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Research Article

Exploring the Antimicrobial Potential of *Calotropis gigantea* Against Pathogens: *Propionibacterium acnes, Vibrio vulnificus, Aeromonas hydrophila* and *Escherichia coli*

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Abstract

Calotropis gigantea, commonly known as giant milkweed, has been traditionally used for its medicinal properties, particularly in treating skin infections and inflammation. This study evaluates the antimicrobial potential of methanol and ethanol extracts from sun-dried and oven-dried leaves and flowers of C. gigantea against key human pathogens, including *Propionibacterium acnes*, *Vibrio vulnificus*, *Aeromonas hydrophila*, and *Escherichia coli*. The disc diffusion method was employed to assess antimicrobial activity, revealing that extracts from oven-dried leaves, particularly those prepared with ethanol, exhibited the strongest antimicrobial activity, notably against *A. hydrophila* and *E. coli*. Phytochemical screening of the extracts identified the presence of terpenoids, flavonoids, phenols, and sterols, compounds known for their antimicrobial and antioxidant properties. Gas chromatography-mass spectrometry (GC-MS) analysis of the ethanol extract revealed 20 bioactive compounds, with Diethyl Phthalate as the predominant compound (61.11%), alongside other compounds with potential antimicrobial and anti-inflammatory activities, such as alpha-tocopherol and 9,12,15-octadecatrienoic acid. The study highlights the efficacy of *C. gigantea* extracts in combating bacterial pathogens and underscores the importance of drying methods and extraction solvents in optimizing antimicrobial properties.

Key words: *Calotropis gigantea, Propionibacterium acnes, Vibrio vulnificus, Aeromonas hydrophila* subsp. *hydrophila* and *Escherichia coli*, Antimicrobial activity

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Introduction

Calotropis gigantea, also known as giant milkweed or crown flower, is a large tree native to tropical regions and valued for its medicinal properties [1]. Traditionally used in herbal remedies, it contains bioactive compounds with notable antibacterial, anti-inflammatory, and analgesic effects. Current clinical studies are exploring the potential applications of its leaves and flowers in treating infections and inflammation. Extracts from this plant show promise as adjunct therapies for bacterial infections and skin diseases, demonstrating strong antibacterial activity against various pathogens. [2]. Although the *C. gigantea* has been employed in treating wounds and ulcers for long, more research would be needed to ascertain the safety and efficiency of the plant in modern medical applications. There is always a possibility of bacterial infections in humans, and that is why timely medical intervention must be sought to counter the risks that particular pathogens present [3]. *Cutibacterium acnes* previously known as *Propionibacterium acnes* is gram-positive bacterium that is resident on human skin, and a primary agent for causing inflammation and follicular plug formation that contribute to development of acne vulgaris. As much as the bacteria is harmless to the skin when consumed in moderation it becomes an aggressor to acne and has adverse effects to mental and skin health. A halophilic, Gram-negative bacterium separated in marine and estuarine surroundings, *V. vulnificus* is highly pathogenic to immuno-compromised individuals or the ones with chronic hepatic disease [4]. Superficial skin infections, septicaemia which is a fatal ailment, gastro-enteritis, and infections arising from consumption of raw or undercooked seafood are frequent [5]. Another Gram-negative bacterium that is freshwater and estuarine environments is *Aeromonas hydrophila* subsp. *hydrophila*. It is linked with wound infections in patients with underlying health complications as well as impacts on aquatic systems [6]. E. coli for short is a Gram-negative facultative anaerobic bacterium that is naturally prevalent in warm-blooded animals and humans including in the gastrointestinal tracts [7]. It may result in severe bacteremia, including meningitis in neonates, gastroenteritis, and acute renal multifocal chronic histological lesions, acute renal multifoil histological lesions, recurrent acute renal multifoil histological lesions, urinary tract infection, and multiple acute hematogenic foci. Large devastating effects on the public health may be observed in case the pathogenic *E. coli* strains get into food and water sources. The primary aim of this study is to evaluate the ability of *C. gigantea* extracts to counter the regarded significant pathogens. The flowers and leaves are sun dried and oven dried and extracts of methanol and ethanol are being used for this research. Some of its aims are to determine the safety and toxicity of the compounds, to compare their efficacy, to identify the MIC and to study the mechanism of action. In addition, the study will identify the active compounds, which are responsible for antibacterial activity, provide important insights for potential clinical applications.

Materials and methods

Collection of plant material

The plant Calotropis gigantea was collected from the Vrikha vihan Pvt Ltd, Bangalore, India. The plant material collected has been identified and authenticated as the leaves and flowers of C. gigantea, which is part of the Asclepiadaceae family (Figure 1).

Figure:1 collected plant material of *Calotropis gigantean*.

The plant exhibits distinct characteristics, including large sessile, obovate leaves and the presence of latex. The flowers are purple with white, and the flower buds are angled. Collection took place in the morning or late afternoon to minimize plant damage and preserve active compounds. Clean, sharp pruning shears were used to cut the plant material, taking only a small portion from each plant. The collected material was washed to remove dirt, then dried by air-drying in a shaded area or using a low-temperature oven.

Preparation of C. gigantea extract

Six different *C. gigantea* extracts were made for this study in order to assess their antimicrobial qualities based on modified protocol [2]. The extracts included in the study were methanol and ethanol extracts from sun-dried leaves, oven-dried leaves, and sun-dried flowers. By analyzing extracts from the plant's leaves and flowers and contrasting the results of sun-versus oven-drying, the research seeks to identify the best conditions for isolating bioactive compounds and evaluates their possible effectiveness against different bacterial pathogens.

Pathogens collection

P. acnes (MTCC 1951), *V. vulnificus* (MTCC 1145), *A. hydrophila* subsp. *hydrophila* (MTCC 1739) and *E. coli* (MTCC 1687) samples were purchased from Institute of Microbial Technology (IMTECH), Chandigarh and stored at 4 ºC. Sample was inoculated in Nutrient broth for overnight aseptically by following the instruction manual. The overnight cultures were reinoculated for 4h for the further use.

Antimicrobial activity assessment

For antimicrobial activity assessment, Disc diffusion method was used based on modified method [8]. The method consists of placing paper disks saturated with antimicrobial agents on a lawn of bacteria seeded on the surface of an agar medium, incubating the plate overnight, and measuring the presence or absence of a zone of inhibition around the disks. Sterilized paper discs were soaked with 20µl of extracts from the stock of 100µg/ml. 100µl of the fresh culture of *P. acne, V. vulnificus, A. hydrophila* subsp.

hydrophila, and *E. coli* were spread on muller hinton agar plate followed by placing of treated discs on it. Plates were kept at 4ºc for 20mins then incubated at 37ºc for overnight.

Plant extract preparation for phytochemical analysis

Extracts of plant material was done based on the modified protocol [9]. Plant Samples such as leaves and flowers were cleaned properly with distilled water and then surface sterilized with 70% alcohol followed by drying of the samples. One part of the leaf samples and flowers were dried under sun and rest were oven dried. After complete drying, the samples were crushed with the help of motor and pestle. The crushed samples were soaked in methanol and ethanol and incubated at room temperature for 72h. Post incubation, the samples were transferred to succinate apparatus for extract preparation. The extracts were stored at 4°c for further uses.

Phytochemical analysis

First screening of extracts for phytochemicals was done using the modified [10] protocol with slight modification. Briefly, Test for Saponins: A part of the extract was taken in test tube and an attempt was made to mix it and shake it vigorously. The ability of the highest recorded formation of stable foam was used as an index of the presence of saponins. In this test we employed the fact that biles which are saponins and surfactants form foams when stirred. Test for terpenoids: To achieve this, the extract was mixed with 2 mL of chloroform in a test tube. To this mixture 2 mL of concentrated sulfuric acid was added cautiously. The test tube containing the reactions mixture was then shaken gently. In addition, a positive result in the presence of terpenoids was noticed as overtime the colour changed from orange to reddish-brown at the contact interface between the two layers. Test for Phenols and Tannins: Acidification of the plant extract was done by adding 2 mL of 2 % ferric chloride (FeCl3) solution to it. Phenols and tannins were taken as indicators and recorded a blue-green or black coloration when the water content was reduced. The validity of this test was based on the fact that phenolic compounds can react with iron salts to form coloured products. Test for Flavonoids: In a test tube a 1 mL stock solution of the plant extract was taken and few drops of diluted NaOH was also added. The solution became a bright yellow color and this was an indication that flavonoids were present in the extract. The color turned non-yellow on the addition of few drops of dilute acid again proved the presence of flavonoids. Test for Sterols: To 1 mL aliquot of the plant extract, few droplet of ethanol was added. After this, while tilting the test tube, 1 mL of the concentrated sulfuric acid was disposed along the walls of the test tube. Any samples showing a violet-green hue was considered to have a positive reaction with sterols because this color change was as a result of the reaction between sterols and sulfuric acid. Test for Quinines: Presence of quinines was checked by adding some drops of the above plant extract into 2ml of concentrated HCl. A precipitate of the yellow colour was formed which further indicated the formation of quinines. Test for Cardiac Glycosides: In the reaction vial, five mL of the plant extract was taken and to it, 2 mL of glacial acetic acid and a drop of ferric chloride solution were also added. The formation of brown ring at the interface was considered as an indication of a positive result. The presence of a violet ring below the brown ring and the green colouration of the acetic acid layer were additional signs of cardiac glycosides. Test for Proteins: Light brown fluid was obtained after some amount of nitric acid was dripped into the plant extract. A solid yellow colouration meant positive results for proteins since the colour reaction denotes structures of proteins.

GC-MS analysis of phytochemicals

The GC-MS analysis of phytochemicals from the ethanol extract of *C. gigantea* followed a standardized protocol [11]. Fresh leaves were collected, air-dried at room temperature, and ground into a fine powder. A measured amount of the powdered plant material was subjected to Soxhlet extraction using 95% ethanol for 6-8 hours. After extraction, the ethanol was evaporated under reduced pressure using a rotary evaporator, leaving behind a concentrated extract. For GC-MS analysis, the extract was dissolved in hexane to achieve a suitable concentration. A 1-2 µL sample was injected into a GC-MS system (Agilent 7890A GC). Chromatographic separation was performed using a DB-5 capillary column (30 m × 0.25 mm × 0.25 µm). The initial column temperature was set to 60°C for 2 minutes, then increased to 280°C at 10°C per minute. The injector temperature was maintained at 250°C, with helium as the carrier gas at 1.0 mL/min. The mass spectrometer operated in electron impact (EI) mode at 70 eV, with the ion source set to 230°C. The mass spectra were analyzed by comparing them with the NIST database, allowing for the identification of various bioactive compounds present in the ethanol extract of C. gigantea.

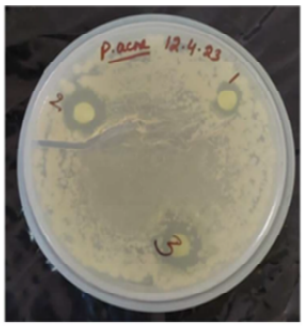
Results

Antimicrobial activity

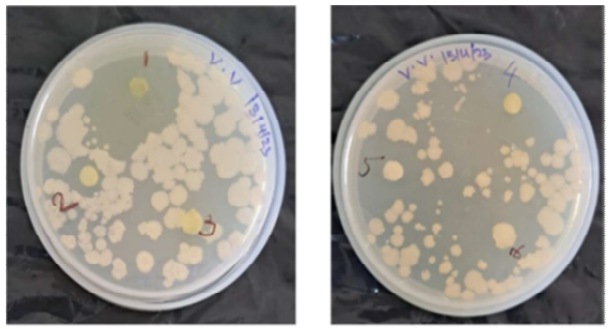
The reinforcing observation was based on the comparison of antimicrobial activity of different types of extracts, different parts of plants and different methods of drying the plants. The sun-dried leaf extracts displayed fair growth inhibition of P. acnes of 4 mm and very good inhibition of *V. Vulnificus* and *E. coli* of 20 mm each. They also exhibited fair zone of inhibition against *A. hydrophila* which was 15mm. On the other hand, ethanol extracts obtained from sun-dried leaves were relatively inactive; the extract did not show any zones of inhibition of *V. vulnificus* and a minimal zone of inhibition (3 mm) against *A. hydrophila* only (Figure 2). The oven-dried leaves' methanol extracts were most effective, particularly against E. coli (0.31 mm), they were however, inactive against *V. vulnificus* (kills 0 mm). The oven-dried leaves ethanol extract was rather broad-spectrum, completely preventing all the tested bacteria such as *A. hydrophila* (21mm) and Escherichia coli (20mm). Other antimicrobial properties from sun-dried flowers including the methanol and ethanol extracts also had a good antimicrobial activity especially on the two organisms *P. acnes* and *V. vulnificus*. But still the percentage inhibition of the ethanol extract of sun-dried flowers was comparatively lesser in case of *A. hydrophila* and E. coli than that of the methanol extract (Figure 3). In general, all the extracts from oven dried leaves were seen to possess better antimicrobial activity but more particularly those prepared using methanol or ethanol.

Phytochemical constituents

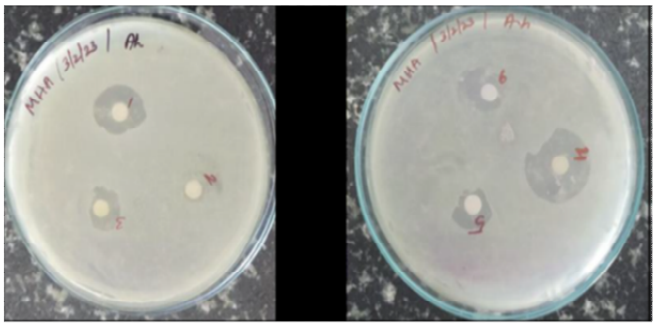
Biochemical tests on the extracts show information on the phytochemical content of the plant and any possible antimicrobial effects. In none of the extracts studied did these plants contain saponins, quinones, cardiac glycosides and anthraquinones. It is interesting to note here that terpenoids was present only in certain extracts and these were the ethanol extracts of the sun-dried leaves and the methanol extracts of the sun-dried flowers of the plant studied here that were active against microbes (Table 1). Total



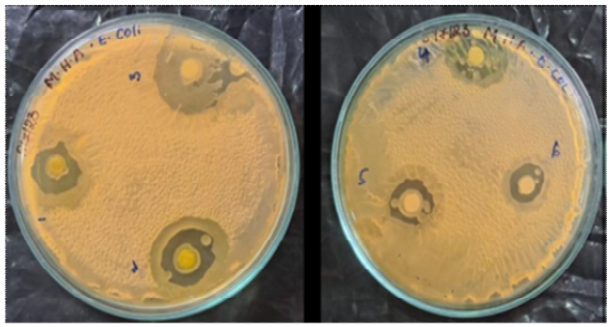
Antimicrobial activity of extracts (1-6) against *Propionibacterium acnes(MTCC 1951)*



Antimicrobial activity of extracts (1-6) against *Vibrio vulnificus (MTCC 1951)*



Antimicrobial activity of extracts (1-6) against *Aeromonas hydrophila*



Antimicrobial activity of extracts (1-6) against *E.coli*

Figure 2: Zone of inhibition formed on the MH Agar of extracts of *C. gigantea* against*Propionibacterium acnes*, *Vibrio vulnificus*, *Aeromonas hydrophila* subsp. *hydrophila* and *Escherichia coli*

Biochemical Analysis	Ethanol Extract			Methanol Extract		
	Leaves		Flowers	Leaves		Flowers
	Sundried	Oven dried		Sundried	Oven dried	
Saponin	-	-	-	-	-	-
Terpenoids	+	-	+	+	-	+
Sterol	+	+	+	+	+	+
Flavanoids	-	-	+	-	-	+
Quinines	-	-	-	-	-	-
Cardiacglycosides	-	-	-	-	-	-
Anthraquinines	-	-	-	-	-	-
Phenols	+	+	+	+	+	+
Protein	-	-	+	-	-	+

Table 1: Phytochemical constituents of various parts of *C. gigantea* with various solvent extraction methods.

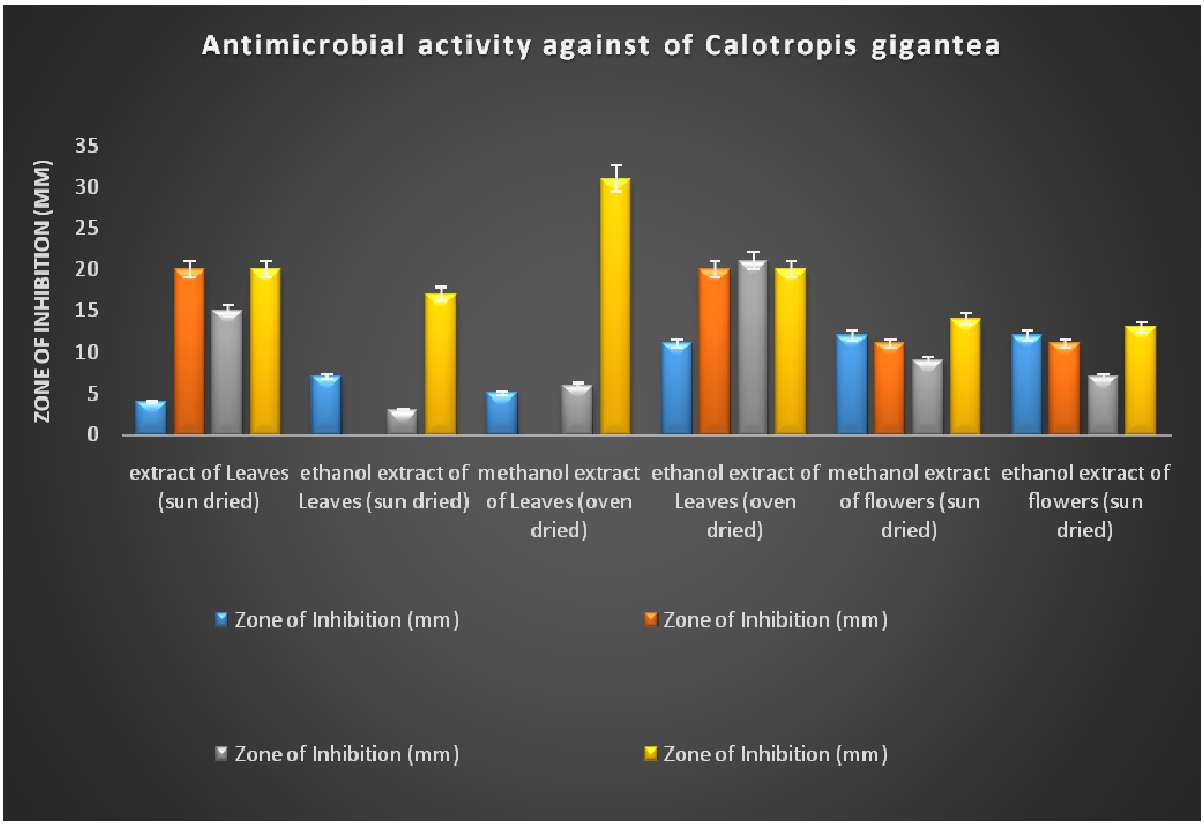


Figure 3. Antimicrobial activity of *C. gigantea* against four human pathogens such *P. acnes*, V. *vulnificus, A.hydrophila* subsp. *hydrophila* and *E. coli.*

sterols were noted in all the extracts and might be attributed to the plant's overall antimicrobial activity. Flavonoids were found only in the methanol extract of sun-dried flower maybe due to improvement of anticandidal activity. Phenols were present in high quantities in all extracts making their antimicrobial potential highly general (Figure 4). The proteins were detected only in methanol extract from sun-dried leaves meaning that their effects on antimicrobial activity are conditioned. The extent to which the extracts are effective in neutralizing bacteria like *P.acnes, V.vulnificus, A. hydrophila* and *E. coli* likewise seems to depend on secondary metabolites like terpenoids and flavonoids as constituents of specific phytochemicals could probably provide more selective attacks on certain pathogens.

GCMS analysis

The GC-MS analysis of the ethanol extract from the C. gigantea plant identified a total of 20 distinct compounds (Figure 5), revealing a diverse profile that may contribute to its traditional medicinal uses. The predominant compound was Diethyl Phthalate (Peak 3), which accounted for a remarkable 61.11% of the total area, indicating its significant presence and potential bioactivity. Other notable compounds included. alpha. -Tocopherol-. beta. -D-mannoside (Peak 20) and 9,12,15-Octadecatrienoic acid (Peak 14), contributing 5.73% and 4.13%, respectively. The extract contained a variety of compounds across different classes, including phenolic, fatty acids, and alcohols, which suggested a range of therapeutic properties. For instance, compounds like 2-Methoxy-4-vinylphenol and n-

Hexadecanoic acid could play roles in antioxidant or anti-inflammatory activities. Several compounds were found in lower concentrations, such as Tridecanal (Peak 5) and Lidocaine (Peak 9), which may have

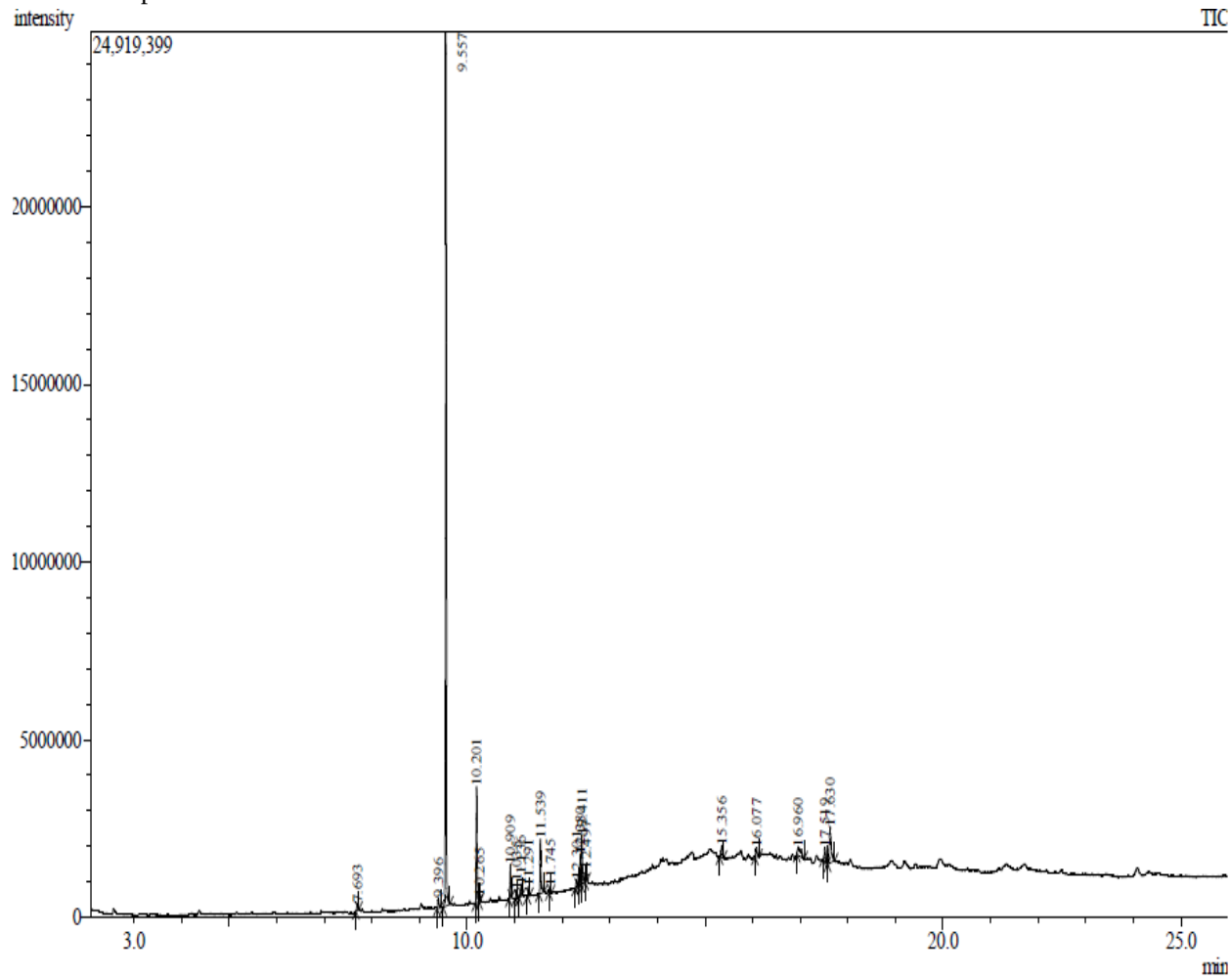


Figure 5: GCMS analysis of phytochemicals present in *C. gigantea*

specific bioactivities or synergistic effects. The results were consistent with previous studies on C. gigantea, which also highlighted the presence of similar bioactive compounds.

Discussion

The results of this study confirm the antimicrobial potential of C. gigantea extracts against significant human pathogens, providing insights into its traditional medicinal applications and the mechanisms behind its bioactivity. The plant's leaves and flowers, when subjected to different drying methods (sun-drying and oven-drying) and extracted using methanol and ethanol, exhibited varying levels of antimicrobial activity, consistent with findings from previous studies on other parts of the plant [2,12]. The *C. gigantea* extracts demonstrated strong antibacterial activity, especially against *V.vulnificus* and *E. coli*, which were among the most sensitive pathogens in this study. Sun-dried leaf extracts exhibited good inhibition against *E. coli* (20 mm) and *V. vulnificus* (20 mm), highlighting their potential in treating gastrointestinal and skin infections caused by these bacteria. The effectiveness of methanol and

ethanol extracts from oven-dried leaves was also evident, with the ethanol extract showing broad-spectrum activity, especially against *A. hydrophila* and *E. coli*. These results are in agreement with previous findings [2], who reported that *C. gigantea* extracts have antimicrobial properties, with its leaves and flowers showing activity against a range of bacterial pathogens. Notably, ethanol extracts from sun-dried leaves were found to be less active, which could be attributed to the loss of some volatile or heat-sensitive bioactive compounds during the drying process [3,13].

Phytochemical analysis further supported the antimicrobial findings, revealing the presence of key bioactive compounds such as terpenoids, flavonoids, phenols, and sterols, which are well-documented for their antimicrobial and antioxidant properties. Terpenoids were found only in specific extracts, particularly the ethanol extracts of sun-dried leaves and methanol extracts of sun-dried flowers. This suggests that certain compounds may be more effectively extracted under specific drying conditions, which is important for optimizing extraction protocols in future studies [14]. Terpenoids are known to exert antimicrobial effects by disrupting microbial membranes, which could explain the observed inhibition of bacteria such as *P. acnes*, *V. vulnificus,* and *A. hydrophila*. The presence of phenolic compounds in all extracts further reinforces the antimicrobial potential of the plant, as phenols are known to possess strong antibacterial and antifungal activity by interacting with bacterial cell walls and proteins [4,15]. Interestingly, while flavonoids were present only in the methanol extract of sun-dried flowers, they may have contributed to the activity observed against *P. acnes*, a pathogen responsible for acne vulgaris [16]. Previous research has highlighted the role of flavonoids in modulating inflammatory pathways and exhibiting antimicrobial effects, particularly in skin infections [5,17]. The presence of proteins in the methanol extract of sun-dried leaves might also explain some of the observed antibacterial effects, as proteins can play a role in the immune response by neutralizing microbial toxins [6,12].

The GC-MS analysis of the ethanol extract revealed a diverse array of bioactive compounds, further supporting the antimicrobial activity observed in this study. Diethyl phthalate was the predominant compound identified, comprising 61.11% of the total extract, which could be responsible for some of the observed biological effects. Other compounds such as alpha-tocopherol and 9,12,15-octadecatrienoic acid have known antioxidant and anti-inflammatory properties, which could complement the antimicrobial effects by reducing oxidative stress in infected tissues [7]. The presence of compounds like 2-methoxy-4-vinylphenol and n-hexadecanoic acid also suggests potential anti-inflammatory and antimicrobial properties, which align with the traditional use of *C. gigantea* in treating inflammatory conditions and skin diseases [11]. The findings from this study underscore the importance of both the choice of plant material and the extraction method in determining the antimicrobial efficacy of plant extracts. Sun-drying and oven-drying methods appeared to impact the levels and types of bioactive compounds extracted, with oven-dried leaves yielding the most potent antimicrobial activity [18]. This highlights the need for standardized protocols in the preparation of plant extracts to maximize their therapeutic potential. Moreover, while the *C. gigantea* extracts demonstrated promising antimicrobial activity, further studies are necessary to evaluate the safety, toxicity, and clinical efficacy of these extracts in human subjects, particularly considering the potential side effects or allergic reactions that may arise from the use of plant-based compounds [4,19]. In conclusion, the antimicrobial potential of *C. gigantea* extracts, particularly those derived from oven-dried leaves and sun-dried flowers, offers promising avenues

for the development of natural antibacterial agents. The presence of bioactive compounds such as terpenoids, flavonoids, and phenols further supports the plant's therapeutic applications. Future research should focus on isolating and characterizing individual bioactive compounds, optimizing extraction techniques, and conducting in vivo studies to better understand the plant's clinical applications in treating skin and systemic infections caused by pathogenic bacteria.

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Conflict of Interest:- None to declare

References

1. Sharma, R., Thakur, G. S., Sanodiya, B. S., Savita, A., Pandey, M., Sharma, A., & Bisen, P. S. (2012). Therapeutic potential of Calotropis procera: A giant milkweed. ISOR J Pharm Bio Sci, 4(2), 42-57.
2. Sharma, M., Tandon, S., Nayak, U. A., Kappadi, D., Rathore, A. S., & Goyal, A. (2017). Calotropis gigantea extract as a potential anticariogenic agents against Streptococcus mutans: An in vivo comparative evaluation. Journal of conservative dentistry : JCD, 20(3), 174–179. https://doi.org/10.4103/JCD.JCD_13_16
3. McLaughlin, J., Watterson, S., Layton, A. M., Bjourson, A. J., Barnard, E., & McDowell, A. (2019). Propionibacterium acnes and Acne Vulgaris: New Insights from the Integration of Population Genetic, Multi-Omic, Biochemical and Host-Microbe Studies. Microorganisms, 7(5),128. https://doi.org/10.3390/microorganisms7050128
4. Yun, N. R., & Kim, D. M. (2018). Vibrio vulnificus infection: a persistent threat to public health. The Korean journal of internal medicine, 33(6), 1070–1078. https://doi.org/10.3904/kjim.2018.159
5. Baker-Austin, C., Oliver, J.D., Alam, M. et al. Vibrio spp. infections. Nat Rev Dis Primers 4, 1–19 (2018). https://doi.org/10.1038/s41572-018-0005-8
6. Semwal, A., Kumar, A., & Kumar, N. (2023). A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. Heliyon, 9(3).
7. Pokharel, P., Dhakal, S., & Dozois, C. M. (2023). The Diversity of Escherichia coli Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. Microorganisms, 11(2), 344. https://doi.org/10.3390/microorganisms11020344
8. Choyam, S., Lokesh, D., Bheemakere Kempaiah, B., and Kammara R (2015) Assessing the antimicrobial activities of Ocins. Front. Microbiol. 6:1034. doi: 10.3389/fmicb.2015.01034
9. Abubakar, A. R., & Haque, M. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of pharmacy & bioallied sciences, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19.
10. Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica, 14(4), 350–356.

https://doi.org/10.26574/maedica.2019.14.4.350

11. Dhivya, R., & Manimegalai, K. (2013). Preliminary phytochemical screening and gc-ms profiling of ethanolic flower extract of Calotropis gigantea Linn.(Apocyanaceae). Journal of Pharmacognosy and Phytochemistry, 2(3), 28-32.
12. Kang, C. G., Hah, D. S., Kim, C. H., Kim, Y. H., Kim, E., & Kim, J. S. (2011). Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicological research, 27, 31-36.
13. Amini, M. H., Ashraf, K., Salim, F., Lim, S. M., Ramasamy, K., Manshoor, N., ... & Ahmad, W. (2021). Important insights from the antimicrobial activity of Calotropis procera. Arabian Journal of Chemistry, 14(7), 103181.
14. González-Hernández, R. A., Valdez-Cruz, N. A., & Trujillo-Roldán, M. A. (2024). Factors that influence the extraction methods of terpenes from natural sources. Chemical Papers, 78(5), 2783-2810.
15. Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants, 9(2), 165.
16. Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., ... &Jaremko, M. (2020). Important flavonoids and their role as a therapeutic agent. Molecules, 25(22), 5243.
17. Hasnat, H., Shompa, S. A., Islam, M. M., Alam, S., Richi, F. T., Emon, N. U., ... & Ahmed, F. (2024). Flavonoids: A treasure house of prospective pharmacological potentials. Heliyon.
18. Yuan, J., Hao, L. J., Wu, G., Wang, S., Duan, J. A., Xie, G. Y., & Qin, M. J. (2015). Effects of drying methods on the phytochemicals contents and antioxidant properties of chrysanthemum flower heads harvested at two developmental stages. Journal of Functional Foods, 19, 786-795.
19. Saddiq, A. A., Tag, H. M., Doleib, N. M., Salman, A. S., & Hagagy, N. (2022). Antimicrobial, antigenotoxicity, and characterization of calotropis procera and its rhizosphere-inhabiting actinobacteria: In vitro and in vivo studies. Molecules, 27(10), 3123.