Anti-Microbial Study of Centella Asiatica Extract Against Pathogenic Bacteria

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***Abstract*—Gotu kola, or *Centella asiatica*, is a traditional medicinal herb that has strong anti-bacterial properties among other pharmacological benefits. Assessing the anti-bacterial activity of the *C. asiatica* leaf extract against a variety of pathogenic microorganisms, including *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, was the aim of this investigation. *Centella asiatica* leaves were macerated in a 90% hydroalcoholic solution for the leaf extraction procedure. The extract was then subjected to a phytochemical examination, and the agar well diffusion method was used to assess the extract's anti-bacterial efficacy. Proteins, triterpenoids, and flavonoids were among the bioactive substances found throughout the screening. For every pathogen examined, the extract showed notable inhibitory zones; the effects varied according to the extract concentration. In contrast to the common antibiotic chloramphenicol, *Centella asiatica* exhibited broad-spectrum anti-bacterial efficacy against B. subtilis and S. aureus. The study's findings supported the traditional use of *Centella asiatica* and opened up new possibilities for therapeutic research by indicating that it would be worthwhile to investigate for possible application as a natural anti-bacterial agent.**

***Index Terms*—*Centella asiatica*, Anti-microbial activity, Pathogenic bacteria, Phytochemicals, Agar well diffusion, Herbal medicine**

1. INDRODUCTION

1.1 Background on Anti-microbial Resistance (AMR)

1.1.1 Rising global concern about anti-microbial resistance

Anti-microbial resistance (AMR) has emerged as a critical global public health threat, with potentially devastating consequences if left unchecked. The World Health Organization's 2014 Global report on AMR surveillance highlighted the extent of this phenomenon worldwide, revealing significant gaps in existing monitoring systems (1). By 2050, AMR could cause up to 10 million deaths annually, significantly increasing mortality rates, treatment failures, and healthcare costs (2). Interestingly, while the COVID-19 pandemic has disrupted research, innovation, and anti-microbial stewardship programs, particularly in low- and middle-income countries, it may have also contributed to decreasing AMR through measures such as lockdowns, social distancing, and improved hygiene practices (3). However, the long-term impact of the pandemic on AMR remains uncertain. In conclusion, addressing AMR requires a multifaceted approach involving various stakeholders. Anti-microbial stewardship programs, led by pharmacists and other healthcare professionals, play a crucial role in reducing inappropriate antibiotic use and improving clinical outcomes (2); (4). Additionally, adopting a 'one-health' strategy that integrates efforts across human, animal, and environmental domains is essential for effective mitigation (5).

1.1.2 Overuse/misuse of antibiotics in medicine and agriculture

The overuse and misuse of antibiotics in both medicine and agriculture have significantly contributed to the global anti-microbial resistance crisis, posing a severe threat to public health (5). This widespread use has led to the prevalence of antibiotics and antibiotic resistance genes (ARGs) in various environments, including agricultural soils, water resources, and food production systems (6) (7) (5).

Interestingly, while antibiotics have revolutionized modern medicine, their excessive use in human activities such as healthcare, food production, and agriculture has resulted in unintended consequences (6) (8). In some regions, like Latin America, the situation is more complex due to the lack of resources, systematic studies, and legislation to control anti-microbial use (6). Additionally, the agricultural sector's large-scale use of antibiotics produces environmental exposures in various reservoirs, selecting for resistant microbes and microbial genes (9). To address this global health threat, many countries have implemented restrictions on antibiotic use as growth promoters and promoted the development of alternatives in human and veterinary medicine and animal farming (10); (11). These alternatives include acidifiers, bacteriophages, enzymes, phytochemicals, probiotics, prebiotics, and anti-microbial peptides (11). The solution to antibiotic resistance will require comprehensive efforts from policymakers in agriculture and the development of alternative therapeutics by experts in diverse fields (12). Understanding the link between human health, animal health, plant health, and the environment is crucial for implementing new regulations and practices aimed at mitigating the spread of antibiotic resistance (8); (7).

1.1.3 Need for alternative therapies, especially from natural sources

The need for alternative therapies, especially from natural sources, has become increasingly apparent in recent years. Complementary and alternative medicine (CAM) encompasses a wide range of practices and therapies outside conventional western medicine, including traditional Chinese medicine, herbal remedies, acupuncture, and mind-body techniques (13); (14). These approaches have gained popularity due to their potential to address complex chronic diseases and fill gaps where conventional treatments may fall short (15). Interestingly, while many natural therapies have been used for centuries, scientific evidence supporting their efficacy and safety is often limited (13); (15). This contradiction highlights the need for further research to validate traditional practices. For instance, some herbal remedies have shown promise in treating conditions like depression, osteoarthritis, and viral respiratory infections, but more rigorous clinical trials are required to establish their effectiveness (16); (17); (18). In conclusion, the growing interest in alternative therapies stems from their potential to complement conventional treatments, address chronic conditions, and offer more holistic approaches to health. However, it is crucial to integrate these therapies responsibly, ensuring their safety and efficacy through scientific research and appropriate regulation (13); (14). As the medical community continues to explore these alternatives, there is a clear need for more comprehensive studies to bridge the gap between traditional wisdom and modern evidence-based medicine.

1.2 Importance of Medicinal Plants in Anti-microbial Research

1.2.1 Historical and ethnomedicinal use of plants to treat infections

Ethnomedicinal studies have documented the widespread use of plants to treat various infections across different cultures and regions. In Pakistan, 52 medicinal plants were reported for treating gastrointestinal infections, with fruits, whole plants, and leaves being the most commonly used parts (19). The Apiaceae family was dominant, and plants like Withania coagulans showed high fidelity levels for treating such ailments. Interestingly, some cultures utilize poisonous plants in their traditional medicine systems. For instance, Tibetan ethnomedicine employs toxic plants like Aconitum pendulum and Strychnos nux-vomica to treat various disorders after proper processing (20). This highlights the complex relationship between toxicity and medicinal properties in traditional healing practices. In conclusion, the historical use of plants for treating infections is deeply rooted in many cultures, with a wide variety of species being utilized. From gastrointestinal ailments in Pakistan to gynecological issues and STIs across different regions (21), plants have played a crucial role in traditional healthcare systems. However, there is a growing concern about the loss of this traditional knowledge, especially among younger generations (22) Further research into the pharmacological properties and safety of these plants is essential to validate their traditional uses and potentially develop new treatments.

1.2.2 Bioactive compounds in plants often serve as a basis for drug discovery

Bioactive compounds derived from plants have long been recognized as valuable sources for drug discovery and development. These compounds, primarily secondary metabolites, have been used to treat various ailments and have led to the creation of numerous medicines, dietary supplements, and commercial products (23). The rich diversity of plant-derived bioactive compounds offers excellent prospects for discovering novel drugs with wide-ranging applications, including antibiotics, agrochemicals, flavors, fragrances, and food preservatives (24) Interestingly, recent research has shown that endophytic fungi associated with medicinal plants can produce similar bioactive compounds as their host plants, potentially offering a more sustainable source for these valuable substances (25); (26). This discovery has opened up new avenues for bio-prospecting and drug development. Additionally, the use of chemical elicitors on hydroponically grown plants has been shown to selectively and reproducibly induce the production of bioactive compounds, dramatically improving the efficiency and reliability of plant extracts in drug discovery while preserving wild species and their habitats (27). In conclusion, while plant-derived bioactive compounds continue to play a crucial role in drug discovery, challenges such as low solubility, instability, and limited availability of source materials persist (28); (29). To address these issues, researchers are exploring innovative approaches, including the use of nanotechnology to enhance bioavailability and stability, as well as the development of more efficient screening and isolation techniques (30); (28). These advancements, combined with the vast potential of plant-derived compounds, underscore the ongoing importance of natural products in modern drug discovery efforts.

1.2.3 Advantages of plant-based anti-microbials: availability, affordability, biocompatibility, lower side effects

Plant-based anti-microbials offer numerous advantages, including wide availability, affordability, high biocompatibility, and lower side effects compared to synthetic alternatives. These natural compounds are gaining popularity in various fields due to their beneficial properties and ease of accessibility (31). The abundance of plant-derived anti-microbials makes them a cost-effective option for large-scale production and use in different applications (32); (33). Interestingly, plant-based anti-microbials demonstrate high biocompatibility and low immunogenicity, making them suitable for use in medical and food applications (33); (34). They also exhibit enhanced anti-microbial activity, antioxidant properties, and anti-inflammatory effects, which contribute to their effectiveness in treating various conditions (33); (31).

Moreover, these natural compounds often have fewer side effects compared to synthetic alternatives, making them a safer option for long-term use (32); (31). In conclusion, plant-based anti-microbials present a promising alternative to synthetic compounds due to their wide availability, affordability, high biocompatibility, and reduced side effects. These advantages make them particularly attractive for use in fields such as medicine, food preservation, and agriculture (34); (35); (36). However, further research is needed to address challenges such as standardization, optimization, and regulatory aspects to fully harness the potential of these natural anti-microbials (35).

1.3 Introduction to *Centella asiatica*

1.3.1 Botanical classification and common names (e.g., Gotu kola)

Centella asiatica (L.) Urban, commonly known as Gotu kola, is a medicinal plant with a rich history in traditional medicine systems (37); (38). It is also referred to as Asiatic pennywort in some literature (Orhan, 2012). The plant belongs to the Apiaceae family and is native to Southeast Asia, where it has been used for centuries in Ayurvedic and traditional Chinese medicine (39)., (38). Interestingly, while most papers consistently use the scientific name *Centella asiatica*, there are slight variations in the author citations. For instance, some papers use *Centella asiatica* (L.) Urban, while others simply use *Centella asiatica* (L.) or just *Centella asiatica* (37); (40); (38). This highlights the importance of standardized nomenclature in scientific literature. In summary, *Centella asiatica* is the accepted scientific name for this plant, with Gotu kola being its most widely recognized common name across various studies. The consistent use of both scientific and common names in research papers facilitates clear communication and helps avoid confusion when discussing this important medicinal plant.

Figure 1: Centella asiatica leaf (41)

1.3.2 Geographic distribution and availability

Geographic distribution and availability of species are influenced by various factors, including environmental conditions, resource availability, and dispersal mechanisms. Studies have shown that both dispersal and niche processes affect species distribution patterns (42). For instance, in tropical montane forests, understorey palm communities are influenced by soil chemical and physical properties, climate, and geographic distance (42). Interestingly, the distribution of resources and species can exhibit spatial heterogeneity even within seemingly uniform habitats. A study on bee communities in Mediterranean scrubland found clear geographical patterns at scales as low as 500- 1000 m, primarily driven by the distribution of floral resources (43). This challenges the assumption that mobile organisms like bees would have homogeneous distributions in uniform habitats. In conclusion, understanding species distribution requires consideration of multiple factors, including environmental variables, resource availability, and dispersal mechanisms. The relationship between habitat use and availability is often non-linear, and models that allow for such complexity perform better in predicting species distributions (44). Furthermore, aggregate community properties, such as total abundance and biomass, can serve as indicators of ecological conditions and environmental change over broad spatial and temporal scales (45).

1.3.3 Traditional medicinal uses: wound healing, anti-inflammatory, cognitive enhancement, etc

Wound healing is a complex process that has been addressed using traditional medicine for centuries. Many plants have demonstrated wound healing properties through mechanisms such as anti-inflammatory, antioxidant, and anti-microbial activities (46); (47). For instance, 65 herbs used in traditional Persian medicine were identified for their wound healing properties, with 40 of them showing at least one of the aforementioned activities (47). Plants like Salvia officinalis, Echium amoenum, and Aloe vera are among the most important medicinal plants used for wound healing in Iran (48). Interestingly, the concept of moist wound healing has been incorporated into traditional medicine practices to accelerate the healing process (46). Additionally, some plants have shown promise in combating multi-resistant organisms, addressing a significant challenge in modern wound care (46). The anti-inflammatory effects of medicinal plants have been extensively studied, with species like Curcuma longa, Zingiber officinale, and Rosmarinus officinalis demonstrating effectiveness in clinical and experimental studies (49). In conclusion, traditional medicinal plants offer a wealth of potential for wound healing and anti-inflammatory treatments. Their diverse mechanisms of action, including antioxidant, anti-microbial, and anti-inflammatory properties, make them valuable resources for developing new therapeutic strategies (50); (51). While the provided context does not specifically address cognitive enhancement, the wide range of applications for medicinal plants in traditional medicine suggests that further research may uncover benefits in this area as well.

1.4 Phytochemistry of *Centella asiatica*

1.4.1 Overview of bioactive constituents: Triterpenoids, Flavonoids, Protein and Amino acid

Bioactive constituents such as triterpenoids, flavonoids, proteins, and amino acids play crucial roles in various biological processes and offer significant health benefits. Triterpenoids and flavonoids are important phytochemicals found in many plant species. Flavonoids, classified into six different groups based on their chemical structures, exhibit remarkable antioxidant potential and various other activities (52). They have been used in traditional medicine for treating numerous ailments, demonstrating anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, anti-cancer, anti- hyperglycemic, and anti-oxidant properties (52). Triterpenoids, along with flavonoids, are present in plants like buckwheat and contribute to its high nutritional value and health benefits (53). Proteins and amino acids are essential biomolecules with diverse functions. There are 22 naturally occurring amino acids, with 20 proteinogenic amino acids serving as building blocks for proteins (54). Biologically active peptides derived from various sources, including casein, fish muscle, and plant protein hydrolysates, have been found to influence intestinal transit, modify nutrient absorption and excretion, and exhibit immunostimulant and anti-hypertensive activity (55). In conclusion, these bioactive constituents offer a wide range of health-promoting properties and have applications in various fields, including biomedicine, agriculture, and food industry. The abundant availability and diverse biological activities of these compounds make them attractive targets for research and development of new therapeutic agents and functional foods (52); (53); (52); (55).

1.4.2 Activities: anti-oxidant, anti-inflammatory, anti-microbial, neuroprotective. Known pharmacological

The reviewed papers highlight several compounds with anti-oxidant, anti-inflammatory, anti- microbial, and neuroprotective properties: exhibits Gastrodin neuroprotective, anti-inflammatory, and anti-oxidant effects, showing efficacy in protecting against neuronal damage and enhancing cognitive function in animal models of neurodegenerative diseases (56). Phycocyanin demonstrates anti-oxidant, anti-inflammatory, and neuroprotective properties, scavenging various free radicals and reducing inflammation markers in experimental models (57). Flavonoids showcase anti-oxidant and anti- microbial activities by neutralizing reactive oxygen species, disrupting bacterial cell membranes, and interfering with fungal growth (58). Interestingly, some compounds exhibit multiple pharmacological activities simultaneously. For instance, thymoquinone displays anti-oxidant, anti-inflammatory, neuroprotective, and anti-bacterial effects, primarily through modulation of the Nrf2 signaling pathway (59). Similarly, propolis demonstrates anti-oxidant, anti-inflammatory, and anti-microbial properties, with its ethyl acetate fraction inhibiting inflammatory markers in vitro and reducing edema in vivo (60). In conclusion, these studies reveal that many natural compounds and their derivatives possess multiple pharmacological activities, often acting through diverse mechanisms. This multifaceted approach to addressing various health conditions underscores the potential of these compounds in developing novel therapeutic strategies for complex diseases involving oxidative stress, inflammation, microbial infections, and neurodegeneration.

1.5 Existing Evidence on Anti-microbial Activity

1.5.1 Summary of previous studies showing anti-microbial potential of *Centella asiatica*

Centella asiatica has demonstrated significant anti-microbial potential in several studies. The plant's extracts have shown activity against various microorganisms, highlighting its potential for therapeutic applications. Research has revealed that *C. asiatica* possesses anti-microbial properties, which are attributed to its bioactive compounds, particularly phenolic compounds and triterpene saponins (61). The ethanolic extract of *C. asiatica* exhibited highly potent anti-bacterial activities against *S. aureus* and *B. cereus*, suggesting its potential use in cosmetic and pharmaceutical applications (62). Additionally, a study on a novel chitosan-based hydrogel containing *C. asiatica* extract demonstrated efficient microbiological activity, indicating its potential for wound healing applications (63). Interestingly, while most studies focus on the plant's anti-microbial effects, some research has explored its potential in other areas. For instance, *C. asiatica* extracts have shown anti-oxidant, anti- inflammatory, and anti-aging effects in cosmetic products (64). This multifaceted approach to studying *Centella asiatica's* properties suggests that its anti-microbial activity may be part of a broader spectrum of therapeutic benefits. In conclusion, the anti-microbial potential of *Centella asiatica* is well-supported by various studies, with evidence of its efficacy against different microorganisms. This property, combined with its other therapeutic effects, makes *Centella asiatica* a promising candidate for further research and development in pharmaceutical and cosmetic applications.

1.5.2 Bacteria that have been previously tested (both Gram-positive and Gram-negative)

Numerous Gram-positive and Gram-negative bacteria have been tested in various studies for their susceptibility to different anti-microbial agents: Gram-positive bacteria commonly tested include *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, and *Bacillus cereus* (65); (67); (65). Other Gram-positive species examined were *Corynebacterium diphtheriae*, *Streptococcus pneumoniae*, *Streptococcus oralis*, and *Streptococcus gordonii* (68); (69).

Gram-negative bacteria frequently tested include *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Salmonella typhimurium*), and Proteus species (65); (67); (66). Additional Gram-negative bacteria studied were *Klebsiella oxytoca*, *Enterobacter cloacae*, and Shigella species (68); (65). Interestingly, some studies found differences in the effectiveness of anti-microbial agents against Gram-positive and Gram-negative bacteria. For instance, nisin in combination with sucrose fatty acid esters showed enhanced activity against Gram-positive bacteria but not against Gram-negative bacteria (66). Similarly, secretory phospholipase A2 from human tears was effective against Gram-positive bacteria but lacked bactericidal activity against Gram-negative organisms in the ionic environment of tears (67). In conclusion, a wide range of both Gram-positive and Gram-negative bacteria have been tested for their susceptibility to various anti-microbial agents, with some studies revealing differential effectiveness based on the bacterial cell wall structure.

1.5.3 Limitations in existing literature (e.g., lack of standardization, limited spectrum testing)

Existing literature across various fields highlights significant limitations, particularly regarding standardization and methodological challenges: In transgender health research, the lack of standardization in lexicon, population definitions, study design, sampling, and measurement hinders evidence-based care and comparability between studies (70). Similarly, freshwater microplastic research faces challenges in methodological standardization, especially in characterization, quality assurance, and quality control procedures (71). Nanotoxicology encounters obstacles in establishing full characterization requirements, standardizing dosimetry, and evaluating kinetic rates of ionic dissolution (72). In mobile network research, limitations exist in managing the explosive increase in data volume and traffic problems (73). Wikipedia citations, suggested as a metric for research impact, face severe limitations due to lack of standardization and incompleteness of references, making them difficult to retrieve and use for research evaluation (74). In psychological research, standardization of scores is common but faces limitations in theoretical interpretation and use with multivariate statistics (75). Interestingly, while efforts are being made to standardize product carbon foot printing (PCF), important differences exist between standards, creating a paradox in securing future standardization (76). In medical imaging, ultrasound for hidradenitis suppurativa has limitations that necessitate additional consideration despite its advantages (77). In conclusion, the lack of standardization and limited spectrum testing are recurring themes across various fields. These limitations hinder comparability between studies, impede evidence-based practices, and create challenges in data interpretation and analysis. Addressing these issues through improved standardization efforts and expanded testing methodologies is crucial for advancing research in these areas.

1.6 Pathogenic Bacteria of Interest

1.6.1 Brief description of the specific pathogenic bacteria tested in your study (e.g., *E. coli*, *S. aureus*, *Bacillus subtilis, Candida albicans*)

The studies described in the provided papers tested a variety of pathogenic bacteria and fungi, with several species appearing consistently across multiple studies: *Escherichia coli* (*E. coli*), a Gram- negative bacterium, was tested in most of the studies (78), 2, 3, 5, 6, 7, 8, 9, 10). *E. coli* is a common cause of urinary tract infections and foodborne illnesses. *Staphylococcus aureus* (*S. aureus*), a Gram- positive

bacterium, was also frequently tested (78), 2, 3, 4, 5, 6, 8, 9). *S. aureus* is known for causing skin infections and can lead to more severe conditions like pneumonia or sepsis. *Bacillus subtilis*, another Gram-positive bacterium, was examined in several studies (79), 3, 5, 6, 7, 8, 9). While not typically pathogenic, *B. subtilis* is often used as a model organism in research. Interestingly, *Candida albicans*, a fungal species, was also commonly tested alongside bacterial pathogens (78), 2, 3, 4, 5, 6,
10). *C. albicans* is an opportunistic pathogen that can cause various fungal infections, particularly in immunocompromised individuals. Carlson and Johnson (1985) specifically highlighted the interaction between *C. albicans* and *S. aureus*, demonstrating how the fungus can protect the bacteria and enhance infection (80). In summary, these studies focused on a range of clinically relevant pathogens, including both Gram-positive and Gram-negative bacteria, as well as fungal species. This diverse selection allows for a comprehensive evaluation of anti-microbial agents against a broad spectrum of potential infectious agents.

1.6.2 Their relevance in clinical settings (e.g., hospital-acquired infections, antibiotic resistance patterns)

Hospital-acquired infections (HAIs) and anti-microbial resistance (AMR) are major global health challenges with significant clinical relevance. HAIs, also known as nosocomial infections, are acquired in healthcare settings and are primarily caused by bacteria such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and Methicillin-resistant *Staphylococcus aureus* (MRSA) (81). These infections are associated with high morbidity and mortality rates, prolonged hospital stays, and increased healthcare costs (82); (83). The prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens in clinical settings is alarmingly high. Studies have reported MDR rates of 40.59% and XDR rates of 24.75% among HAI pathogens (82). This increasing incidence of AMR poses a significant challenge in treating and preventing infections, particularly in surgical settings where antibiotic prophylaxis is routinely used to prevent surgical site infections (84). To address these challenges, healthcare institutions are implementing anti-microbial stewardship programs (ASP) and strengthening infection prevention and control (IPC) measures. These strategies include surveillance of antibiotic resistance patterns, prudent anti-microbial use, timely handwashing, aseptic techniques, and minimizing the use of invasive devices (83); (85). Additionally, novel approaches such as nanotechnology are being explored for more potent and sensitive methods of detecting and treating bacterial infections in clinical settings (81).

1.7 Objectives of the Study

1.7.1 Primary aim: To evaluate the anti-microbial effect of *Centella asiatica* extract against selected pathogenic bacteria

Centella asiatica extract has demonstrated significant anti-microbial activity against various pathogenic bacteria. Studies have shown that the dichloromethane: methanolic extract of *C. asiatica* exhibited anti-bacterial effects against human pathogens including *Salmonella typhi*, *Escherichia coli*, *Shigella sonnei*, *Bacillus subtilis*, and *Staphylococcus aureus* (86). The extract showed dose- and time- dependent anti-bacterial properties in time-kill kinetic studies. Interestingly, the ethanolic extract of
C. asiatica was found to be highly potent specifically against *S. aureus* and *B. cereus* (62). This extract contained phenolic compounds like sinapic acid, catechin, quercetin, and hesperidin, with hesperidin being the predominant phenolic compound. Additionally, cosmetic formulations containing *C. asiatica* extract demonstrated anti-microbial properties (64); (87). In conclusion, *C. asiatica* extract shows promising anti-microbial activity against several pathogenic bacteria, particularly gram- positive strains like *S. aureus*. The anti-bacterial effects are attributed to the presence of various bioactive compounds including phenolics, flavonoids, terpenoids, and saponins (86); (62). These findings suggest that *C. asiatica* extract could be a valuable source of natural anti-microbial agents for pharmaceutical and cosmetic applications. However, further research is needed to isolate specific active compounds and evaluate their individual anti-microbial efficacy.

1.7.2 Secondary objectives: Possibly compare effects on Gram-positive vs. Gram-negative bacteria, explore dose-response, identify mechanisms, etc.

Gram-negative and Gram-positive bacteria exhibit distinct responses to various anti-bacterial agents and immune mechanisms, primarily due to differences in their cell wall structures (88). Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, are generally more resistant to antibiotics than Gram-positive bacteria due to their outer membrane and active efflux pumps (89). However, the potency of antibiotics varies significantly among different Gram-negative pathogens, suggesting major mechanistic differences in how antibiotics penetrate permeability barriers (89). Interestingly, some compounds show selective anti-bacterial activity against Gram-negative bacteria. For instance, carbon dots derived from *Artemisia argyi* leaves exhibited 100% bactericidal efficiency against Gram-negative bacteria at 150 µg ml.-1, while having no significant effect on Gram-positive bacteria (90). This selectivity is attributed to the ability of these compounds to damage only the cell walls of Gram-negative bacteria and inhibit cell wall-related enzymes by altering their secondary structure (90). In conclusion, the differences in susceptibility between Gram-positive and Gram- negative bacteria to various anti-bacterial agents are primarily due to their distinct cell wall structures and permeability barriers. The outer membrane and efflux pumps in Gram-negative bacteria play a dominant role in their resistance, with the outer membrane being more critical in *E. coli* and *P. aeruginosa*, while efflux dominates in *A. baumannii* (89). These findings highlight the importance of considering both barriers when optimizing antibiotics for favorable outer membrane permeability, efflux evasion, or both (91).

2. EVALUATION AND ANALYSIS OF CENTELLA ASIATICA LEAF EXTRACTS

Centella asiatica leaf extracts have been extensively studied for their phytochemical composition and bioactive properties. The ethanolic extract of *C. asiatica* contains various phenolic compounds, including sinapic acid, catechin, quercetin, p-coumaric acid, hesperidin, eugenol, and hesperetin, with hesperetin being the predominant phenolic compound (62). Additionally, the leaves contain significant amounts of triterpenes, particularly madecassoside and asiaticoside (92). Interestingly, the extraction method and plant part used can significantly influence the composition and bioactivity of *C. asiatica* extracts. (93).

2.1 Materials & Methods

2.1.1 Study Area and Population:

The current research was conducted at Birbhum Pharmacy School, which is a part of Maulana Abul Kalam Azad University of Technology (MAKAUT), West Bengal. Dubrajpur is a city in Birbhum district, West Bengal, India. Dubrajpur is a place of cultural background and historical value. Geographically, it's situated roughly around latitude 23.8169° North to longitude 87.4201° East. The area is heavily networked by rail and road, with Dubrajpur railway station being one of the major transport stations. Based on the recent census statistics, the population of Birbhum district exceeds 3.5 million, with Bengali as the dominant language, followed by Hindi and Santali. The economy of the region is driven primarily by agriculture, small industries, and education.

2.1.2 Collection of *Centella asiatica*

Centella asiatica, also known as Gotu kola, is a medicinal plant with extensive therapeutic value, particularly in Southeast Asian countries and traditional Chinese medicine (5). The plant contains active compounds, primarily pentacyclic triterpenes, including asiaticoside, madecassoside, asiatic acid, and madecassic acid (37); (71). *C. asiatica* has been used for centuries to treat various conditions, with its most notable effects on neurological and skin diseases (Sun et al., 2020). It has shown efficacy in improving the treatment of small wounds, hypertrophic scars, burns, psoriasis, and scleroderma (37). The plant's mechanism of action involves promoting fibroblast proliferation, increasing collagen synthesis, and improving the tensile strength of newly formed skin (37); (94). Interestingly, *C. asiatica* extract has also demonstrated anti-bacterial activity and potential applications in food packaging to prevent food loss (92); (94).

2.1.3 Extraction

Extraction is a key step in phytochemical research, where bioactive constituents are separated from the plant tissue through selective solvents. The objective of the step is to extract the secondary metabolites responsible for the pharmacological activity observed.

2.1.4 Method of Extraction

Centella asiatica leaves can be extracted using various methods, each with its own advantages and effects on the yield and properties of bioactive compounds. Conventional methods like maceration and heat-air drying have been used, but modern techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical CO2 extraction are gaining popularity due to their efficiency and environmental friendliness (95); (96). For instance, MAE with 80% ethanol at 100 watts for 7.5 minutes yielded the highest triterpenoid content, while UAE with 80% ethanol at 48°C for 50 minutes also produced significant results (96). These green extraction methods consumed 54- 59% less energy compared to conventional maceration. Interestingly the choice of extraction method can significantly impact the bioactive compound yield and anti-oxidant activity. Freeze-drying the leaf parts before ethanol extraction resulted in higher anti-oxidant capacity, primarily influenced by triterpenoids like madecassoside and asiaticoside (93). Additionally, supercritical CO2 extraction with a cosolvent as a pretreatment step has been shown to solve color-fading problems in modern formulations while improving in vitro wound healing activity (97). In conclusion, while traditional methods are still used, advanced extraction techniques like MAE, UAE, and supercritical CO2 extraction offer improved efficiency, yield, and retention of bioactive compounds from *Centella asiatica* leaves. The choice of method should be based on the desired outcome, such as maximizing specific compounds or enhancing particular bioactivities.

2.2 Experimental Section:

2.2.1 Plant materials:

The selected plant *C. asiatica* leaves were collected from Dubrajpur city under Birbhum district in West Bengal, India (23.8169°, 87.4201°). Uprooted entire plants, leaf are separated and washed out in tap water, dried in the shade at room temperature for 7 days. After drying it is grinded into finely powdered form and stored.

Figure 2: *Centella asiatica* dried leaf powder

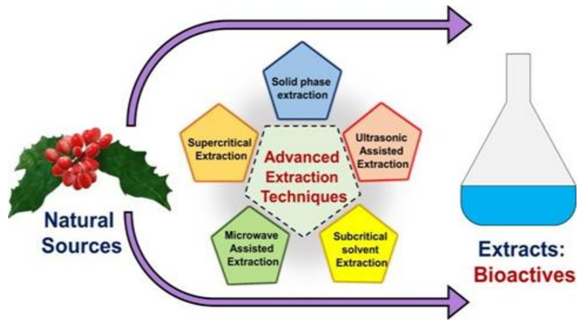


Figure 3: Extraction Process of *Centella asiatica* leafs (98)

2.2.2 Chemicals:

All chemicals were acquired from the BIRBHUM PHARMACY SCHOOL the chemicals are listed below Chloroform, Mayer's Reagent, Dragondroff's Reagent, Wagner's Reagent, Dilute Ammonia Solution, Hydrochloric, Sulfuric acid, Distilled water, Lead Acetate, Glacial Acetic acid.

2.2.3 Preparation of extracts:

Fresh leaves of *Centella asiatica* were collected from a local herbal garden and authenticated by an experienced botanist. To create a 90% hydroalcoholic solvent, ethanol and distilled water were combined in a 90:10 (v/v) ratio. For the extraction process, 100 grams of the dried and powdered *Centella asiatica* was placed in a clean, dry amber glass container. Following this, 1000 ml. of the freshly prepared 90% hydroalcoholic solvent was added, maintaining a drug-to-solvent ratio of 1:10. The container was securely sealed and left at room temperature for 7 days. During this period, the mixture was stirred manually two to three times daily to ensure adequate maceration and to enhance the extraction of active compounds. After the maceration period concluded, the mixture was first filtered through muslin cloth and subsequently through Whatman No. 1 filter paper to obtain a clear extract. The filtrate was concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 40–45°C, in order to remove the solvent and produce a semi-solid extract. Finally, the concentrated extract was transferred into a clean, amber glass container and stored at 4°C in the refrigerator until required for phytochemical screening and formulation development.

3. PHYTOCHEMICAL SCREENING

Phytochemical screening of plant extracts is an important step in the identification of bioactive compounds and the evaluation of their potential medicinal value. Several studies have used the method to screen a variety of plant species and identify a number of phytochemicals with varied biological activities. Phytochemical screening of *Centella asiatica* has revealed a diverse array of bioactive compounds, including tannins, flavonoids, terpenoids, saponins, and steroids (99). Notably, alkaloids were not detected in the plant extracts. The presence of these phytochemicals contributes to the plant's medicinal properties and potential applications in various industries. Interestingly, different extraction methods can yield varying results in terms of phytochemical composition and bioactivity. For instance, ultrasonic-assisted extraction was found to produce the highest recovery and anti-oxidant activity, with a scavenging activity of 79% (99). Additionally, the choice of extraction method can significantly impact the retention of bioactive compounds, which is crucial for maintaining the herb's medicinal properties (100) In conclusion, *C. asiatica* is rich in phenolic compounds, with hesperetin being the predominant phenolic compound identified in one study (99). Other important phytochemicals include isoprenoids (sesquiterpenes, plant sterols, pentacyclic triterpenoids, and saponins) and phenylpropanoid derivatives (eugenol derivatives, caffeoylquinic acids, and flavonoids) (102).

3.1 Evaluation of Alkaloids

Minute quantity of extract free from solvent i.e. chloroform, alcohol and water were taken and stirred primarily with a little drop of dil. HCL followed by filtration. The filtrate collected was subjected to treatment with an array of alkaloid reagents. Chemical tests performed for the presence of alkaloids were given below.

Sl no.	Test	Method	Result
1.	Mayer's Test	1ml. of extract was mixed with 1ml. of Mayer's reagent.	Appearance of cream colour precipitate which indicates the presence of alkaloids.
2.	Dragendroff's Test	1ml. of extract was mixed with 1ml. of Dragendroff's reagent.	Appearance of orange to reddish colour precipitate which ensure the presence of alkaloids.
3.	Wagner's Test	1ml. of extract was mixed with 1ml. of Dragendroff's reagent.	Appearance of reddish brown colour precipitate which indicates the presence of alkaloids.

Table 1: Chemical test performed for the presence of Alkaloid.

3.2 Evaluation of Glycosides

Small amount of extract was dissolved in distilled water separately followed by filtration. Then the filtrate is treated with dil. HCl and were hydrolyzed for 2 to 4 hours on water bath different preliminary tests were performed for the presence of glycosides. Chemical tests performed for the presence of cardiac glycoside are given below.

Sl no.	Test	Method	Result
1.	Borntrager's Test	1ml. of extract was mixed with 1ml. of chloroform, after separation of chloroform layer dilute ammonia was added the layer of Chloroform.	Pink in colour which indicates the presence of o – anthraquinone glycosides.
2.	Keller-Killani test	Adding a solution of 2ml. glacial acetic acid, 1% ferric chloride, and 1ml. concentrated sulfuric acid to the 1ml. extract.	Reddish brown Colour.

Table 2: Chemical test performed for the presence of Glycosides.

3.3 Evaluation of Protein and Amino Acid

Chemical test performed for the presence of phenolic compound and tannins are given below.

Sl no	Test	Method	Result
1.	Millon's Test	Approximately 2 ml. of the extract was taken in a test tube, and a few drops of Millon's reagent were added. The mixture was then gently heated in a water bath for a few minutes.	The formation of a white precipitate that turned brick red upon further heating confirmed the presence of proteins, particularly those containing the amino acid.

Table 3: Chemical test performed for the presence of Protein and Amino Acid.

3.4 Evaluation of Flavonoids

Chemical test performed for the presence of flavonoids are given below.

Sl no	Test	Method	Result
1.	Lead Acetate Test	Approximately 2–3 ml. of the plant extract was taken in a clean test tube, and to this, 1 ml. of 10% lead acetate solution was added. The mixture was then gently shaken and allowed to stand for a few minutes.	The appearance of a yellow precipitate indicated the presence of flavonoid compounds in the extract.
2.	Ferric Chloride Test	A small amount of the extract (about 2–3 ml.) was taken in a test tube. To this, a few drops of freshly prepared 5% ferric chloride ($FeCl_3$) solution were added. The mixture was gently shaken and observed for any color change.	The development of a dark green, bluish- black, or deep blue color indicated the presence of phenolic compounds, which may include flavonoids.

Table 4: Chemical test performed for the presence of Flavonoids.

3.5 Evaluation of Saponins

Chemical test performed for the presence of saponins are given below.

Sl no	Test	Method	Result
1.	Foam Test	The extract was diluted with 1ml. of distilled water and was shaken vigorously.	Formation of persistent foam up to 10 min which indicates the presence of Saponin.

Table 5: Chemical test performed for the presence of Saponin.

4.7 Evaluation of Carbohydrates

Chemical test performed for the presence of carbohydrates are given below.

Sl no	Test	Method	Result
1.	Molisch test	A sample containing a potential carbohydrate is mixed with a solution of α-naphthol in ethanol (Molisch's reagent). Concentrated sulfuric acid is slowly added along the inner wall of the test tube, forming a layer below the sample.	Observe the interface for the formation of a purple ring.
2.	Fehling's Test	1ml. of extract was mixed with 1ml. of Fehling A and 1ml. of Fehling B Solution.	Appearance of blue black/green black colour which indicates the presence of carbohydrates.

Table 6: Chemical test performed for the presence of Carbohydrate.

4.8 Evaluation of Triterpenoids

Sl no	Test	Method	Result
1.	Salkowski's test	About 2 ml. of the plant extract was mixed with 2 ml. of chloroform in a clean test tube. To this mixture, a few drops of concentrated sulfuric acid (H_2SO_4) were carefully added by the side of the test tube to form a separate layer.	The formation of a golden-yellow coloration at the bottom of the test tube indicated the presence of triterpenoids.

Table 7: Chemical test performed for the presence of Triterpenoid.

4.9 Evaluation of Tropane Alkaloid

Sl no	Test	Method	Result
1.	Vitali morin test	A small quantity of the ethanolic leaf extract was evaporated to dryness. The residue was treated with a few drops of fuming nitric acid and evaporated again to dryness. Upon addition of acetone and a drop of alcoholic potassium hydroxide, the appearance of a violet coloration indicated the presence of tropane alkaloids.	A violet color confirmed the presence of tropane alkaloids.

Table 8: Chemical test performed for the presence of Tropane Alkaloids.

4. COMPARATIVE ANTI-MICROBIAL EVALUATION OF CENTELLA ASIATICA EXTRACT AND CHLORAMPHENICOL USING AGAR WELL DIFFUSION METHOD

4.1 Microorganism species used:

The bacterial strains (ATCC, CDL, Kolkata) used were *E. coli* (ATCC 8739), *B. Subtilis* (ATCC 6633), *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* are popular test microorganisms used for anti-microbial testing based on their varied properties and clinical significance. These microorganisms belong to various classes of pathogens that include Gram- negative bacteria (*E. coli*), Gram-positive bacteria (*B. subtilis* and *S. aureus*), and fungi (*C. albicans*), and hence they are suitable to assess the broad-spectrum anti-microbial activity of compounds. *E. coli*, *B. subtilis*, *C. albicans*, and *S. aureus* for anti-microbial screening of *Centella asiatica* would yield an extensive evaluation of its possible anti-microbial activity. This would enable testing of the plant extract's efficacy against a variety of clinically important pathogens, including both bacterial and fungal pathogens. Yet, it should be noted that the anti-microbial effect can differ based on the compounds in the *Centella asiatica* extract and their modes of action against various microorganisms.

4.2 Preparation of Nutrient Agar Media:

4.2.1 Nutrient Agar:

A great agar medium for verifying purity prior to biochemical or serological testing is nutritional agar, which has nutrients that are appropriate for the subculturing of a broad variety of bacteria. Besides, the addition of agar solidifies nutrient agar, which makes it suitable for the cultivation of microorganisms.

4.2.2 Preparation nutrient agar:

Suspend 28g of nutrient agar powder in 1L of distilled water. Mix and dissolve them completely. Sterilize by autoclaving at 121°C for 15 minutes. Pour the liquid into the petri dish and wait for the medium to solidify.

4.2.3 Storage condition and shelf life for nutrient agar:

Store the dehydrated medium at 10-30°C. Once the nutrient agar is prepared in the petri dish, stored at 2-8°C.

Typical Formula	Nutrient Agar (Gm/Litre)
Beef Extract	5gm
Distilled water	100ml.
Peptone	5 gm
Sodium Chloride	3 gm
Agar	25 gm

Table 9: Composition of Nutrient Agar

4.3 Inoculation of Bacterial Suspensions:

1. Sterile petri plates are taken and numbered as 1,2,3,4,5,6,7&8
2. The nutrient agar media which is cooled to 45° C after autoclaving, is poured on the sterile petri plates to form a thick layer (6 mm) and allowed to rest undisturbed for 2 hours.
3. After 2 hours when the media solidifies, from test tube 0.5 ml. of bacterial suspension is taken and inoculated in petri plate (1), in an aseptic condition (laminar air-flow chamber).
4. In the same way inoculation in done in petri plate (2,3,4,5,6,7,8) from suspension of test tube 2,3,4,5,6,7 & 8.
5. In the petri plates hole are bored with cork borers and the plated are placed in BOD incubator to incubate for 72 hours.

4.4 Preparation of Test and Standard Solutions

The crude extract of *Centella asiatica* was dissolved in distilled water and diluted with sterile distilled water to obtain concentrations of 5, 10, 15, and 20 mg/ml. Similarly, standard Chloramphenicol was prepared at the same concentrations (5,10, 15 and 20 mg/ml.) using sterile distilled water. All solutions were freshly prepared before each experiment.

4.5 Agar Well Diffusion Method

The anti-microbial activity of the *Centella asiatica* extract and the standard Chloramphenicol was tested by the agar well diffusion method. Eight sterile Petri plates were prepared for each of the microbial strains tested—four for the plant extract (test group) and four for the standard drug (Chloramphenicol). Sterile nutrient agar was employed for bacterial cultures, whereas Sabouraud dextrose agar (SDA) was employed for *Candida albicans*. About 20 ml. of melted agar medium was filled in every 90 mm Petri dish aseptically and let to set. After setting, the agar surfaces were seeded by swabbing them uniformly with 100 µL of the calibrated microbial inoculum that had been diluted to achieve the 0.5 McFarland turbidity standard. Following inoculation, four 6 mm equidistant wells were aseptically drilled in every agar plate with the help of a sterile cork borer. For the test plates, 100 µL of every concentration of the extract of *Centella asiatica* (5,10, 15 and 20 mg/ml.) was slowly pipetted into the wells using a micropipette. In the same way, in the plates of the standard group, 100 µL of each respective concentration of Chloramphenicol (5,10, 15 and 20 mg/ml.) was placed into the wells. The plates were left to stand at room temperature for one hour to allow the diffusion of the test and standard solutions into the surrounding agar. After the diffusion period, the plates seeded with bacterial strains were incubated at 37 °C for 24 hours, whereas the plates seeded with *Candida albicans* were incubated at 28 °C for 48 hours. Post- incubation, the anti-microbial activity was determined by determining the zones of inhibition diameter (in millimeters) around each well with a ruler or Vernier caliper. All the tests were carried out in triplicate, and the mean zone of inhibition was determined for every concentration of both the plant extract and the standard drug.

4.6 Determination of Zone of Inhibition

The Zone of Inhibition (ZOI) is a crucial measure in antibiotic susceptibility testing, particularly in the disk diffusion method. It is defined as the circular area around an antibiotic disk where bacterial growth is inhibited (101). The size of this zone can be used to determine the susceptibility of bacteria to specific antibiotics. Several studies have explored methods to improve the accuracy and efficiency of ZOI determination. A rapid (6-7 hr.) modified Kirby-Bauer disk-susceptibility method using tetrazolium dyes has been developed to enhance the delineation between growth areas and inhibition zones (102). This method showed excellent correlation with the standard Kirby-Bauer test and demonstrated comparable reproducibility. Interestingly, the relationship between ZOI and antibiotic concentration is not always straightforward. An equation has been derived to express the size of the inhibition zone diameter as a function of the disk content of antibiotic, allowing for the calculation of regression line constants for correlating zone diameter with minimum inhibitory concentration (MIC) using a single reference strain (56).

5. DISCUSSION

Qualitative Phytochemical Screening of *Centella asiatica* leaf (aqueous) extract Aqueous extract of *Centella asiatica* leaf shows positive for Millon's Test, Lead acetate Test, Salkowski's Test, Ferric Chloride Test.

Meanwhile the Test for Alkaloids (by Wagner's Test), Test for Carbohydrate (by Molisch Test), Test of Glycosides (by Keller-Killani Test), Test for Steroids has been marked negative.

Sl no.	Tests	Extracts Used	Water
1.	Alkaloids	Mayer's Test	Negative
		Dragendroff's Test	Negative
		Wagner's Test	Negative
2.	Glycosides	Borntrager's Test	Negative
		Modified Borntrager's Test	Negative
		Keller-Killani test	Negative
3.	Proteins and Amino Acids	Millon's Test	Positive
4.	Test of Flavonoids	Lead Acetate Test	Positive
		Ferric Chloride Test	Positive
5.	Vitali Morin Test		Negative
6.	Test for Saponins		Negative
7.	Test for Proteins		Negative
8.	Test for Steroids		Negative
9.	Test for Triterpenoids	Salkowski's test	Positive
10.	Carbohydrates	Molisch test	Negative
		Fehling's Test	Negative

Table 10: Result of Qualitative Phytochemical Screening.

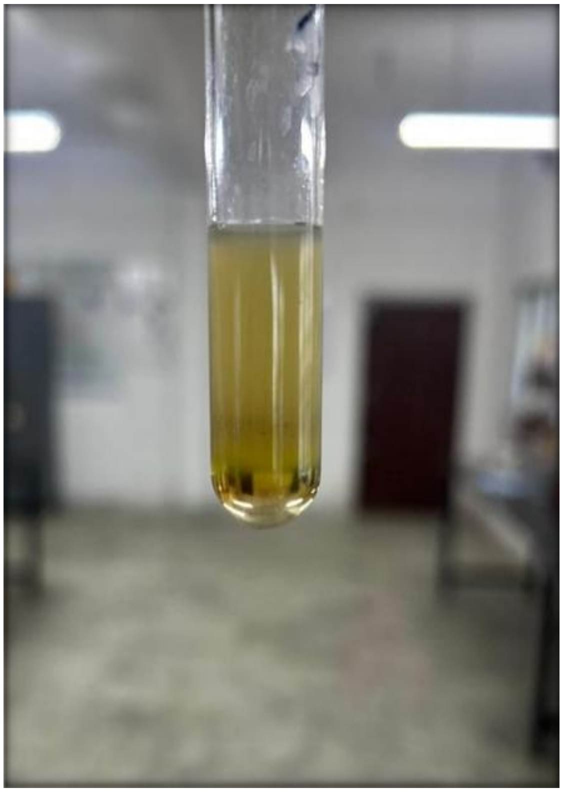


Figure 4: Salkowski's Test

Figure 6: Lead Acetate Test

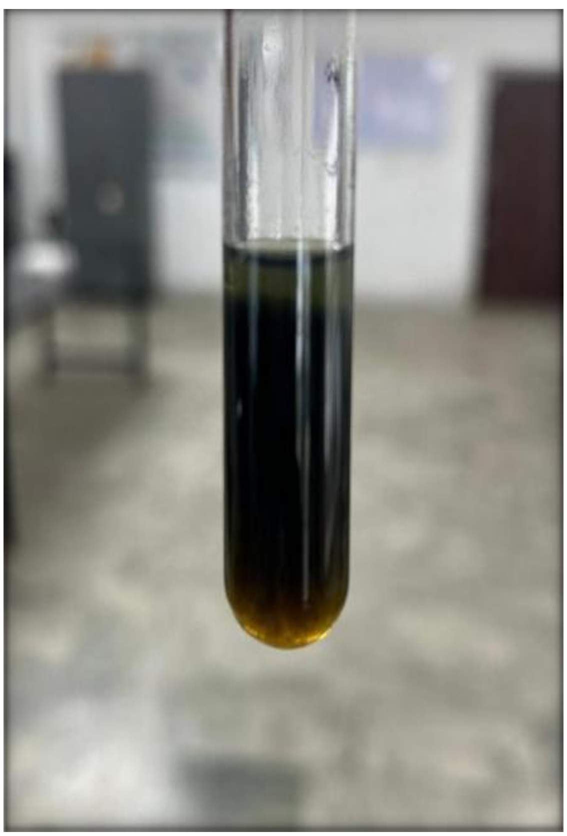


Figure 5: Ferric Chloride Test

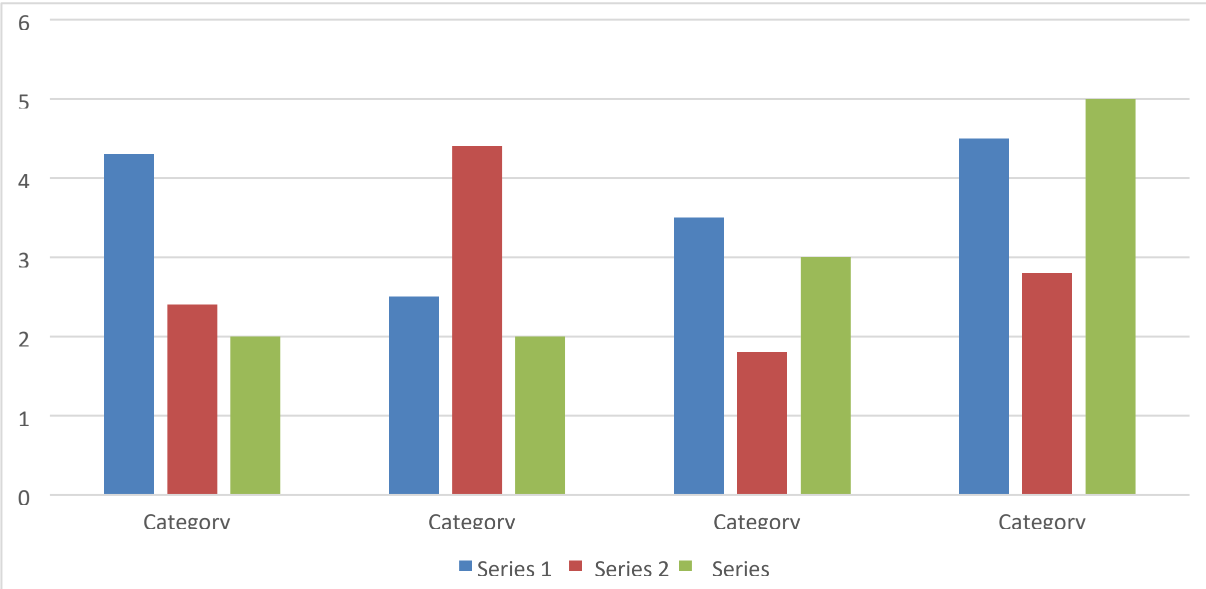
Figure 7: Millon's Test

5.1 Evaluation of Anti-microbial Activity of Chloramphenicol Standard Solution. The zone of inhibition produced by Chloramphenicol standard solution is found to be 30mm with concentration 5mg/ml. & 15mg/ml. for *Bacillus subtilis* (Table 11) (Figure 9). The zone of inhibition produced by Chloramphenicol standard solution is found to be 52mm with concentration 5mg/ml. for *Candida albicans* (Table 11) (Figure 9). The zone of inhibition produced by Chloramphenicol standard solution is found to be 50mm with concentration 15mg/ml. for *Staphylococcus aureus*. (Table 11) (Figure 9). The zone of inhibition produced by Chloramphenicol standard solution is found to be 45mm with concentration 10mg/ml. for *E. coli* (Table 11) (Figure 9).

5.2 Evaluation of Anti-microbial Activity of Aqueous extract of *Centella asiatica* The zone of inhibition produced by *Centella asiatica* solution is found to be 40mm with concentration 10mg/ml. for *Bacillus subtilis* (Table 11) (Figure 10). The zone of inhibition produced by Chloramphenicol standard solution is found to be 58mm with concentration 5mg/ml. for *Candida albicans* (Table 11) (Figure 10). The zone of inhibition produced by Chloramphenicol standard solution is found to be 48mm with concentration 10mg/ml. for *Staphylococcus aureus*. (Table 11) (Figure 10). The zone of inhibition produced by Chloramphenicol standard solution is found to be 40mm with concentration 20mg/ml. for *E. coli* (Table 11) (Figure 10).

Sl. No	icroorganism species	Concentration (mg/ml.)	Zone of inhibition(mm)	
			Centella asiatica leaf Extract (mm)	oramphenicol (mm)
1.	*Bacillus subtilis*	5	40	50
		10	30	32
		15	25	21
		20	20	30
2.	*Candida albicans*	5	60	45
		10	30	50
		15	35	20
		20	25	21
3.	*aphylococcus aureus*	5	55	45
		10	35	40
		15	30	35
		20	25	20
4.	*E. coli*	5	36	55
		10	50	60
		15	50	20
		20	30	40

Table 11: Anti-microbial activity of leaf extract of *Centella asiatica* and Chloramphenicol (Standard) against Pathogenic microorganisms.



	Series 1	Series 2	Series 3
Bacillus subtilis (Category 1)	4.3	2.4	2
Candida albicans (Category 2)	2.5	4.4	2
Staphylococcus aureus (Category 3)	3.5	1.8	3
E. coli (Category 4)	4.5	2.8	5

Graph 1: Anti-microbial Effective Concentration on isolated Standard microorganism with *Centella asiatica L*. and Chloramphenicol.

Figure 8: Growth of *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, and Staphylococcus aureus without anti-microbial agent.

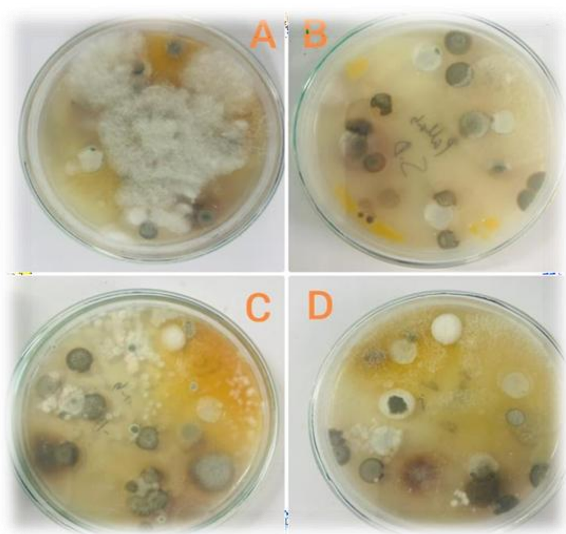


Figure 9: Zone of Inhibition produced by Chloramphenicol (Standard) against A: *Bacillus subtilis*, B: *Candida albicans*, C: *Staphylococcus aureus* and D: *E. coli.*

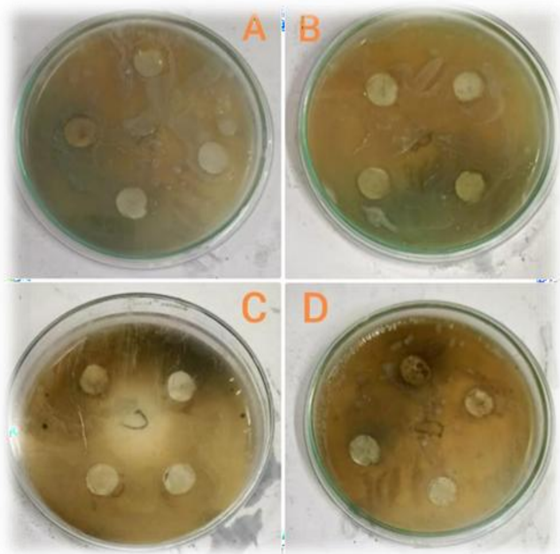


Figure 10: Zone of Inhibition produced by extract of *Centella asiatica* L. against A: *Bacillus subtilis*, B: *Candida albicans*, C: *Staphylococcus aureus* and D: *E. coli*.

6. RESULTS

The study evaluated the anti-microbial effect of *Centella asiatica* leaf extracts against pathogenic microorganisms including Bacillus subtilis, Candida albicans, Staphylococcus aureus, and Escherichia coli, using the agar well diffusion method. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, triterpenoids, and proteins, which contribute to its anti-microbial properties. The asqueous extract of *Centella asiatica* demonstrated significant inhibitory activity, with zones of inhibition ranging from 20–60 mm, depending on the concentration (5–20 mg/mL) and microbial strain. Notably, the extract exhibited strong efficacy against *Candida albicans* (60 mm at 5 mg/mL) and *Staphylococcus aureus* (55 mm at 5 mg/mL), comparable to or even exceeding the activity of the standard antibiotic chloramphenicol in some cases. These findings suggest that *Centella asiatica* possesses potent broad-spectrum anti-microbial properties, supporting its traditional use in treating infections and highlighting its potential as a natural alternative to conventional antibiotics. Further research is needed to isolate the active compounds and optimize their therapeutic applications.

7. CONCLUSION

The ability of *Centella asiatica* leaf extract to suppress the effects of both Gram-positive and Gram- negative pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*, is one of the study's most significant anti-microbial qualities. Furthermore, a number of phytochemical analyses revealed some of the primary constituents in the extracts, including proteins, triterpenoids, and flavonoids, which most likely aided in *Centella asiatica*'s anti-bacterial capability. In particular, the extract showed concentration-determined inhibition for *S. aureus* and *B. subtilis* that was comparable to that of the common antibiotic chloramphenicol. The results presented in this study confirm the traditional use of *C. asiatica* to treat microbiological infections and suggest a potential natural remedy for antibiotic resistance. Its possible therapeutic use and safety for human consumption require more research, including compound isolation, mechanistic studies of action, and clinical trial efficacy.

The results of this research substantiate the conventional medicinal application of *Centella asiatica* and identify it as a possible source of anti-microbial compounds from nature. Especially remarkable is the wide-spectrum activity of the extract, which indicates the presence of a number of bioactive compounds operating synergistically. These findings form a firm scientific rationale for the continued study of *Centella asiatica* for the purpose of developing plant-derived anti-microbial drugs, particularly in view of the emerging worldwide menace of antibiotic resistance. The future would involve the isolation and characterization of single phytoconstituents and evaluation of their mechanism of action, cytotoxicity, and therapeutic potential.

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