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Examination of Phytochemical Composition using GC-MS and Assessment of Antibacterial Efficacy of Indian Geranium Oil against *Aeromonas Caviae*

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Abstract
Geranium oil, extracted from the leaves and flowers of the geranium plant, holds a significant position for its potential health benefits. This essential oil has found utility in both traditional and alternative medicinal practices, showcasing its versatility across diverse healthcare methodologies. This study systematically analyzed the chemical composition of geranium oil, identifying a diverse array of compounds. Notably, Aminoacetonitrile, 3-Nitro-2-methyl propene, and 2,6-Octadiene (E,E) emerged as predominant components in the analysis. These compounds, including acetic anhydride, serve as vital precursors in pharmaceutical drug synthesis and other organic compound formations. The antibacterial efficacy of geranium oil against Aeromonas caviae was evaluated, revealing notable inhibitory effects of 17 mm at an elevated concentration of 30 µg/µl. The mechanisms underlying this antibacterial activity may be attributed to bioactive compounds present in geranium oil, such as citronellol, geraniol, and linalool, each known for their antimicrobial properties. Further research is essential to precisely elucidate these mechanisms and comprehensively evaluate the safety and efficacy of geranium oil for potential therapeutic applications.

Keywords: Geranium Oil, *Pelargonium graveolens*, Chemical Composition, Antibacterial Activity, *Aeromonas caviae*, Bioactive Compounds

Introduction

Phytochemicals, bioactive compounds derived from plants, have attracted considerable interest for their possible impacts on health and overall wellness (Dillard and German; Hai; Park et al.; Szakiel et al.; Singh et al.; da Silva et al.; Abbas et al.; Süntar; Kumari and Deka; Agrawal et al.). These compounds demonstrate a wide range of properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Agrawal et al.). Indian Geranium oil, derived from the Pelargonium graveolens plant, is well-known for its unique and aromatic properties. With a rich history in traditional medicine, this essential oil has been utilized for various therapeutic applications over time (Boukhris et al.; Benazir et al.; Asgarpanah and Ramezanloo; Verma et al.; Hamidpour et al.; Pandey et al.; Mazeed et al; Sandasi et al.). However, there is a lack of scientific data regarding the phytochemical makeup and antibacterial attributes of Indian Geranium oil against pathogens. This study's main objective is to conduct a thorough phytochemical examination of Indian Geranium oil and assess its effectiveness against *Aeromonas caviae*, a Gram-negative bacterium commonly associated with infectious diseases in humans and animals (Callister and Agger; Vila et al.; Kimura et al.; Ghatak et al.; Kumar et al.; Cardozo et al.; Majeed et al.). Investigating the phytochemical composition of Indian Geranium oil and its potential antibacterial effects on this pathogenic bacterium could significantly contribute to the advancement of innovative therapeutic methods. These findings might encourage the use of natural products in developing effective strategies against bacterial infections. The precise objectives of this study include: The goal is to utilize advanced analytical methods, notably gas chromatography-mass spectrometry (GC-MS), to analyze the chemical composition of Indian Geranium oil. This analytical approach aims to identify and measure the primary phytochemical compounds present in the oil, such as terpenes, phenols, flavonoids, and various other bioactive elements. The aim is to evaluate the antibacterial effectiveness of Indian Geranium oil against *Aeromonas caviae* using well-established microbiological methods. This assessment will entail measuring the antibacterial activity through the determination of the zone of inhibition, employing the agar well diffusion method as the standard procedure. The study aims to uncover the mechanisms underlying the antibacterial properties of Indian Geranium oil. This investigation will analyze the oil's potential effects on various aspects, including the integrity of the bacterial cell membrane, the formation of biofilms, and specific enzymatic activities related to *Aeromonas caviae*. By achieving these objectives, the research aims to provide a comprehensive understanding of the phytochemical composition of Indian Geranium oil and its antibacterial capabilities against *Aeromonas caviae*. The findings of this study have the potential to contribute to the development of natural alternatives for combating bacterial infections, with broader implications for the fields of medicine, pharmaceuticals, and natural product research.

Materials and Methods

All chemicals, solvents, and essential oils utilized in the research were obtained from trusted and established chemical suppliers. For the qualitative phytochemical analysis, the methodology described by Rathore et al. was followed to screen Indian geranium oil. This involved a systematic procedure aimed at identifying and quantifying the diverse bioactive compounds within the oil (Rathore et al.).

Qualitative Phytochemical Analysis

The phytochemical screening of Indian geranium oil followed the methodology outlined by Rathore. This process involved a systematic examination to identify and quantify various bioactive compounds present in the oil (Rathore et al.). For the tests conducted: To test for acids, Millon's Test was performed by adding five drops of Millon's reagent to 1.0 ml of the oil. The mixture underwent heating on a water bath for 5 minutes followed by cooling. Subsequently, 1% sodium nitrite solution was added. The formation of a red color indicated the presence of acids. For alkaloids, Mayer's Test was carried out by adding 2.0 ml of concentrated hydrochloric acid and a few drops of Mayer's reagent to 2.0 ml of the oil. The emergence of a green color or the formation of a white precipitate signified the presence of alkaloids. To test for

anthocyanin and betacyanin, the Sodium Hydroxide Test was conducted. This involved adding 1.0 ml of 2N sodium hydroxide to 2.0 ml of oil and heating the mixture for 5 minutes at 100°C. The presence of anthocyanin was indicated by the formation of a bluish-green color, while the presence of betacyanin was denoted by the appearance of a yellow color. For carbohydrates, Molisch's Test was performed by adding 1.0 ml of Molisch's reagent and a few drops of concentrated sulfuric acid to 2.0 ml of oil. The formation of a purple or reddish ring in the solution indicated the presence of carbohydrates.

Several tests were conducted to determine the presence of specific phytochemical compounds in the oil. The Ferric Chloride Test was employed to detect Cardiac Glycosides, where 2.0 ml of glacial acetic acid and a few drops of 5% ferric chloride were added to 0.5 ml of oil. This mixture was then under-layered with 1.0 ml of concentrated sodium hydroxide, and the formation of a brown ring at the interface indicated the presence of cardiac glycosides. To identify Coumarins, the Sodium Hydroxide Test was performed by adding 1.0 ml of 10% sodium hydroxide to 1.0 ml of oil, with the development of a yellow color signifying their presence. Flavonoids were detected using the Sulphuric Acid Test, where 1.0 ml of oil was treated with concentrated sulphuric acid, and the formation of an orange color indicated their presence. Lastly, the presence of Glycosides was determined using the Sulphuric Acid Test, where 1.0 ml of glacial acetic acid, 5% ferric chloride, and concentrated sulphuric acid were added to 2.0 ml of oil, with a resulting greenish-blue color indicating their presence.

The Ferric Chloride Test, employed to detect Phenols, involved adding 2.0 ml of distilled water to 1.0 ml of oil, followed by a few drops of 10% ferric chloride. The formation of a blue or green color indicated the presence of phenols. For Protein detection, the Ninhydrin Test was performed by adding a few drops of 0.2% ninhydrin to 2.0 ml of oil and heating for 5 minutes, with a resulting blue color indicating the presence of proteins. Quinones were identified using the Sulphuric Acid Test, where 1.0 ml of oil was treated with 1.0 ml of concentrated sodium hydroxide, and the formation of a red color indicated their presence. Lastly, the Foam Test was utilized to detect Saponins, involving the combination of 1.0 ml of oil with 5.0 ml of distilled water. The resulting mixture was vigorously shaken for 15 minutes in a graduated cylinder, and the presence of a 1.0 cm layer of foam indicated the presence of saponins.

The Iodine Test was utilized to detect Starch, where a few drops of iodine solution were added to 2.0 ml of oil, and the emergence of a blue-purple color indicated its presence. Steroids were identified using the Salkowski Test, involving the addition of 2.0 ml of chloroform and a few drops of concentrated sulphuric acid to 5.0 ml of oil, with a resulting red color indicating their presence. Tannins were detected through the Ferric Chloride Test, where 2.0 ml of 5% ferric chloride was added to 1.0 ml of oil, and the formation of a dark blue or greenish-black color indicated their presence. Terpenoids were identified using the Sulphuric Acid Test, where 2.0 ml of chloroform was added to 0.5 ml of oil, followed by the careful addition of concentrated sodium hydroxide, and the formation of a red-brown color at the interface indicated their presence. Lastly, the Liebermann-Burchard's Test (LB test) was employed to detect Triterpenoids, where a few drops of Liebermann-Burchard's reagent were added to 1.5 ml of oil, and the appearance of a blue-green color indicated their presence.

Spectral Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS spectral analysis was employed to discern the aromatic compounds present in the Indian geranium oil samples. The analysis was conducted using an Agilent 7890 gas chromatograph interfaced with a 240 mass selective detector and an ion trap. Interpretation of the results was facilitated by the extensive National Institute of Standards and Technology (NIST) database, housing over 62,000 patterns. By comparing the spectrum of the unknown component with the spectra of known components stored in the NIST library, the name, molecular weight, and structure of the constituents within the tested materials could be determined.

Culture Media, Inoculums Preparation and Antimicrobial Activity

Nutrient agar and broth sourced from Himedia,

India, were utilized as the growth media for bacterial strains. Bacterial cultures were inoculated into nutrient broth using sterilized loops and then incubated at 37ºC for 72 hours. Following incubation, antibacterial analysis was performed using the standard agar well diffusion method to assess the antibacterial activity of essential oils. This assessment included measuring the zone of inhibition against the target microorganisms. Ciprofloxacin served as the benchmark antibacterial agent in the experiment for reference purposes (Yang et al.). This method provided a comprehensive evaluation of the antibacterial efficacy of the essential oils against the specified test microorganisms.

For the experiment, *Aeromonas caviae* was selected as the bacterial species, with stock cultures obtained from Microlabs, Institute of Research and Technology, situated in Vellore, Tamil Nadu, India. The stock cultures were meticulously handled and maintained under controlled conditions. In preparation for the antibacterial analysis, the microorganisms were cultured overnight at 37ºC in Mueller-Hinton Broth, maintaining a pH of 7.4. Mueller-Hinton Broth is widely employed for standardized bacterial growth in microbiological research, and the specified pH ensures optimal conditions for bacterial proliferation. The overnight incubation allowed the bacteria to reach the logarithmic growth phase, establishing a robust and standardized starting point for subsequent experiments. This careful cultivation and handling of bacterial cultures from a reputable source, alongside the utilization of standardized growth conditions, contribute to the reliability and consistency of the experimental framework for evaluating the antibacterial efficacy of essential oils.

Results and Discussion

Geranium oil, extracted from the leaves and flowers of the geranium plant (Pelargonium graveolens), is renowned for its diverse traditional and alternative medicinal applications, attributed to its potential health-promoting properties. The complex chemical composition of geranium oil can vary depending on factors such as plant species, geographical origin, and extraction methods. Analysis of geranium oil reveals the presence of various compound classes, including alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, and triterpenoids (see Table 1). Let's embark on a detailed exploration of each of these compound groups.

Alkaloids, natural compounds with notable physiological impacts on the human body, exhibit diverse biological activities in geranium oil, albeit with variations in specific types. The alkaloids found in geranium oil are recognized for their antimicrobial, anti-inflammatory, and analgesic properties (Kaur and Arora; Komakech et al.; Bouyahya et al.). Flavonoids, categorized as plant secondary metabolites, are renowned for their antioxidant and anti-inflammatory characteristics. These compounds, responsible for the vivid hues in numerous fruits and flowers, play a role in geranium oil by contributing to its potential antioxidant activity. This contribution may aid in safeguarding against oxidative stress and certain chronic diseases (Kris-Etherton and Keen; Grassi et al.; Zhang et al.; Maiti et al.; García-Sánchez et al.). Phenols, aromatic compounds recognized for their robust antioxidant and antimicrobial qualities, play a role in the potential therapeutic effects of geranium oil. Within geranium oil, phenols contribute by aiding in the neutralization of detrimental free radicals, thereby mitigating oxidative damage in the body. Additionally, research indicates that phenols exhibit antiseptic and antibacterial properties (Kachur and Suntres).

Saponins, natural surfactant compounds found in many plants, possess foaming and emulsifying properties, making them valuable in various cosmetic and pharmaceutical applications. Within geranium oil, saponins may enhance its emulsifying abilities, aiding in effective blending with other substances (Kregiel et al.; Sharma et al.). Tannins, a group of polyphenolic compounds abundant in plants, exhibit astringent qualities and are commonly used in traditional medicine for their antiviral, antibacterial, and antifungal effects. In geranium oil, tannins could potentially provide similar benefits, contributing to its antimicrobial properties (Dahl et al.; El-Saadony et al.; Hou et al.; Jia et al.). Terpenoids encompass a diverse group of compounds widely found in nature, including essential oils, and are responsible for the unique aromas and flavors associated with

many plants. In geranium oil, terpenoids likely contribute to its distinct fragrance. Additionally, research has explored the potential therapeutic effects of terpenoids, including anti-inflammatory, antimicrobial, and analgesic properties (Prakash et al.; Bouyahya et al.). Triterpenoids, a subclass of terpenoids, often exhibit unique bioactivities. Known for their anti-inflammatory and antioxidant properties, these compounds in geranium oil may contribute to potential anti-inflammatory effects and protection against oxidative stress (Li et al.; Lee et al.). It's important to note that while the presence of these compounds in geranium oil has been documented, their precise composition and concentrations can vary based on several factors. Together, these constituents play a role in the potential therapeutic benefits of geranium oil, including antimicrobial, anti-inflammatory, and antioxidant effects. However, further research is necessary to fully understand the mechanisms of action and specific health advantages associated with geranium oil and its components.

Table 1 Qualitative Phytochemical Analysis of Essential Oils

Phytocompounds	Geranium Indian oil
Acids	-
Alkaloids	+
Anthocyanins and Betacyanins	-
Carbohydrates	-
Cardiac Glycosides	-
Coumarins	-
Flavonoids	+
Glycosides	-
Phenols	+
Proteins	-
Quinones	-
Saponins	+
Starch	-
Steroids	-
Tannins	+
Terpenoids	+
Triterpenoids	+

Where + denotes presence of phytocompounds and - denotes absence of phytocompounds.

Antibacterial Activity of Geranium Oil Indian variety against *Aeromonas caviae*

The antibacterial test results display the zone of inhibition, demonstrating the effects of various concentrations of essential oil treatments on specific bacterial strains. The zone of inhibition represents the clear area surrounding a disc or well in a culture medium treated with a particular substance. In this study, the substances examined include DMSO (Dimethyl sulfoxide) as a negative control, Ciprofloxacin (an antibiotic) as a positive control10 µg/µl, and different concentrations of Geranium oil. This clear zone indicates the successful inhibition of bacterial growth in that specific region, offering a visual representation of the antibiotic's ability to suppress bacterial proliferation.

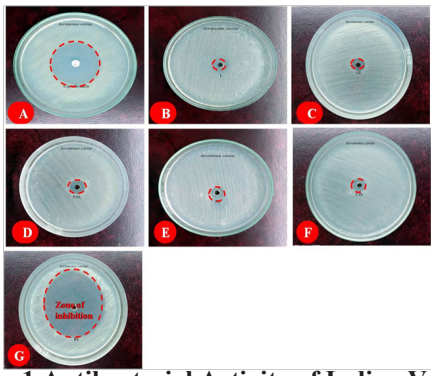


Figure 1 Antibacterial Activity of Indian Variety Geranium Oil against *Aeromonas caviae* using Different Concentrations
A - Ciprofloxacin control (10 µg/µl), B - 5 µg/µl of Geranium oil, C - 10 µg/µl of Geranium oil, D - 15 µg/µl of Geranium oil, E - 20 µg/µl of Geranium oil, F - 25 µg/µl of Geranium oil and G - 30 µg/µl of Geranium oil
The red dotted circle on the plate indicates the zone of inhibition against *Aeromonas caviae*

In the assessment of the Indian variety of Geranium oil against the bacteria *Aeromonas caviae*, concentrations ranging from 5 µg/µl to 30 µg/µl were investigated. Mean zones of inhibition were recorded at 6, 8, 9, 11, 12, and 17 mm for concentrations of 5, 10, 15, 20, 25, and 30 µg/µl, respectively. These results were notably impressive, demonstrating comparability with the standard reference antibiotic. The mean zone of inhibition for Geranium oil ranged from 6 to 17 mm.In vitro findings further elucidate

that the phytochemicals present in Geranium oil induced inhibitory effects like those observed with Ciprofloxacin (see Fig. 1 and 2). This suggests that at the specified concentrations, Geranium oil exhibits significant antibacterial activity against *Aeromonas caviae*. The documented results underscore the potential of Geranium oil as an effective agent in inhibiting the growth of the tested bacteria, with concentrations showing noteworthy antimicrobial effects comparable to a well-established antibiotic. However, at a concentration of 30 µg/µl, Geranium oil demonstrated a substantial inhibitory effect with a zone of inhibition measuring 17 mm. This indicates that at this higher concentration, Geranium oil was able to effectively inhibit the growth of *Aeromonas caviae* bacteria. On the contrary, the negative control, DMSO, exhibited no noticeable antimicrobial effect against the tested bacterial strains. In contrast, the positive control, Ciprofloxacin (10 µg/µl), demonstrated a zone of inhibition measuring 15 mm. Geranium oil, evaluated at various concentrations, displayed robust antimicrobial effects against the targeted bacterial strains, with higher concentrations yielding larger zones of inhibition. The mechanism of action for Geranium oil's antibacterial activity may involve the presence of certain bioactive compounds within the oil. Geranium oil contains various components, including citronellol, geraniol, and linalool, which possess antimicrobial properties. These compounds may interfere with the bacterial cell membrane, disrupt vital cellular processes, or inhibit enzyme activity, leading to the inhibition of bacterial growth (Swamy et al.). It is important to note that further studies and experiments would be needed to determine the precise mechanisms responsible for Geranium oil's antibacterial activity against *Aeromonas caviae* and to assess its safety and efficacy for therapeutic use.

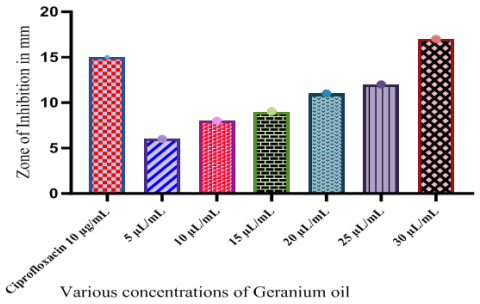


Figure 2 Antibacterial activity of Indian variety of Geranium oilagainst Aeromonascaviae using different concentration

A - Ciproflaxin Positive control 10 µg/µl, B - 5 µg/µl of Geranium oil, C - 10 µg/µl of Geranium oil, D - 15 µg/µl of Geranium oil, E- 20 µg/µl of Geranium oil, F- 25 µg/µl of Geranium oil, and G- 30 µg/µl

Gas Chromatography-Mass Spectrometry (GC-MS) Examination

The chemical profile of the Indian variety of Geranium oil extract was determined using Gas Chromatography-Mass Spectrometry (GC-MS) data and compared with known compounds stored in the National Institute of Standards and Technology (NIST) library associated with the GC-MS. A total of 39 chemical structures were identified through this analysis. Among these compounds, nine demonstrated a high level of bioactive compounds.

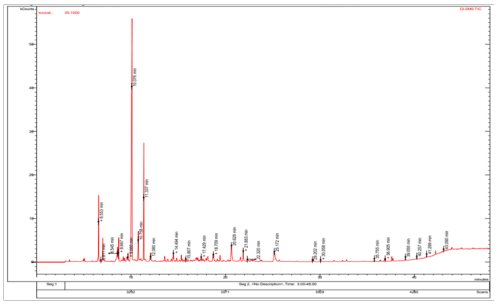


Figure 3 The Chromatogram obtained through Gas Chromatography-Mass Spectrometry (GC-MS) analysis reveals the Composition of the Essential Oil extracted from Geranium oil Indian Variety

Table 2 presents comprehensive information on these nine identified phytocompounds, including retention time, compound name, molecular formula,

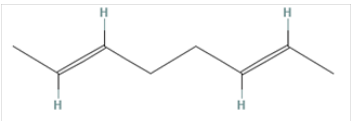
molecular weight, percent area, and PubChem ID, specifically in relation to their binding affinity with the target protein against *Aeromonas caviae*. To visually represent the chemical composition, the GC-MS chromatogram of the Geranium oil Indian variety extract is depicted in Figure 3. This chromatogram provides a graphical representation of the various compounds present in the Geranium oil, highlighting their respective retention times and relative abundance.

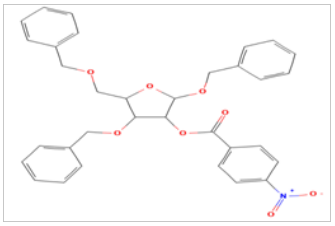
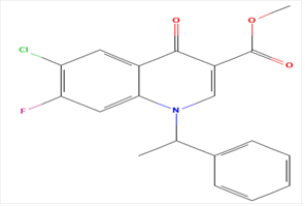
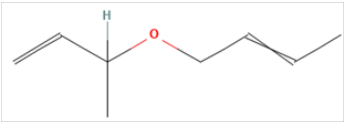
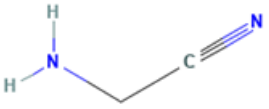
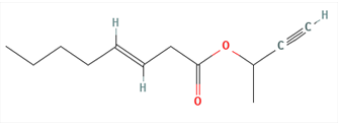
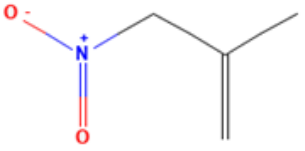
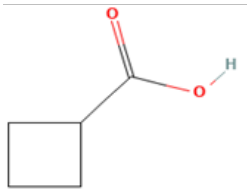
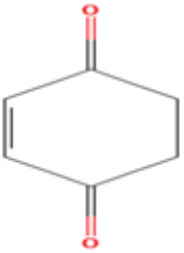
The results from Table 2 and 3 highlight the identification of key compounds in the Indian variety of Geranium oil extract through GC-MS analysis. Among these, the following compounds displayed notable attributes: 2,6-Octadiene, (E,E), 2-p-Nitrobenzoyl-1,3,5-tribenzyl-alpha-d-ribose, 6-Chloro-7-fluoro-4-oxo-1-(1-phenylethyl)-1,4-dihydroquinoline-3-carboxylic acid, methyl ester, 1-Butene, 3-(2-butenyloxy), Aminoacetonitrile, Oct-3-enoic acid, but-3-yn-2-yl ester, 3-Nitro-2-methyl propene, Cyclobutanecarboxylic acid, 2-Cyclohexene-1,4-dione. The retention times for these compounds are recorded as follows: 6.553 (6.828%), 6.997 (2.509%), 8.545 (2.082%), 8.667 (2.497%), 10.076 (42.628%), 10.758 (3.928%), 11.337 (10.860%), 20.628 (3.547%), and 25.172 (3.627%). These retention times provide information about the duration each compound is retained during the chromatographic separation. To better understand the structural characteristics of these identified compounds, their 2D structures are illustrated in Table 3. This visual representation allows for a more in-depth examination of the molecular arrangements and bonding patterns, providing valuable insights into the chemical composition of the compounds responsible for the observed biological activity in Geranium oil (Kriek et al.; Truelove et al.; Braun and Devant; Wei et al.; Paizs et al.; Cho et al.; Swamy et al.). It serves as a starting material for the synthesis of numerous pharmaceutical drugs and other organic compounds. Pharmaceutical synthesis involves the creation of new drugs or the production of existing drugs on a larger scale.

Table 2 Phytochemical Compounds Identified through GC-MS Analysis of Geranium Oil Indian variety Oil Extract

S. No	Compound name	RT	Molecular formula	Molecular Weight	Area %	Amount
1	2,6-Octadiene, (E,E)	6.553	C_8H_{14}	110.20	43396	6.828
2	2-p-Nitrobenzoyl-1,3,5-tribenzyl-alpha-d-ribose	6.997	$C_{33}H_{31}NO_8$	569.6	15946	2.509
3	6-Chloro-7-fluoro-4-oxo-1-(1-phenylethyl)-1,4-dihydroquinoline-3-carboxylic acid, methyl ester	8.545	$C_{19}H_{15}C_1FNO_3$	359.8	13233	2.082
4	1-Butene, 3-(2-butenyloxy)-	8.667	$C_8H_{14}O$	126.20	15871	2.497
5	Aminoacetonitrile	10.076	$C_2H_4N_2$	56.07	270949	42.628
6	Oct-3-enoic acid, but-3-yn-2 -yl ester	10.758	$C_{12}H_{18}O_2$	194.27	24968	3.928
7	3-Nitro-2-methyl propene	11.337	$C_4H_7NO_2$	101.10	69024	10.860
8	Cyclobutanecarboxylic acid	20.628	$C_5H_8O_2$	100.12	22542	3.547
9	2-Cyclohexene-1,4-dione	25.172	$C_6H_6O_2$	110.11	23055	3.627

Table 3 The 2D molecular composition of the predominant phytochemicals in Geranium oil Indian variety extract as determined by GC-MS analysis

S. No	PubChem (CID)	Compound Name and Biological Activity	2D Structure of Phytocompounds
1	5364393	2,6-Octadiene, (E,E) Antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, proteus mirabilis, Pseudomonas aeruginosa* and *Salmonella typhimurium* (Ololade et al.)	

2.	542798	2-p-Nitrobenzoyl-1,3,5-tribenzyl-alpha-d-ribose Anti-thrombotic agents (Oso et al.)	
3.	570411	6-Chloro-7-fluoro-4-oxo-1-(1-phenylethyl)-1,4-dihydroquinoline-3-carboxylic acid, methyl ester Antibacterial agent (Yousuf et al.)	
4.	543223	1-Butene, 3-(2-butenyloxy) Anticancer activity (Olaoye et al.)	
5.	10901	Aminoacetonitrile Preventive activity against Fusarium Diseases (Kirino et al.)	
6.	91692446	Oct-3-enoic acid, but-3-yn-2 -yl ester No biological activity	
7.	259637	3-Nitro-2-methyl propene Antibacterial activity (Dong et al.)	
8.	19494	Cyclobutane carboxylic acid Antibacterial activity (Kharlamova et al.)	
9.	138275	2-Cyclohexene-1,4-dione Antimicrobial activity (Hossain et al.)	

Conclusion

Geranium oil derived from Pelargonium graveolens offers potential health benefits owing to its intricate chemical composition. Main compounds such as aminoacetonitrile, 3-Nitro-2-methyl propene, and 2,6-Octadiene (E,E) have industrial applications as starting materials for pharmaceutical synthesis.

In the assessment of antibacterial activity against *Aeromonas caviae*, geranium oil demonstrated significant inhibitory effects at a concentration of 30 µg/µl. The antimicrobial properties of geranium oil are likely attributed to bioactive compounds such as citronellol, geraniol, and linalool. However, further research is warranted to elucidate the precise mechanisms responsible for the antibacterial activity and to assess the safety and efficacy of geranium oil for therapeutic use.

Declarations

Ethics Approval Not applicable

Funding No funding and worked with online and freely available software's.

Conflict of Interests The authors declare that they have no conflict of interests.

Availability of Data and Materials All the data generated or analyzed during this study are included in this article.

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