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RESEARCH ARTICLE

Investigating the therapeutic potential of *Celosia cristata via* GC-MS characterization and *in silico* docking

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Abstract

Celosia cristata, an annual shrub belonging to the family Amaranthaceae, is widely cultivated in India for its vibrant flowers. This study investigates the GC-MS profiling and antibacterial activity of *Celosia cristata* flower extract. Antibacterial efficacy of the extracts was tested against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method at concentrations ranging from 10–50 µL. The extract exhibited moderate antibacterial activity, with inhibition zones of 10 –15 mm against *E. coli*. GC-MS analysis identified 25 major phytochemical constituents, namely Hentriacontane (19.52 %), Benzoic acid, 4-ethoxy-, ethyl ester (11.87 %), Heptacosanol (10.97 %), Cyclotetracosane (6.01 %) and Butane, 2-phenyl-3-(trimethylsilyloxy) (1.57 %). Many of these compounds are known for their antioxidant, antimicrobial and anti-inflammatory properties. Further, molecular docking studies revealed that diphenyl sulfone may have potential inhibitory activity against *E. coli* haemolysin E (1QOY). Collectively, these findings highlight the therapeutic potential of *Celosia cristata* in pharmaceutical applications and antimicrobial drug development.

Keywords: antioxidant; *Celosia cristata*; docking; flower; GC-MS

Introduction

Celosia cristata, an annual shrub naturally grown in the tropical regions belonging to the Amaranthaceae family, has been widely recognized for its vibrant aesthetic appeal and medicinal properties. Traditionally, its flowers have been used in various cultures to treat ailments such as diarrhoea, inflammation and wounds. The leaves and flowers are rich in bioactive and nutritionally important compounds (1). Gas Chromatography & Mass Spectrometry (GC-MS) is a robust analytical technique used for the identification and quantification of volatile and semi-volatile organic compounds and it plays a crucial role in plant defence (2). In agriculture, *C. cristata* is appreciated for its ability to thrive in diverse environments and its role in enhancing soil fertility through its nitrogen-fixing properties. Previous studies have primarily focused on *Celosia argentea* emphasizing the development of tissue culture media for growth induction and yield enhancement (2, 3). Further *in vitro* and *in vivo* studies (4) have been conducted on *Celosia plumosa* to enhance growth (5), establish cell suspension cultures (6) and improve disease resistance (7), whereas limited research has been conducted on the chemical composition of *Celosia cristata* flowers. Meanwhile, the emergence of antibiotic resistance in pathogens such as the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli*, has developed resistance to several antibiotics, including methicillin. As a promising alternative, the identification of plant-derived natural products with antibacterial activity is gaining increasing attention. Hence, this study utilizes GC-MS and molecular docking to complement the antibacterial activity, identify bioactive compounds and evaluate their antibacterial potential through possible interactions between the tested compounds and bacterial target proteins.

Materials and Methods

The flower samples were collected from the TNAU Botanical Garden, Department of Floriculture and Landscaping, TNAU, Coimbatore, in 2024. The region experiences a subtropical to tropical climate, with temperatures ranging from 28 to 32 °C and humidity levels between 60 % and 75 %. To ensure optimal phytochemical preservation, the flowers were harvested early in the morning and shade-dried at room temperature (25 °C) for 4 to 6 days to preserve heat-sensitive secondary metabolites. After drying, the flowers were ground into a fine

powder and stored in airtight containers until analysis. Then samples were promptly transported to the laboratory in sealed, moisture-proof containers to prevent environmental degradation.

Sample preparation for GC-MS

For extraction, 10 g of powdered flower material was soaked in 100 mL of ethanol (99.9 %). The extraction was carried out by maceration for 72 hrs with periodic shaking and was subsequently filtered using Whatman No. 1 filter paper to remove the insoluble residues. The filtrate was concentrated to a viscous crude extract using a rotary evaporator under reduced pressure at 40 °C. The final extract was stored at 4 °C in amber-coloured vials to prevent photodegradation. The analysis was performed using an Agilent Technologies GC-MS system (Model 7890B GC coupled with 5977A MSD). Helium was use as the carrier gas at the flow rate was 1.0 mL/min. 1 µL aliquot of the prepared sample was injected in spitless mode. The initial oven temperature was set at 50 °C for 2 mins, then ramped to 300 °C at 10 °C/min.

Antibacterial Activity Assessment

Bacterial growth was assesses using the Kirby-Bauer method. Firstly, five isolated colonies from an agar plate culture were selected. A loop was used for transferring the selected colonies into a tube containing 4 to 5 mL of Muller-Hinton broth, which was then incubated at 35 °C until it reached the desired turbidity. The turbidity of the actively growing broth culture was then adjusted to achieve a bacterial concentration of approximately 1 to 2 × 10^8 CFU/mL for *S. aureus* and *E. coli*. For inoculation, a sterile cotton swab was dipped into the adjusted bacterial suspension and gently pressed against the inner wall of the tube to remove excess inoculum. The dried surface of a Muller-Hinton agar plate was inoculated by streaking the swab evenly across the sterile agar in three directions, ensuring uniform distribution. The plate was then left undisturbed for 3 to 5 minutes to absorb excess moisture before placing the drug -impregnated disks. A well of 6 mm in diameter was then excised in the agar and filled with 10-50µL of either the standard antibiotic (gentamicin) or the sample. to ensure complete diffusion, these plates were incubated at 37 °C for 24 hrs in an inverted position.

Molecular docking

Molecular docking was performed using the protein target 1QOY (Hemolysin E). Haemolysin E (HlyE) is a pore-forming toxin present in *E. coli* that facilitates host cell invasion by creating transmembrane pores, that cause cell lysis and immune evasion. By promoting tissue penetration and inflammation, it breaks down epithelial barriers and promotes systemic infection, thereby increasing the pathogenicity of *E. coli*, particularly in cases of extraintestinal infections. The chemical compounds identified *via* GC-MS analysis, namely Diphenyl sulfone, 9,12-Octadecadienoic acid (Z, Z)-, Tetrasiloxane, decamethyl-, Butane, 2-phenyl-3-(trimethylsilyloxy)- and Cyclotrisiloxane, hexamethyl, were selected as ligand based on their documented antibacterial effects as presented in Table 1. The Auto Dock vina module in PyRx 0.8 was employed in this study to carry out molecular docking (8). Target proteins were uploaded and transformed into macromolecules using the "make macromolecule" option in PyRx. The binding sites of target proteins were identified using the Computed Atlas of Surface Topography of Proteins (CASTp) (9). Additionally, AutoDock4 and Autogrid4 parameter files were used for grid layout and docking.

Result and Discussion

Phytochemical Composition of GC-MS

The Library Search Report identified the compounds using the NIST08 database. Table 1 represents the primary identified compounds along with their area percentage, retention times and their potential biological relevance. The GC-MS analysis of *C. cristata* flowers revealed a diverse range of secondary

Table 1. Compounds identified through GC-MS in *Celosia cristata* flower extract

Name of the Compound & Molecular formula	Retention time	Activity	Reference
Benzoic acid, 4-ethoxy-, ethyl ester $C_{11}H_{14}O_3$	10.064	Antioxidant activity	(15)
Ar-tumerone $C_{15}H_{20}O$	11.208	Antimicrobial& Antidiabetic activity	(16)
Diphenyl sulfone $C_{12}H_{10}O_2S$	13.164	Antifungal& Antibacterial activity	(17)
9,12-Octadecadienoic acid (Z,Z)- $C_{18}H_{32}O_2$	14.330	Antioxidant & Antibacterial activity	(18)
2-Chloroethyl linoleate $C_{20}H_{35}ClO_2$	14.330	Antioxidant activity	(19)
2-Methyl-7-phenylindole $C_{15}H_{13}N$	17.352	Antioxidant & Anticancer activity	(20)
Cyclotetracosane $C_{24}H_{48}$	17.474	Antifungal activity	(21)
1-Heptacosanol $C_{27}H_{56}O$	17.474	Antioxidant, Anti-Inflammatory & Antimicrobial	(22)
2,4,6-Cycloheptatrien-1-one $C_7H_6O_2$	17.885	Anti Inflammatory& Antioxidant activity	(23)
Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy]silane $C_9H_{22}O_3Si$	17.885	Antioxidant activity	(24)
1,1,1,3,5,5,5-Heptamethyltrisiloxane $C_7H_{21}O_2Si_3$	18.007	Antioxidant& Anti-Inflammatory activity	(25)
Tetrasiloxane, decamethyl- $C_{10}H_{30}O_3Si_4$	18.118	Antibacterial activity	(26)
2-Methyl-7-phenylindole $C_{15}H_{13}N$	18.174	Antioxidant & Anticancer activity	(27)
Butane, 2-phenyl-3-(trimethylsilyloxy)- $C_7H_{16}Si$	18.241	Antibacterial activity	(28)
Octacosane $C_{28}H_{58}$	18.463	Antioxidant activity	(29)
1-Heptacosanol $C_{27}H_{56}O$	18.729	Antioxidant and Anti-Inflammatory Activity	(21)
1-Docosanol, methyl ether $C_{23}H_{48}O$	18.729	Antimicrobial activity	(30)
Cyclotrisiloxane, hexamethyl- $C_6H_{18}O_3Si_3$	19.252	Antioxidant & Antibacterial activity	(31)
Triacontane $C_{30}H_{62}$	20.007	Antihepatotoxic activity	(32)
Eicosane $C_{20}H_{42}$	20.363	Anti-Inflammatory activity	(33)
Tetracosane $C_{24}H_{50}$	20.363	Antimicrobial activity	(10)
Hentriacontane $C_{31}H_{64}$	20.363	Anti-Inflammatory	(34)
dl-.alpha.-Tocopherol $C_{29}H_{50}O_2$	20.751	Antioxidant activity	(35)
4-Methyl-2-trimethylsilyloxy-acetophenone $C_{12}H_{18}O_2Si$	22.818	Antioxidant& Anti-Inflammatory activity	(36)

metabolites, including alcohols, esters, acids and phenolic compounds. Notable compounds such as Benzoic acid, 4-ethoxy -, ethyl ester (10.064), Ar-tumerone (11.208), Diphenyl sulfone (13.164), 2-Chloroethyl linoleate (14.330**),** Cyclotetracosane (17.474), 2,4,6-Cycloheptatrien-1-one (17.885), Tetrasiloxane, decamethyl-(18.118), Cyclotrisiloxane, hexamethyl-(19.252), Eicosane (20.363), 4-Methyl-2-trimethylsilyloxy-acetophenone (22.818) were identified, which are known for their significant bioactive properties (Fig. 1). The presence of these metabolites align with reports from other medicinal plants, where similar compounds have been linked to antioxidant, antimicrobial and anti-inflammatory activities (10). Comparative studies on other floral species, such as *Calendula officinalis*, have also reported a high prevalence of phenolic and furan derivatives, suggesting a shared biochemical pathway for secondary metabolite synthesis (11). The identified metabolites in *C. cristata* flowers underscore their pharmacological and ecological significance, associated with potent antioxidant and antimicrobial properties. This highlights, *C. cristata* as a promising candidate for pharmaceutical applications targeting oxidative stress-related disorders (12). Among the identified compounds, 2-Methoxy-4-vinylphenol, a phenolic compound, exhibits both anti-carcinogenic and anti-inflammatory properties, which could be leveraged in the development of anti-inflammatory drugs (13). The presence of these compound enhances the utility of *C. cristata* in pharmaceutical formulations (14).

Antibacterial Potential

Antimicrobial resistance to drugs poses a significant challenge to the treatment of different diseases caused by pathogens (14). The antibacterial activity of *C. cristata* can also contribute to its ecological defence against pathogens. The selection of crude extracts for screening offers greater potential than testing pure compounds isolated from natural products. The antibacterial activity of the flower extract was assessed against two bacterial strains: *S. aureus* and *E. coli*, using the zone of inhibition (mm) as the evaluation parameter. The extract demonstrated a notable inhibitory effect against *E. coli*, with the zone of inhibition increasing dose-dependently from 11 mm at 10 µL to 15 mm at 50 µL (Fig. 2). In contrast, *S. aureus* exhibited resistance at lower concentrations (10-40 µL), with only a mild inhibition zone of 10 mm observed at the highest concentration (50 µl) (Fig. 3). These findings indicate that *C. cristata* flower extract possesses selective antibacterial potential, particularly effective against *E. coli*. This selective inhibition may be attributed to the presence of specific bioactive compounds identified through GC-MS analysis, such as Diphenyl sulfone, 9,12-Octadecadienoic acid, Tetrasiloxane decamethyl and Butane, 2-phenyl-3-(trimethylsilyloxy) which are known to possess antibacterial properties. Consistent with previous studies on the antibacterial effects of plant extracts, including jasmine flower (15), marigold leaves (16) and tuberose flowers (17), the current results further support the potential of botanical sources as natural antibacterial agents against various pathogenic bacteria (18), particularly *E. coli*.

Molecular docking

A ligand's affinity for its target protein is indicated by its binding score, with greater negative value indicating a stronger binding. With the lowest binding score (-4.5), diphenyl sulfone demonstrated the highest interaction with *E. coli* Haemolysin E (1QOY). In contrast, the binding score of 9,12-Octadecadienoic

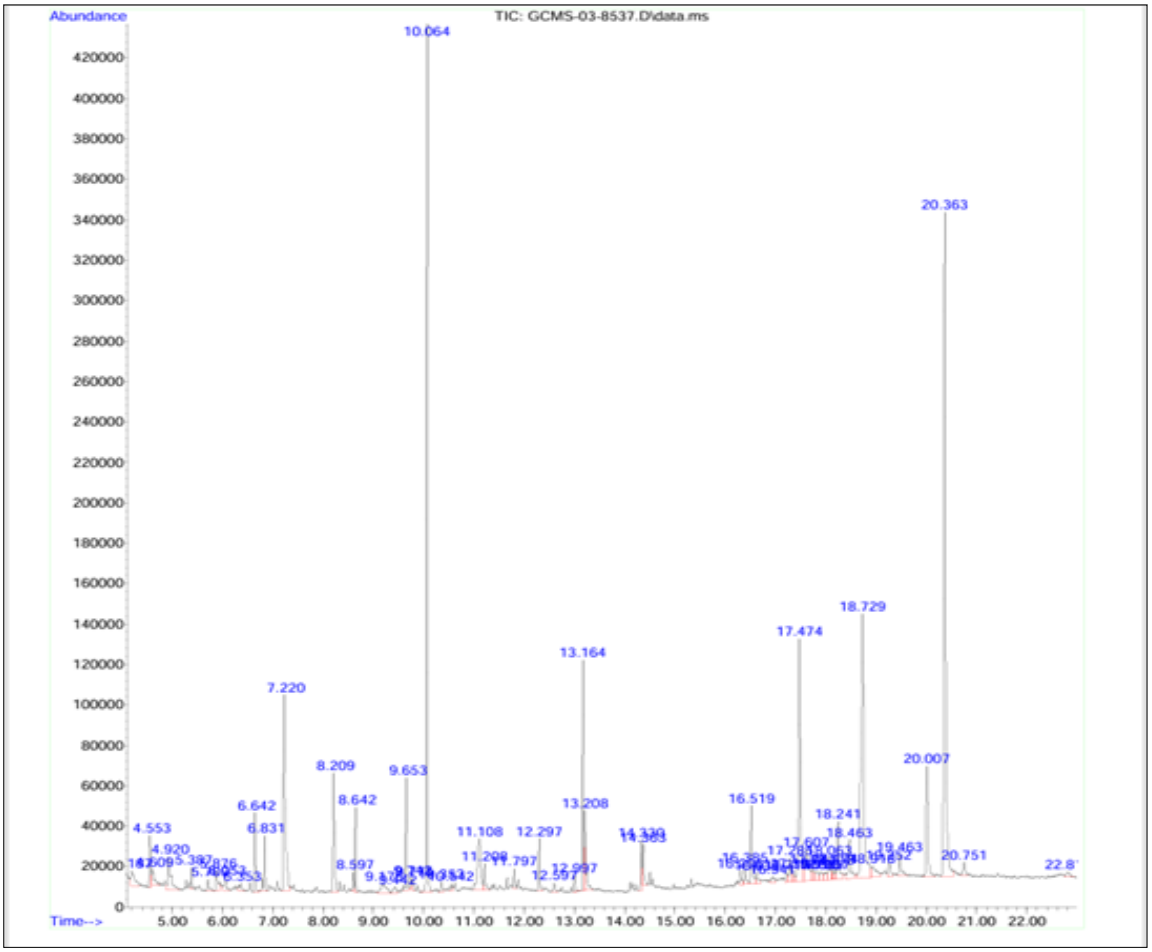


Fig. 1. GC-MS chromatogram of *Celosia cristata* flower extract.

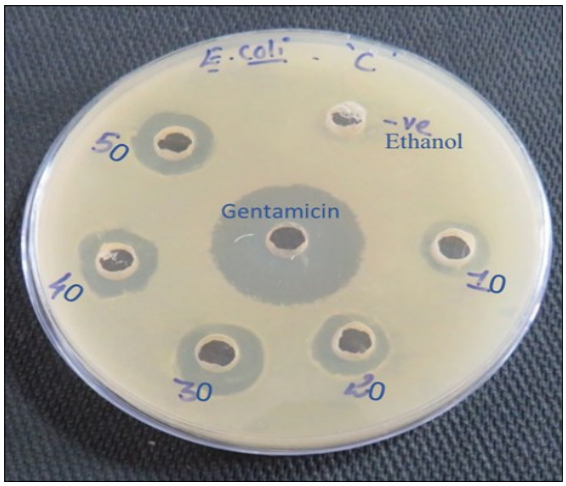


Fig. 2. Antibacterial activity of *C. cristata* flower against *E. coli* (10 - 50 are concentration in µL).

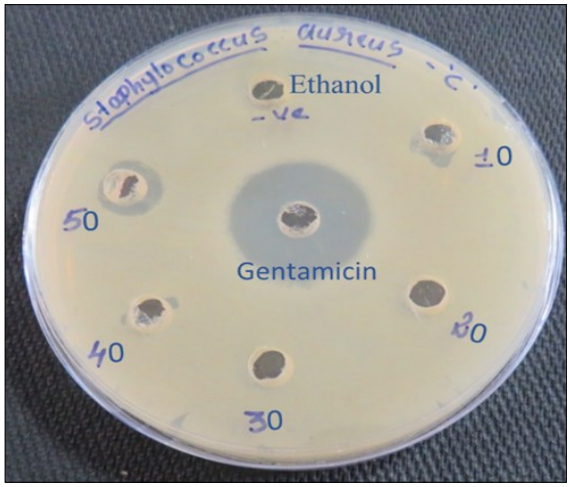
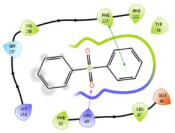
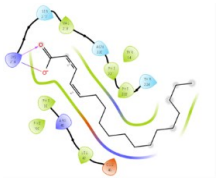
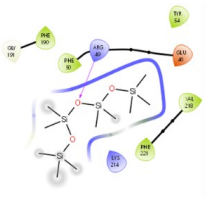
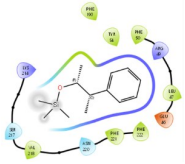
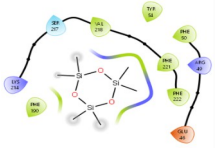


Fig. 3. Antibacterial activity of *C. cristata* flower against *S. aureus* (10 - 50 are concentration in µL).

acid was positive (0.3), suggesting a weak or unfavourable interaction with the target. The binding potential of tetrasiloxane, decamethyl and butane derivatives is moderate, with scores ranging from -2.9 to -3.8. Cyclotrisiloxane, hexamethyl-, recorded a score of -3.1, which indicates a weak interaction (Table 2). The stronger binding of diphenyl sulfone is supported by a variety of interaction types, including hydrophobic (π-alkyl) interactions with PHE221 and TYR54, as well as a hydrogen bond with ARG49. The chemical compounds namely tetrasiloxane and butane derivative did not exhibit hydrogen bonding but maintained hydrophobic interactions, which can still contribute to binding, although generally weaker than hydrogen bonds. Despite hydrogen bonding with LYC214, 9,12-Octadecadienoic acid, primarily

Fig. 2. Antibacterial activity of *C. cristata* flower against *E. coli* (10 - 50 are concentration in µL).

Ligand	Docking details	Hemolysin E 1QOY	Docking images
Diphenyl sulfone	Binding score (G-Score)	-4.5	
	Conventional H bond	ARG49	
	Alkyl and pi= alkyl6	PHE:221 TYR:54	
9,12-Octadecadienoic acid (Z,Z)-	Binding score (G-Score)	0.3	
	Conventional H bond	LYC214	
	Alkyl and pi= alkyl6	PHE:221 GLU:46	
Tetrasiloxane, decamethyl-	Binding score (G-Score)	-2.93	
	Conventional H bond	ARG49	
	Alkyl and pi= alkyl6	PHE221	
Butane, 2-phenyl-3-(trimethylsilyloxy)-	Binding score (G-Score)	-3.8	
	Conventional H bond	-	
	Alkyl and pi= alkyl6	SER217	
Cyclotrisiloxane, hexamethyl-	Binding score (G-Score)	-3.1	
	Conventional H bond	-	
	Alkyl and pi= alkyl6	LYS:214 ARG:49	

exhibited hydrophobic interactions, which can contribute to binding, even though they are typically weaker than hydrogen bonds. Among the tested compounds, diphenyl sulfone emerged as the most promising candidate for inhibiting *E. coli* Hemolysin E, indicating is significance in developing anti-bacterial agents. Compounds with moderate scores (butane derivative, siloxanes) may serve as leads for further optimization, especially if they demonstrate favourable bioavailability or synthetic accessibility. These results are in accordance with the previous report indicating that diphenyl sulfone complexes possess antibacterial activity against both Gram-positive and Gram-negative bacteria, including *E. coli*, *Klebsiella pneumoniae* and *S. aureus*. These findings also highlights the potential of sulfone derivatives in antimicrobial drug development (37).

Conclusion

This study reports the presence of a diverse array of bioactive phytochemicals in *C. cristata* flowers. These constituents are recognized for their antioxidant, antimicrobial and anti-inflammatory properties, supporting the plant's potential in pharmaceutical, nutraceutical and cosmetic applications. Antibacterial assays revealed selective activity against *Escherichia coli*, with inhibition zones increasing in size with higher extract concentrations, suggesting a dose-dependent effect. Molecular docking results validated the antibacterial efficacy of diphenyl sulfone, against *E. coli*, underscoring its potential as a lead compound for further antibacterial drug development. Overall, the phytochemical composition, antibacterial efficacy and docking analysis establish *C. cristata* as a promising natural source for developing therapeutics against microbial infections and oxidative stress.

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Authors' contributions

SS collected and analyzed the data and draft manuscript. RC reviewed and corrected the manuscript, while MG, AR, PM, PG and VS provided valuable technical aspects and suggestions to enhance the manuscript's quality.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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