Identification of Antibacterial and Antioxidant Activity of *Syzigium Cumini* Leaves Extracted Essential Oil Against Diarrhoea

1. **Usman Minhas***, Pakistan Council of Scientific and Industrial Research (PCSIR)
2. **Hajra Sohail*** , Lahore College for Women University
3. **Dr. Kiran Fatima***, Assistant Professor, Rawalpindi Medical University
4. **Malik Ali Raza,** The University of Lahore
5. **Maria Akhtar,** The University of Lahore
6. **Muhammad Furqan Arshad,** Government College University, GCU, Lahore
7. **Samreen Sadiq*,** Jiangsu University of Science and Technology,Zhenjiang, China
8. **Iltaf Khan,** Jiangsu University of Science and Technology,Zhenjiang, China
9. **Shoaib Khan,** Northeast Agricultural University, Harbin, China
10. **Faiza Saleem (Corresponding Author),** Lahore College for Women University, Faiza.saleem@lcwu.edu.pk

Abstract

Syzigium cumini (Family: Myrtaceae) is an evergreen glabrous tree indigenous to tropical and subtropical areas of Asia. This plant has numerous bioactive compounds such as anthocyanin, quercetin, glycoside, kaempferol, antimellin, and myrecetin. By employing the agar-well diffusion method, this study will examine the high antibacterial activity of extracted essential oils from leaves against multiple bacteria that cause diarrhoea. *S. cumini* oil extract had effective potential against *E. coli, S. aureus, Salmonella typhi, Klebseila,* and *B. subtilis.* As a result, it will be employed as an anti-diarrheal agent, antibacterial and antioxidant ingredient in different pharmaceutical and health products. Gas Chromatography-Mass Spectrometry will be applied to identify the oil extract components that cause antibacterial and antioxidant action.

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the relationship between antioxidant activity and the bioactive elements of *S. cumini.*

Keyword: antioxidant, elements, pharmaceutical, diffusion, antibacterial

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Introduction

S. cumini is also known as Eugenia jambolan, black plum, or Jamun. Its trees yield oblong or ellipsoid raspberries every year. Its extract has antibacterial, antioxidant, anti-diarrheal, anti-cancer, anti-inflammatory, anti-diabetic, anti-HIV, anti-ulcerogenic and anti-fungal properties. According to Shaheen *et al.*, (2019) these extracts are obtained from a variety of plant parts, including flowers, buds, bark, leaves and wood. *S. cumini* essential oils are complex chemical

compounds with 20-60 changeable components at varying quantities. However, two to three significant components (e.g., β -ocimene, α -Pinene, and bocimene) are present in greater abundance (20-70%) than other trace quantities.

Essential oils are a highly intriguing class of antibacterial and antioxidant substances in plants. They are organic liquids distinguished by aromatic notes in plant materials such as wood, roots, bark, leaves, flowers, seeds, and fruits. It includes a wide variety of phytocompounds that have several antibacterial targets. The distinct hydrophobicity of essential oils gives them the capacity to interact with lipids on bacteria's cell membranes, increasing the permeability of the cell membrane and upsetting the pathogens' cell structure (Song *et al.*, 2018). In the extraction of essential oils, two methods are primarily used; solvent extraction and azeotropic techniques (hydro diffusion, hydro-distillation and steam distillation) and among them best approach for isolating essential oils is hydro-distillation.

Material and methods

3.1. Essential Oil Extraction from *Syzygium Cumini* Leaves

For the extraction of essential oils from leaves, a steam distillation technique was used (Geed *et al.*, 2014). Half of the 500ml flat-bottomed flask; is filled with extract powder and distilled water The distilled apparatus is then put together, heated, and stirred; a thorough stirring is necessary to avoid overheating. After a few minutes, the extract begins to boil, and vapors containing volatile compounds ascend through the system. The oil is immiscible in water (co-distilled), which results in the formation of two phases.

Oil developed a layer over water in a receiving flask at the end of the procedure. For separating oil from water, another process is liquid-liquid extraction. It is based on the solubility of its component constituents, as demonstrated by the two unmixed liquors (organic solvent and water). In this oil separation procedure, hexane was used as an organic solvent.

3.2. Estimation of Antioxidant Activity in *Syzygium Cumini* Leaves Extract

S. cumini is an excellent source of antioxidants. Secondary metabolites obtained from plant components include antioxidants such as carotenoids, flavonoids, benzoic acid, ascorbic acid, cinnamic acid, and tocopherols. The most effective antioxidants are beta carotene and ascorbic acid. Antioxidant metabolism produces free radicals and reactive oxygen species, which cause oxidative stress. This stress is effective in the treatment multiple disorders, including diarrhea, heart disease, degenerative neurological diseases, and cancer (Shahnawaz et al., 2010).

Procedure

The radical scavenging activity of DPPH was identified using the Brand-Williams method. BHT (butylated hydroxytoluene) stock solution was made by dissolving 0.01g in 100 ml methanol. By measuring absorbance, %age antioxidant activity was computed and a graph of BHT against the concentration on the x-axis and %age antioxidant activity on the y-axis was created. The

antioxidant BHT was utilised as a positive control. DPPH was used to dilute each sample. 3ml of DPPH was added to various volumes of Syzigium cumini leaves extract ranging from 10µl-50µl.

Calculation of % Antioxidant Activity

$$\% \text{ Antioxidant activity} = \frac{\text{Abs (blank)- Abs (Sample)}}{\text{Abs (Blank)}} \times 100$$

3.3. Analysis of Essential Oil Constituents

GC-MS was used for detecting essential oil components (Sharma *et al.*, 2020). It showed all the major and minor constituents present in leaves extract e.g. e globulol, caryophyllene, δ-cadinene, α-pinene, β-eudesmol, β-pinene, γ-cadinene, camphene, α-terpinenol, camphor, α-muurolene, epicubenol, α-copaene, viridiflorene, β-guanine, β-bourbonene, terpinen-4-ol, endo-borneol, levoverbenone, isobornyl acetate,oxygenated & non-oxygenated sesquiterpenes, monoterpene hydrocarbons, bicyclic-monoterpenoid, terpenoid ketones, alkene alcohol. The concentrated proportion of detected elements will be estimated using a standard procedure and calibration curves generated by doing GC-MS analysis on authentic substances.

3.4. Antibacterial Activity of *Syzygium cumini* Leaves Extracted Essential Oil

3.4.1. Antibacterial Activity

Essential oils isolated from *S. cumini* leaves are shown to have antibacterial activity against Gram-positive and Gram-negative bacteria. All extracts revealed inhibition zones, but the methanol-based extract exhibited the greatest ZOI (zone of inhibition), measuring 18-24mm against *Bacillus* species. The essential antibacterial activity of *S. cumini* extracts is due to the reduction of cell membrane permeability.

Cell wall breakdown, cytoplasmic membrane damage and membrane protein damage, cytoplasm coagulation, and proton depletion out of the cell have all been described as modes of anti-bacterial action of essential oils in bacterial cells. The mechanism of essential oils is dependent on their concentration, as lesser concentrations block enzymes while higher concentrations precipitate protein from the cell wall. Figure 3.1.

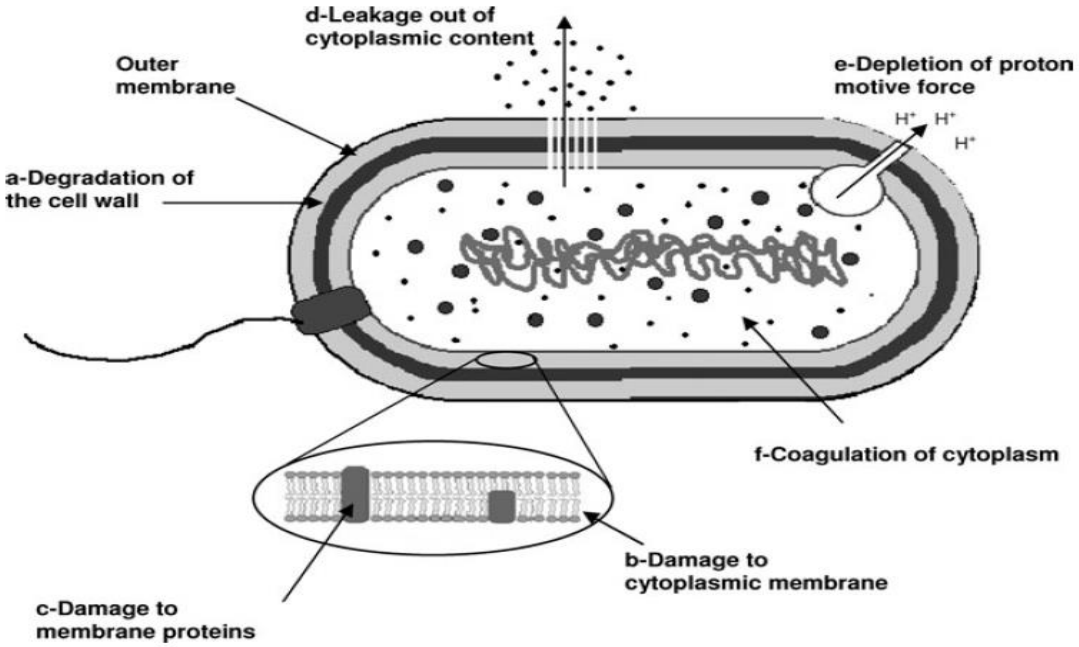

Figure 3.1: Mechanisms of action of essential oils and their components in a bacterial cell

3.4.2. Media and Inoculation Preparation

Under aseptic conditions, sterile nutrient agar plates were made and inoculated with the spread plate method.

3.4.3. Agar Well Diffusion Method

According to Heggers (1990), the antibacterial activity of *S. cumini* leaves' essential oil was examined using the agar well diffusion method. 100µl of essential oil were poured into each well, and ethanol was used as a control. The antibacterial activity was carried out against six different bacterial strains, including *E. coli. Salmonella typhi*, *Klebsiella*, *S. aureus*, *Pseudomonas*, and *Bacillus subtilis*. After 24 hours of incubation, the antibacterial activity of an essential oil was measured in terms of the diameter of the inhibitory zone (in mm).

Results

4.1. Antioxidant Activity (DPPH Assay)

DPPH is a stable free radical, exist in purple color and transforms into the yellow color (non-radical form) when abstracting one of his electrons. So it is widely used to measure the electron-donating capacity of any antioxidant under the assay conditions. The % antioxidant activity of *S.cumini* leaves extract was found to be 28.84%, 42.30%, 56.41%, 71.79% and 84.61% at different range of concentration from 10-50 µg/ml (Fig 4.1). The IC50 value also showed the antioxidant potency of *S. cumini* leaves extract as lower the IC50 value, higher the antioxidant effect. % radical scavenging activity was plotted against the concentration of particular substance having antioxidant activity to gets IC50 value. IC50 value defined the minimum amount of

antioxidant that scavenge the 50% of free radicals in the assay system as it is inversely proportional to the antioxidant potency (Table 4.1).

Figure 4.1: Comparison Between % Antioxidant Activity of *Syzygium cumini* Leaves Extract and BHT

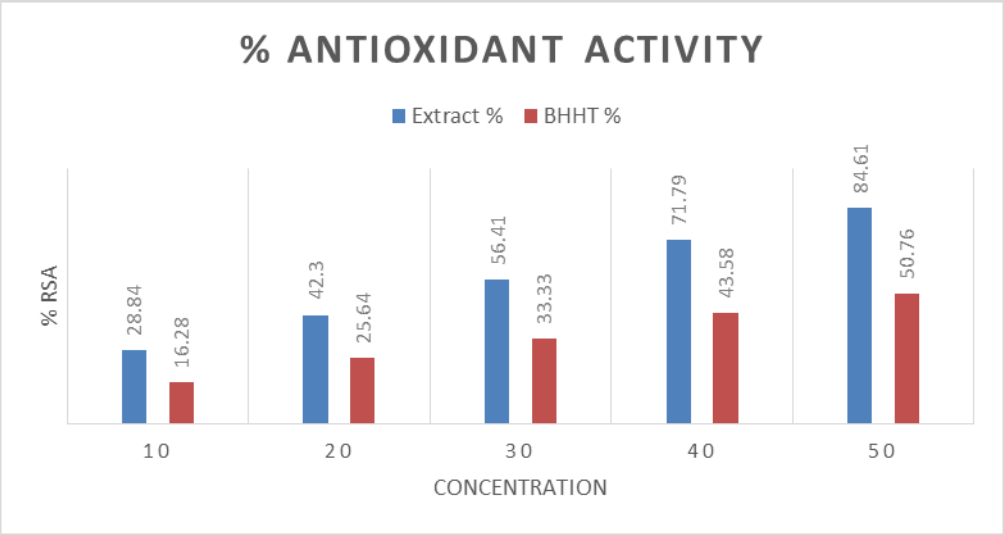

Table 4.1: % Radical Scavenging Activity and IC50 Values of *S.cumini* Leaves Extract

Calculation of % Radical Scavenging and IC50 from DPPH Assay			
Absorbance Measurement Data			
Concentration µg/ml	**Sample**	**% RSA**	**IC50**
10	0.555	28.84	-3.17733816
20	0.45	42.3	3.913351769
30	0.34	56.41	11.00404169
40	0.22	71.79	18.09473162
50	0.12	84.61	25.18542154
Control	0.778		

4.2. Essential Oil Examined Through GC-MS

Table 4.2: Chemical Composition of Essential Oil Examined Through GC-MS

No.	Compound	Retention time (RT)	Molecular Formula	Peak Area %
1	α-Pinene	3.293	C10H16	13.2
2	D-Li1monene	5.047	C10H16	4.2
3	Benzene, 1,3-bis(1,1-dimethylethyl)	7.171	C15H24O	10.2

4	Acetic acid, 1,7,7-trimethyl-bicyclo(2,2,1)hept-2-yl ester	7.729	C12H20O2	3.6
5	Caryophyllene	8.531	C15H24	9.02
6	Humulene	8.836	C15H24	5.34
7	Naphthalene	9.284	C10H8	6.7
8	Caryophyllene oxid	9.994	C15H24O	7.2
9	12-Oxabicyclo, 3,7-diene, 1,5,5,8-tetramethy	10.204	C15H24O	4.3
10	β-Ocimene	10.349	C10H16	6.2
11	n-Hexadecanoic acid (Palmitic acid)	11.771	C16H32O2	12.44
12	Phytol	12.332	C20H40O	10.5
13	9,12-Octadecadienoic acid (Linoleic acid)	12.768	C18H32O2	4.6

Figure 4.2: GC-MS Chromatogram of Essential Oil of *S.cumini* Leaves Extract

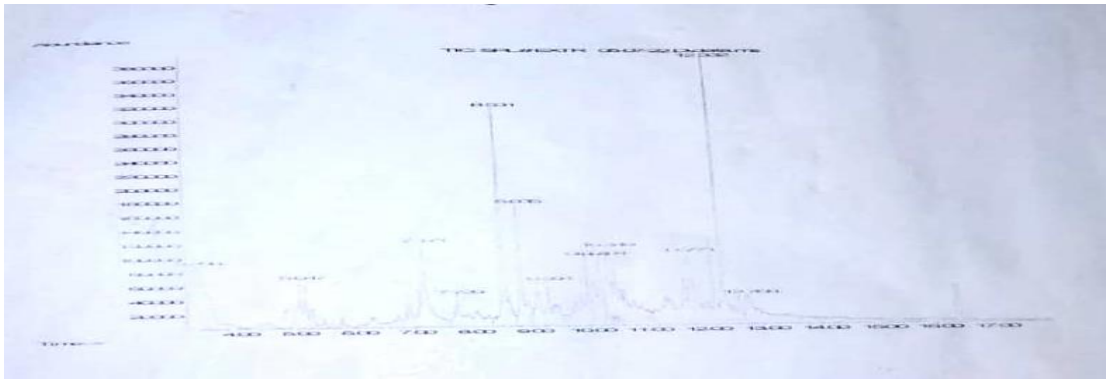

Figure 4.3 shows the essential oil was found to contain a total of thirteen components, with a total concentration of 97.8%, according to the GC-MS study. α -pinene (13.2%), n-Hexadecanoic acid (12.44%), phytol (10.5%), and benzene, 1,3-bis(1,1-dimethylethyl) (10.2%) were the main ingredients that were found and had a concentration of over 10 percent. Caryophyllene (9.2%), Caryophyllene oxid (7.2%), Naphthalene (6.7%), β -ocimene (6.2%), and Humulene (5.34%) were the components with an intermediate concentration (10.00 - 5.00%). Linoleic acid (4.6%), 12-Oxabicyclo, 3,7-diene, 1,5,5,8-tetramethy (4.3%), D-limonene (4.2%), and acetic acid (3.6%) were the components found in small concentration (5.00%). Among the 95.66 % identified compounds, α-pinene, camphene, β- pinene were monoterpene hydrocarbons amounting to 13.62. Among the 97.8% identified constituents, α-pinene, β-ocimene and D-limonene were monoterpenes hydrocarbons amounting to 23%. Phytol and linoleic acid were diterpenes (15.1%), naphthalene and palmitic acid was polycyclic aromatic hydrocarbon (19.14%) ; while 12-Oxabicyclo, 3,7-diene, 1,5,5,8-tetramethy and caryophyllene oxide, Benzene, 1,3-bis(1,1-dimethylethyl) were characterized as oxygenated

sesquiterpenes (21.7%). Two compounds namely caryophyllene and humulene were hydrocarbon sesquiterpenes (14.36%).

4.3. Antibacterial Activity of Essential Oil:

Antibacterial activity of *S. cumini* leaves extracted essential oil showed remarkable results against pathogenic bacterial strains i.e., *E.coli, Salmonella, B.subtilis,* and *S.aureus*. Essential oil were rich in variable phytochemical constituents i.e., α-pinene, β-ocimene, phytol and D-limonene that have antibacterial and antioxidant properties. Essential oil showed resistance against *Salmonella* with 16mm zone of inhibition (ZOI), intermediate results against *E.coli* and *B.subtilis* with 14mm (ZOI). Whereas essential oil were sensitive towards *S.aureus* with minimum zone diameter inhibition i.e., 12mm.

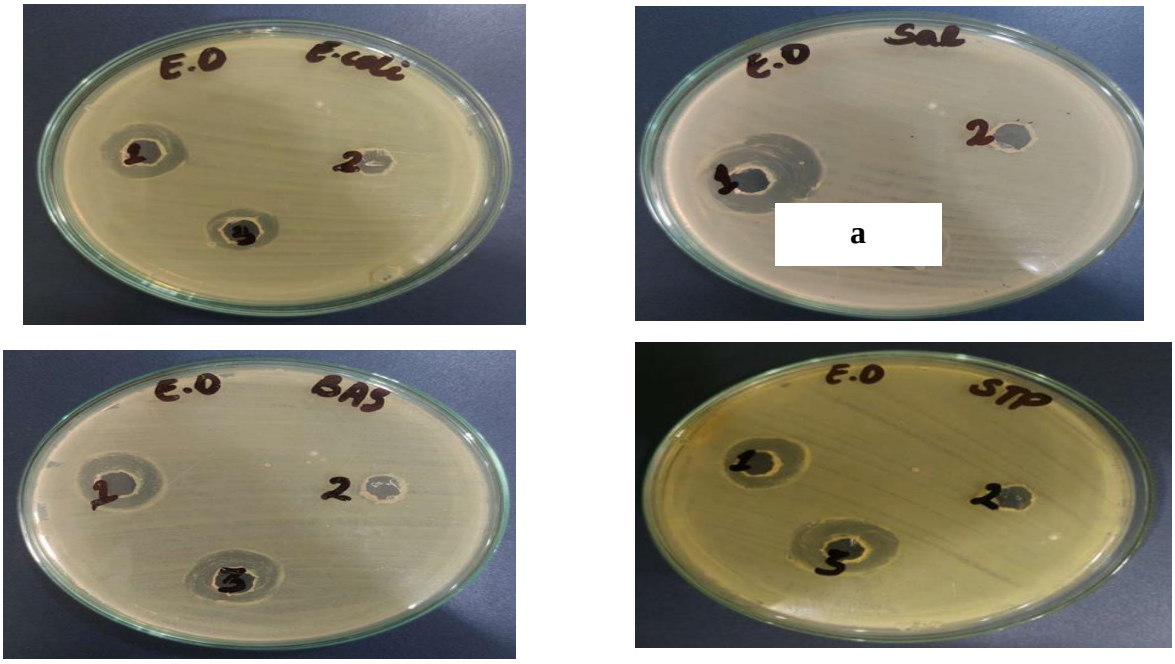

Figure 4.3: Antibacterial Activity of *S.cumini* Extracted Essential Oil against Bacterial Strains (a) *E.coli* (b) *Salmonela sp.* (c) *B.subtilis* and (d) *S.aureu*

Discussion

Many researchers have discovered that the essential oil produced from *S. cumini* fresh leaves has variety of chemical components and antioxidants. Essential oil is a complex blend of oxygenate monoterpenes, hydrocarbon monoterpenes, sesquiterpenes, and diterpenes. According to GC-MS analysis, the largest component discovered was α -Pinene (13.2%), which exhibited several biological properties such as anti-bacterial, anti-oxidant, anti-coagulant, gastro-protective, and anti-cancer. n-Hexadecanoic acid (12.44%), also known as palmitic acid, was the other main ingredient. It is a saturated fatty acid that occurs naturally in palm oil and is used as a dietary supplement. Caryophyllene oxid (7.2%) was found in moderate concentrations and was employed as a pheromone to attract insects such as green lacewings and has anti-cancer and antioxidant properties.

Mohamed *et al.*, (2013) found that the essential oil of *S. cumini* has antibacterial properties against gram-positive and gram-negative bacteria such as *E.coli* and *B.subtilis*. The greatest inhibition zone for essential oil against *B.subtilis* was 14mm. Essential oil derived from S. *cumini* leaves shown significant antibacterial action against pathogenic bacterial strains.

The current study found that *S. cumini* essential oil showed exceptional antibacterial and antioxidant properties against four pathogenic bacteria that cause diarrhea (*E.coli*, *B.subtilis, Salmonella*, and *S.aureus*). Antibiotic resistance is now a serious global healthcare issue. Treatment of bacterial infections with efficient first-line antibiotics pushed to second-line or third-line medications, which have become more expensive, time-consuming, and dangerous operations. Plants can serve as antimicrobial agents and boost antibiotic activity (Moussaoui *et al.*, 2016).

Conclusion

This study discovered the use of *S.cumini* essential oil in combination with multi-resistant drugs against diarrhea causing bacteria. The essential oil of *S.cumini* has antibacterial and antioxidant activities in the investigation. The presence of multiple beneficial compounds with antibacterial or antioxidant activity against *E.coli* and *Pseudomonas* sp. was revealed by GC-MS. Overall, the study revealed that *S.cumini* and oil were effective against diarrhea causing bacteria.

Referesnces

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