. Ramadhan, "Plant description, phytochemical constituents and bioactivities of *Syzygium* genus: A review," *Open Chemistry*, vol. 18, no. 1, pp. 1256-1281, 2020.
- [18] V. Chavan, T. K. Chache, and V. Yadav, "Simultaneous quantification of gallic acid, myricetin and quercetin in the extract of *Syzygium cumini* plant and its formulation using HPLC," *Asian J. Pediatr. Pract.*, vol. 5, no. 1, pp. 85-88, 2019.
- [19] G. Akowuah, I. Zhari, I. Norhayati, A. Sadikun, and S. Khamsah, "Sinensetin, eupatorin, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia," *Food Chemistry*, vol. 87, no. 4, pp. 559-566, 2004.
- [20] K. S. De Bona *et al.*, "*Syzygium cumini* extract decrease adenosine deaminase, 5' nucleotidase activities and oxidative damage in platelets of diabetic patients," *Cellular Physiology and Biochemistry*, vol. 26, no. 4-5, pp. 729-738, 2010.
- [21] R. S. Rita and E. Sy, "*Syzygium cumini* leaves extract from West Sumatra Indonesia alleviate oxidative stress by decreasing malondialdehyde level and enhancing catalase activity in rat induced by lead acetate," *Pharmacognosy Journal*, vol. 13, no. 6, 2021.



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