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Isolation of Bioactive compounds from the essential oil of Jambolan (*Syzygium cumini)* and Invitro evaluation of biological potential

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Abstract

The essential oil from leaves of the *Syzygium cumini* is the subject of this essay, which concentrates on its chemical makeup, antioxidant properties, cytotoxicity (as measured by the mortality and hemolysis of brine shrimp) and antibacterial properties. According to the results of the GC-MS study, 39 substances were discovered. Among these, α-pinene (22.29%), (E)-ocimene (11.68%), D-limonene (7.96%) and α-terpineol (5.26%) are the most noticeable. The free radical scavenging assay (33.17 g/mL), β-carotene in linoleic acid, and percent inhibition in linoleic acid (54.9%) systems were used to test the *Syzygium cumini* essential oil's antioxidant activity. Oil's antibacterial activity was tested using two-gramme-negative bacteria, four-gramme-positive bacteria, and four pathogenic fungi. The most susceptible fungi and bacteria were *Aspergillus niger* and *Streptococcus Aureus*, with MIC values of 2.03 and 3.83 mg/mL, respectively. The LC50 value of the *S.cumini* leaf essential oil was 29.45 µg/mL for cytotoxicity against *Artemia salina* nauplii, and the minimum hemolytic activity was 4.26% at a dosage of 0.5 mg/mL.

Keywords: *Syzgium cumini*, essential oil, *Artemia salina*, hemolytic assay, probit analysis

Highlights

- Essential oil extraction from *Syzygium cumini* using Hydrodistillation extraction techniques.
- Bioactive components of essential oil were identified using gas chromatography-mass spectrometry.
- Essential oil and a high percentage of bioactive compounds showed good antioxidant and antimicrobial activities.
- Isolated bioactive compounds in essential oil had excellent cytotoxic and haemolytic potential.
- The extracted essential oil can be used in functional food, nutra-pharmaceutical, and insecticide industries.

1. Introduction

A significant dual role of free radicals and oxidants in food systems that may be toxic or beneficial. Free radicals are typically an essential physiology component and can be found as reactive, transient oxygen and nitrogen species (Di Meo & Venditti, 2020; Kruk et al.,2019). Due to oxidative stress brought on by an unbalanced antioxidant defence system in people, these reactive species are overproduced (Bhatti et al. 2022; Alemany-Cosme et al. 2021).

Despite many food preservation technologies, industrialists and customers are still concerned about how quickly food and other eatables deteriorate. Despite these preservation techniques, food sickness and shorter shelf life are caused by microbial contamination and lipid peroxidation. Additionally, it causes various food-borne illnesses and financial losses (Zubair et al., 2022; Gallo, 2020). Because of this, people are looking for natural ingredients like extracts and essential oils that can be utilized as food preservatives (Rahardiyan et al., 2020). Di Meo & Venditti, 2020).

Antioxidants have been overused in the food sector to extend the shelf life of foods containing polyunsaturated fatty acids. Antioxidants can be a very efficient substitute for storing food and reducing the oxidation rate (Gulcin, 2020; Pateiro et al., 2021). Due to their harmful impact on health, the frequent use of synthetic antioxidants is currently prohibited. Therefore, identifying efficient non-carcinogenic antioxidants that can safeguard the human body against free radicals and their deadly consequences through which they become accountable for chronic diseases has been of significant interest to scientists (Chen et al., 2021).

Numerous sources of plant-based antioxidants have been investigated during the investigation that has already been conducted. Additionally, these natural antioxidants can be synthesized into functional meals and nutraceuticals to reduce oxidative damage in our bodies. Scientists are paying attention to essential oils because they can preserve food, pharmaceuticals, complementary treatments, and natural cures (Anjos da Silva et al., 2023). They have also demonstrated several pharmacological properties, including hepatoprotective, antidiabetic, spasmolytic, antibacterial, anti-carcinogenic and antiviral activities. The behaviour of these against bacteria and oxidants, which demonstrated its effect on food security, is another factor drawing attention to them (Drinić et al.2020)

The Jambolan (*S.cumini*) tree is a sizable, leafy, evergreen plant indigenous to Pakistan, India, China, Indonesia and the West Indies and grown in tropical climates. Jambolan leaf essential oils have a variety of uses along with medicinal uses, including flavour in meat items, alcoholic beverages, and perfumery goods. Numerous studies have been conducted on the biological effects of Jambolan leaf essential oil (Sharmeen et al, 2021; Sarma et al., 2020; Elyemni et al.,2019).

The objective of the current study was to determine the composition and other characteristics of the hydro-distilled

essential oil of *S. cumini*, including its antioxidant, cytotoxic, and antibacterial capabilities.

2. Materials and Methods

2.1 Plant collection

The *S. cumini* leaves sample was collected from the Botanical Garden, University of Agriculture Faisalabad, Pakistan. Fresh material was treated right away to obtain its essential oil.

2.2 Extraction of Essential Oil

Bright green colour leaves of Jambolin were collected, washed and weighed. By boiling the plant material in water, the hydrodistillation process was employed to extract essential oils (Drinić et al., 2020; Sarma et al., 2020; Elyemni et al.,2019). 1000 g of fresh leaves were immersed in water and boiled for 3 hours. A separatory funnel was used to separate the oil because of its hydrophobic nature, which caused it to form a separate layer. Additionally, anhydrous sodium sulphate was used to eliminate the water content of the oil, followed by filtration and storage of the filtrate at 4 °C in a sealed glass bottle.

2.3. Analysis of Essential Oil

Different physical parameters like colour, refractive index, solubility and oil density were determined using standard methods [3-5]. A Perkin Elmer Clarus 600 GC with an ULTRA 1 column filled with 100% dimethyl polysiloxane (sheet thickness 0.25 m, 30 m 0.25 id) and a Perkin Elmer Clarus 600C MS with an FID detector were used to perform the GC-MS analysis of the *S.cumini* essential oil.

2.4. Antioxidant assay

The antioxidant activity of *S.cumini* essential oil was discovered with a few modest adjustments. The level of oxidation inhibition was calculated (Mahulette et al., 2020; Bogdan et al., 2021). The IC50 value (g/ml) was used to measure antioxidant activity; the lower the IC50 value, the greater the antioxidant activity (Missouri et al., 2020; Ikeda et al.,2021). Oxidation was measured using the thiocyanate technique (Elazzouzi et al., 2020). Inhibition of the linoleic acid emulsion system with a small adjustment allowed researchers to measure antioxidant activity (Ikeda et al.,2021).

2.5. Antimicrobial assays

Bacillus subtilis, *Staphylococcus aureus*, 2 gram-negative bacteria, *Escherichia coli*, *Pasteurella multocida* and 4 pathogenic fungi, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger* and *Ganoderma lucidum*, were tested for antimicrobial activity against the sample. While fungal strains were cultured for 48 hours at 28°C using potato dextrose agar, bacterial strains were grown overnight at 37°C in nutritional agar. The disc diffusion test was used to assess the antibacterial properties of *S.cumini* essential oil (Aimad et al.,2021; Krishnan et al.,2019; Abdollahzadeh et al., 2021). After mixing and autoclaving Potato Dextrose Agar and Nutrient Agar, 100 µL/100 mL of inocula were added to a medium, transferred to Petri plates, and hardened. The filter paper disc containing 10 µL sample was deposited and incubated for 24 and 48 hours at 37 and 28 degrees Celsius. Using a zone reader, inhibitory zone diameters were measured in millimetres. Results were contrasted with antibacterial drugs such as *Rifampicin* and *Flucoanazole* (Hekmati et al., 2020; Choi et al.,2021; Sun et al.,2021).

The MIC, or the concentration that prevents microorganism growth, was calculated using a microdilution broth susceptibility experiment (Sun et al., 2019; Tibbits et al.,2022; Schön et al., 2020). A 96-well microliter plate generated a series of essential oil dilutions, 160µ L of nutrient broth, potato dextrose broth for bacteria and fungi, and 20µ L of test solution. The next stage was performing an inoculation onto the microplates at a concentration of 105 CFU/mL (as verified by the viable count of a standard microbe suspension). For bacteria, the plates were incubated for 24 hours at 37°C, and for fungus, for 48 hours at 28°C. The antibacterial reference utilized was rifampicin, and the antifungal reference used was fluconazole. The well bottom had a white 'pellet' that represented growth, and the computations were done in mg/mL(Tibbits et al.,2022; Schön et al., 2020; Schug et al., 2020; Kavanagh et al., 2019; Silaban et al., 2022)

2.6. Haemolytic and cytotoxic effects

The brine prawn lethality test was conducted according to the protocol (Sharma &. Kharel 2019; Greco et al. 2020). The hemolytic activity was evaluated using human and bovine erythrocytes (Bastos et al.,2022; Santos-Sánchez et al., 2019). Following are the calculations for the hemolysis:

Data from the brine shrimp lethality assay were organized by dose, subject count, and number of lethal events for

$$\%\,Haemolysis = \left(\frac{\mathrm{Hb_{ABS}}}{\mathrm{Hb100\,\%_{ABS}}}\right) \times 100$$

statistical analysis. Results contain 95% fiducial limits for all statistically significant data.

3. Results and Discussion

3.1: Yield of essential oil

The oil was light brown and had a yield of 1.57%. Similar calculations were made for additional physical variables like density (0.83 g/cm^3) and refractive index (1.29300).

3.2: GC-MS Analysis of Essential Oil

Results of GC-MS analysis showed (Table 1) 39 components, accounting for roughly 98% of the total weight of the *S.cumini* essential oil sample. The primary constituents of the oil were discovered to include α-pinene (22.29%), (E)-ocimene (11.68%), D-limonene (7.96%), α-terpineol (5.26%), β-Caryophyllene (4.39%) and α-Humulene (2.59%). About 54.32% of the oil's composition was made up of hydrocarbon monoterpenes, followed by oxygenated monoterpenes (10.17%), hydrocarbon sesquiterpenes (5.16%), and oxygenated sesquiterpenes (4.30%).

Table 1. GC /MS analysis of essential oil from *Syzigium cumini* leaves

No	Compound	RT	% age area
1.	*α*-Pinene	6.45	20.97
2.	Camphene	6.755	1.74
3.	Myrcene	8.065	3.78
4.	o-Cymene	9.07	1.12
5.	D-Limonene	9.24	7.96
6.	*β*-(*E*)-Ocimene	9.585	11.68
7.	*α*-(*E*)-Ocimene	9.86	4.7
8.	*γ*-Terpinene	10.145	0.36
9.	Isoterpinolene	11.045	0.84
10.	Fenchol	11.835	0.35
11.	Neo-allo-ocimene	12.305	0.46
12.	4(10)-Thujen-3-ol	12.625	0.2
13.	Thujol	12.91	0.19
14.	(E)-Borneol	13.465	0.31
15.	Terpinen-4-ol	13.815	0.41
16.	Crypton	14.11	0.48
17.	*α*-Terpineol	14.295	5.26
18.	(E)-Myrtenol	14.43	0.18
19.	Carveol	14.605	0.03
20.	(+)-Verbenone	14.815	0.22
21.	Fenchyl acetate	15.085	0.62
22.	Phellandral	16.755	1.34
23.	Bornyl acetate	17.045	2.23
24.	(+)-*E*-Pinocarvyl acetate	17.335	0.06
25.	(+)-*Z*-Verbenol, acetate	17.445	0.17
26.	Carvacrol	17.595	0.09
27.	*α*-Copaene	19.57	0.04
28.	(*E*)-*β*-Caryophyllene	20.825	4.39
29.	*α*-Humulene	21.72	2.59
30.	Alloaromadendrene	21.895	0.07
31.	Eudesma-4(14),11-diene	22.585	0.18
32.	*α*-Selinene	22.785	0.03
33.	Palustrol	24.72	0.34
34.	(+)-Spathulenol	24.93	1.1
35.	Caryophyllene oxide	25.065	2.2
36.	Epiglobulol	25.48	0.64
37.	Humulenol-II	26.235	0.27

38.	Longipinocarveol, trans-	26.34	0.3
39.	(*E*)-Guai-11-en-10-ol	26.685	0.33

3.3: Antioxidant potential of *S.cumini* essential oil

DPPH• has been utilized as a valuable instrument for assessing the antioxidant capability of *S.Cumini* oil due to its stable free radical, the. The underlying mechanism is either the transfer of a hydrogen atom to DPPH or the addition of an electron, which neutralizes it in a reaction between DPPH and an antioxidant (Alemany-Cosme et al., 2021; Gulcin et al., 2020; Pateiro et al. 2021). The shift in colour of DPPH from purple to yellow, which was evaluated in terms of absorption taken by spectrophotometer at 515 nm, made it apparent that the concentration had decreased. The stable, purple-coloured radical DPPH was converted into its reduced form, which was yellow, by the *S.cumini* essential oil under investigation.

Compared to BHT, which had an IC_{50} of 2.53 g/mL, the *S.cumini* sample in the current investigation had a good antioxidant potential with an IC50 of 19.83 µg/mL. The essential leaf oil from *S.cumini* reportedly has stronger antioxidant activity, according to *Rabeque* (2022). It was thought that the presence of α-pinene, the major component of *S.cumini* essential oil, which increases the activity of the entire oil, is supposed to be responsible for this potential.

The antioxidant capacity of *S.cumini* was also examined by *S. Temesgen* (Xiao et al., 2020) using DPPH and linoleic acid Inhibition tests. The thiocyanate method was employed to examine the antioxidant activity of the samples from *S.cumini* essential oil. This method inhibits the linoleic acid oxidation pathway. Unsaturated fatty acid linoleic acid oxidizes ferrous and ferric ions using various peroxides, forming a combination with thiocyanate ions derived from ammonium thiocyanate as a result (Ali et al.,2021; Lowry et al.,2020).

Absorbance was measured by spectrophotometer at 500 nm, and the concentration of the produced complex was ascertained. High absorbance values indicate that more peroxides are formed throughout the process, which lowers the oil's antioxidant activity. Compared to BHT, whose inhibition was 79%, all essential oil doses demonstrated considerable inhibition, ranging from 17% to 58%. The highest inhibition for the sample at concentrations of 50µ L/mL and 10µ L/mL is 62% and 12 %, respectively. The percent inhibition increased from 81.8% to 93.3% with changing concentrations from 20-150 µg/mL (Loucif et al.2020).

The bleaching of β-carotene by *S.cumini* essential oil as an antioxidant in the linoleic acid system is depicted in Figure 1. Antioxidants prevent the system's β-carotene from being destroyed by linoleate or other free radicals by neutralizing them (Uddin et al., 2021; Farahmandfar et al .2019). The absence of antioxidants rapidly causes β-carotene to be colourless (Szwajgier et al., 2021). The rate of linoleic acid oxidation and antioxidant activity is directly correlated with the decline in β-carotene absorption. The control displayed the maximum colour depletion in the absence of a sample (Nonato et al., 2022; Theofanous et al., 2022; Temesgen et al., 2022)

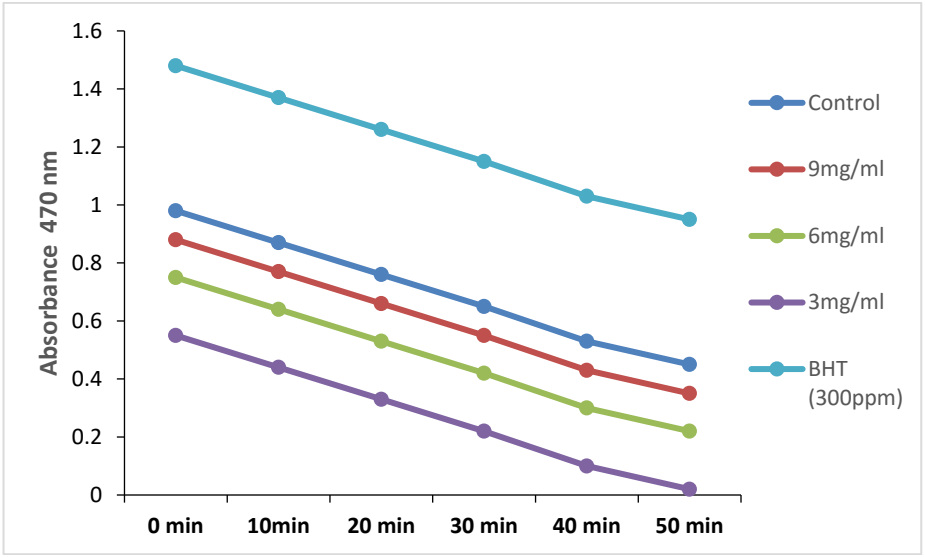

Figure 1. Inhibition of linoleic acid peroxidation by Beta carotene bleaching method of *S. cumini* essential oil

3.4. Antimicrobial potential of *S.cumini* essential oil

The antimicrobial activity of the *S.cumini* essential oil was tested using eight pathogenic microorganisms. Figures 2 to 5 display the findings of the minimum antibacterial and antifungal potential of *S.cumini* essential oil made using the disc diffusion method and MBC and MFC measurement by microdilution broth assays. According to the

findings, *S.cumini* essential oil exhibits exceptional activity compared to antibiotics. *S. aureus* and *E. coli* were the most susceptible bacteria, with the biggest inhibition zones (11.7 mm and 11.3 mm) and the lowest MIC values (3.83 mg/mL and 2.9 mg/mL), respectively. The most sensitive and dynamic outcome has been shown by *A. niger* had the greatest inhibition zone (17.5 mm) and the lowest MIC value (2.03 mg/mL). Against *A.alternata*, *A. flavus* and *G.lucidum*, moderate inhibition zones of *S.cumini* essential oil were found. Setty et al. (2022) have reported similar findings. They discussed the testing of *Myristica fragrans'* mace, meat extracts, and seeds against various pathogen strains. Regarding action against the pathogens, the flesh extract was superior to seed and mace.

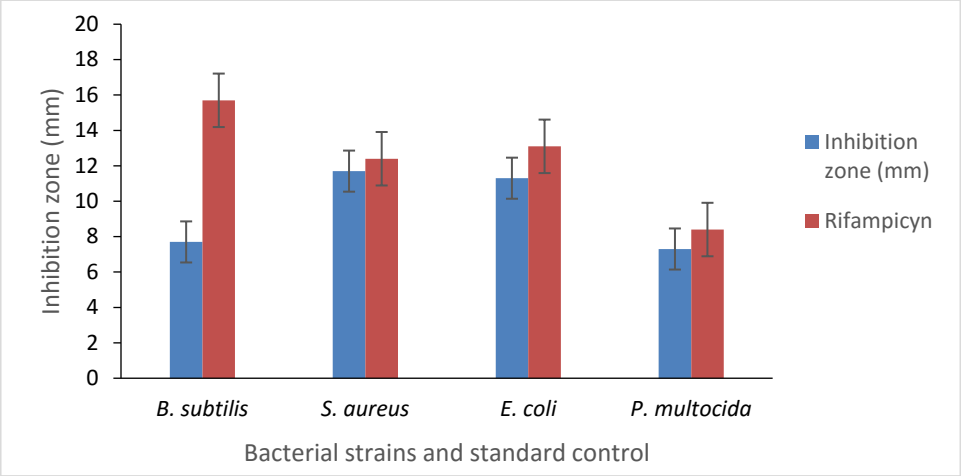

Figure 2. Antibacterial activity of *S.cumini* essential oil.

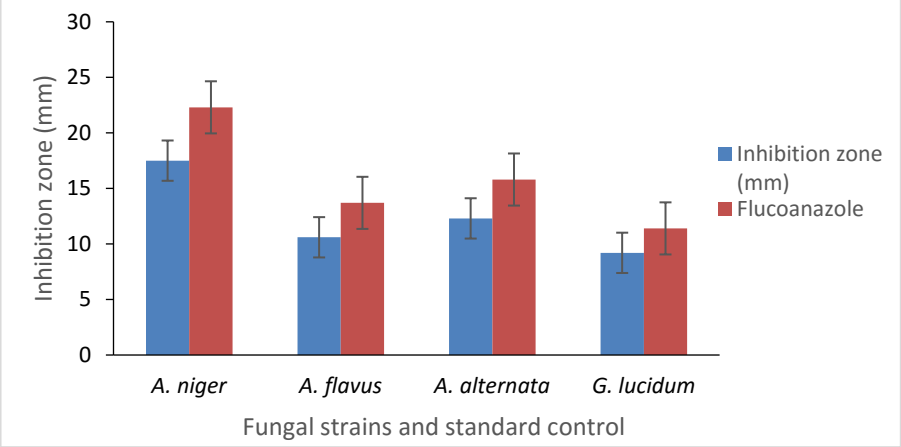

Figure 3. Antifungal activity of *S.cumini* essential oil.

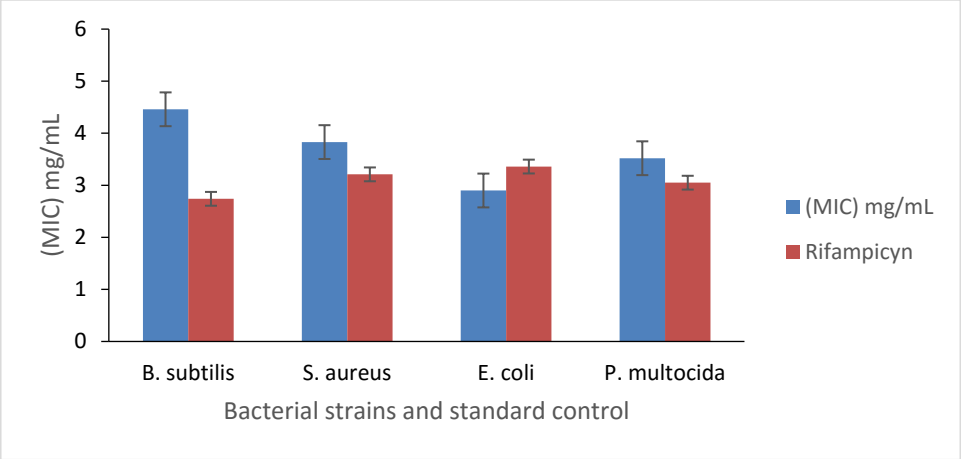

Figure 4. The minimum bactericidal concentration of *S.cumini* essential oil.

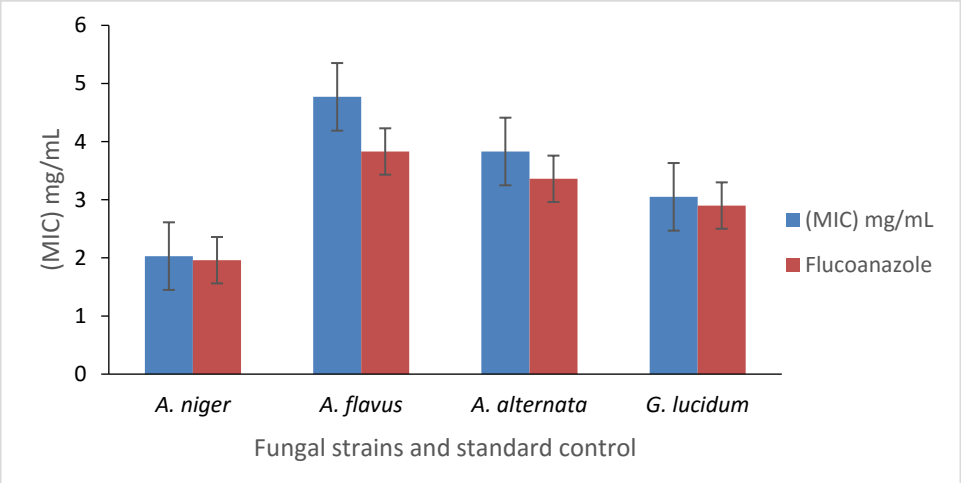

Figure 5. The minimum fungicidal concentration of *S.cumini* essential oil.

3.5. Haemolytic and cytotoxic potential of *S.cumini* essential oil

With LC50 values of 29.45 µg/mL and moderate activity in contrast to other essential oils, *S. cumini* essential oil has emerged as a superior choice for the brine shrimp lethality test. The presence of D limonene and α-β pienene in higher amounts may explain this distinct behaviour. Significant outcomes were discovered to exist. Another investigation was published for the seed and rind fragrant oils of *A. subulatum* (Nepali cardamom). Both showed moderate activity in the test for cytotoxic behaviour, with LC50 values of 25.2 and 13.1 g/mL, respectively (He et al.,2019)

Results of hemolytic activity showed that 15mg/mL of essential oil of *S.cumini* had the most hemolytic activity, while 5 mg/mL had the lowest. Bovine and human erythrocytes had maximum values of 8.24% and 9.63%, respectively, whereas these two species' lowest values were 0.29% and 0.43%, respectively. Analyzing a drug's hemolytic potential is crucial. This could serve as a valuable cytotoxicity indicator for assessing the viability of pharmacological applications (Ni, et al.,2019; Mehmood et al.,2019). The S.cumini essential oil displayed less cytotoxicity than other plant oils and extracts. This unusual characteristic suggests its usefulness for pharmacological purposes, especially at lower dosages.

4. Conclusions

The essential oil was extracted from *S.cumini* from the Faisalabad district of Pakistan (1.57 g/100 g). It was shown to be enriched with α-pienene (22.29%) by GC-MS analysis. *Syzygium cumini* oil's antioxidant and antibacterial properties were assessed using various tests. Compared to the control, the results showed it had good potential as an antioxidant and antibacterial agent. Studies on cytotoxicity revealed that it has no cytotoxic impact at lower levels, making it appropriate for use as a medication.

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Conflict of Interest

The authors say they have no competing interests.

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