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Review Article

Centella asiatica in Surakarta City, Indonesia: A Comprehensive Review of Discovery, Genetic Diversity, Antimicrobial Potential, and Cultivation

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Abstract

Centella asiatica, commonly known as gotu kola, is a medicinal plant with a long history of use in traditional medicine. This literature review provides a comprehensive overview of recent research on *Centella asiatica* in Surakarta City, Indonesia. It focuses on four key areas: discovery, genetic diversity, antimicrobal potential, and cultivation.

The review begins by outlining the importance of accurately identifying *Centella asiatica* species using DNA barcoding techniques. It then explores the genetic diversity of recently discovered or uncommon species of *Centella asiatica* within the Surakarta City District, emphasizing the importance of genetic variation for adaptability, resilience, and the advancement of improved varieties.

Moving on to the antimicrobal potential of *Centella asiatica*, the review examines the efficacy of *Centella asiatica* extracts against MRSA strains, emphasizing its therapeutic potential for medical applications. Finally, the review explores techniques for large-scale cultivation of *Centella asiatica*, with a particular focus on the Temporary Immersion System (TIS) as a promising approach for increased biomass production, optimal growth, and spatial efficiency.

This literature review synthesizes the current knowledge on *Centella asiatica* in Surakarta City, Indonesia, and identifies promising avenues for future research and practical applications.

Keywords: Surakarta Centella Diversity; Antimicrobal Potential; Cultivation; DNA Barcoding

Introduction

Highlighting the Historical Significance of *Centella asiatica*

Centella asiatica, commonly recognized as Gotu kola, holds profound historical significance deeply embedded in traditional medicinal systems, notably Ayurveda and Traditional Chinese Medicine (TCM). Its historical usage is extensively documented in ancient Chinese medical texts such as "Su Wen Shi" and "Zheng Lei Ben Cao" from the Song Dynasty, highlighting its enduring significance in Chinese medicinal practices [1]. Renowned for its versatile therapeutic applications in TCM, *Centella asiatica* is characterized as bitter, cold, and non-toxic. It proves invaluable in addressing conditions such as fever and various dermatological ailments [1].

Beyond its role in Chinese medicine, *Centella asiatica* is known for its traditional association with wound healing. It has been shown to be effective in promoting the formation of granulation tissue and expediting the overall healing process. The recognition of its historical importance has had a significant impact on current uses, leading scientists to examine its potential protective effects and study active components, such as asiatic acid and asiaticoside, especially in relation to cardiovascular diseases [2].

Scientific inquiry unveils the intricate biochemistry underlying the medicinal values, encompassing a diverse range of phytoconstituents such as terpenes, phenols, vitamins, minerals, polyacetylene, and fatty acids. Noteworthy are triterpene saponins and their derivatives, which have emerged as pivotal contributors to the bio-

activities of *Centella asiatica*. These compounds underscore the plant's role in treating a wide range of chronic disorders, including Alzheimer's disease, varicose veins, and hypertonic scars [1]. The comprehensive exploration of *Centella asiatica*'s multifaceted contributions reflects the convergence of ancient wisdom and modern scientific rigor, laying the groundwork for a more profound comprehension of its therapeutic potential in contemporary healthcare.

Emphasize its modern pharmaceutical potential

In its transition from historical significance to contemporary relevance, *Centella asiatica* has emerged as a focal point in modern pharmaceutical research, supported by rigorous scientific inquiry. With deep roots in traditional Chinese medicine [1], recent studies have validated its substantial positive impact on neurological and dermatological conditions. This marks a significant trajectory towards integrating it into pharmaceutical applications [1].

The detailed examination of *Centella asiatica* uncovers distinct pharmaceutical properties and compounds, particularly triterpenes like asiatic acid, asiaticoside, madecassic acid, and madecassoside. These compounds, meticulously identified, not only showcase the potential of the herb but also lay the foundation for the development of healthcare products with cardioprotective, antiatherosclerotic, antihypertensive, antihyperlipidemic, antidiabetic, antioxidant, and anti-inflammatory effects [2]. This identification and understanding of key components significantly contribute to the evolving landscape of pharmaceutical innovation.

The integration of *Centella asiatica* into the pharmaceutical industry is exemplified by its pharmacological activities, which are derived from major bioactive triterpene glycosides collectively known as centellosides. This unique classification positions *Centella asiatica* as a cornerstone in the development of innovative treatments and therapies, as emphasized by groundbreaking research. Beyond the realm of triterpenes, the study of *Centella asiatica* phenolics (CAP) adds another dimension of potential, especially in terms of their antiglycative properties, which implies potential nutraceutical applications. This multifaceted approach expands the horizons of *Centella asiatica*'s utility, contributing to the versatility of its applications in the pharmaceutical field.

Moreover, recent pharmaceutical research highlights the crucial role of *Centella asiatica* in addressing current healthcare challenges, particularly in the field of neuroprotection. Compounds such as triterpenoid saponins, elucidated in studies , demonstrate significant neuroprotective effects. These findings not only enhance the scientific understanding of *Centella asiatica* but also establish it as a valuable resource in the development of innovative healthcare solutions that tackle the complexities of modern medical challenges.

In summary, the combination of historical wisdom and modern scientific rigor emphasizes the significant pharmaceutical potential of *Centella asiatica*. The diverse array of compounds found in *Centella asiatica*, ranging from triterpenes to phenolics, offers a promising opportunity for the development of advanced healthcare solutions. This firmly establishes *Centella asiatica* as a valuable and dynamic resource in the ongoing pursuit of innovative pharmaceutical interventions.

The objectives

The research introduction focuses on outlining specific objectives designed to address key inquiries in the study of *Centella asiatica* in Surakarta City. The primary objective is to identify new species of *Centella asiatica* using DNA barcoding, a technique known for its speed and accuracy in plant identification, as highlighted in the influential study by Khwanchum., *et al*. [3]. This research aim is intricately linked to the imperative of accurate identification, as discussed in the aforementioned paper, thus highlighting the importance of Objective 1 in improving the reliability of medicinal plants in the region [32].

The second objective of the study is to uncover the genetic diversity of recently discovered or uncommon *Centella asiatica* species within the Surakarta City District. The importance of genetic diversity is highlighted in scholar and Padmalatha and Prasad [4], with each study emphasizing its crucial role in providing adaptability, resilience, and the potential for developing improved varieties. Objective 2 aligns seamlessly with the broader perspectives presented in these academic contributions, thereby enhancing the understanding of genetic variation in *Centella asiatica* [4].

Shifting the focus to the antimicrobial potential of *Centella asiatica*, the third objective aims to identify new or uncommon species that demonstrate anti-MRSA activity. This pursuit is based on the documented antimicrobial properties of *Centella asiatica*, which were notably highlighted by Idris and Nadzir [5]. Their research underscores the efficacy of *Centella asiatica* extracts, particularly the methanolic extract, against MRSA strains, thereby emphasizing

the therapeutic potential for medical applications [5]. Objective 3 thus acts as a crucial bridge, connecting traditional medicinal knowledge with contemporary antimicrobial applications.

Lastly, the fourth objective aims to develop a technique for large-scale cultivation, taking inspiration from the benefits outlined in the research of Mathavaraj and Sabu [5]. The temporary immersion system (TIS) is presented as a promising approach, offering increased biomass production, optimal growth, and spatial efficiency. This objective (Objective 4) aligns seamlessly with the need for organized commercial cultivation, genotype-environmental evaluation, documentation of genetic variation, and genetic mapping, as proposed by Mathavaraj and Sabu [8]. This multifaceted approach not only advances scientific knowledge but also contributes to the practical applications of *Centella asiatica* in large-scale cultivation scenarios.

Justify the significance of the rare *Centella asiatica* species

The exploration and scrutiny of rare species within the *Centella asiatica* genus hold profound significance within the realm of medicinal applications, as underscored by scholarly perspectives presented in the works of Sun., *et al*. [1], Arribas-López., *et al*. [6]. These rare botanical entities not only significantly contribute to the knowledge base of *Centella asiatica*'s medicinal potential but also expand its applications in the therapeutic landscape, addressing a wide range of diseases [1]. The unique chemical compositions and pharmacological properties found in these rare species offer the exciting possibility of discovering new bioactive compounds. These compounds may be characterized by higher concentrations of specific triterpenoids or other active constituents that enhance their medicinal properties [1]. Moreover, selecting rare species plays a pivotal role in the development of various processed food products, elevating their micronutrient profile and bolstering their antioxidant potential.

Delving into the phytochemical profiles of these rare species of *Centella asiatica*, as discussed in the scholarly works highlights their inherent significance. These botanical specimens may contain unique combinations of polyphenols, flavonoids, and fatty acids, enhancing their bioactivity and expanding their potential medicinal uses. The understanding of the phytochemical composition of these substances is of utmost importance. It serves as a crucial factor in optimizing the methods of extraction, identification, and quantification, thus ensuring the maximum utilization of their medicinal potential [6]. Additionally, the innovative approach to novel drug delivery systems, such as phytosomes, has the potential to increase the bioavailability of active compounds from these rare species, resulting in improved therapeutic effectiveness.

Identifying Rare *Centella asiatica* species

Overview of *Centella asiatica* and its common species

In the relentless pursuit of identifying rare species of *Centella asiatica*, a fundamental aspect lies in comprehending the intricate characteristics of the plant, meticulously explained in scholarly contributions such as [6]. *Centella asiatica*, commonly known as Gotu Kola, displays unique characteristics. It is characterized by small, circular leaves that measure between 2-6 cm in diameter. The leaves have a smooth texture and are arranged in a peltate manner, attached to the stem at the central axis of the leaf. This nuanced portrayal serves as a crucial element in the identification of the plant and holds paramount importance in traditional medicinal practices. The leaves, enriched with bioactive compounds such as triterpenoids, flavonoids, and phenolic acids, highlight the potential health benefits of the plant, including cognitive enhancement, wound healing, and anti-inflammatory effects. Further reinforcing this understanding, Sadik and Rifqah Amalia Anwar [6] emphasize the therapeutic properties of *Centella asiatica*'s leaves. They describe the leaves as small, round-shaped, green, smooth, and slightly glossy. The leaves are arranged alternately along the stem in a palmate pattern.

Delving into the realm of common *Centella asiatica* species, their prevalence, and distribution, pertinent elucidations are presented in scholarly works by Prasad., *et al*. [7] and Vinolina and Sigalingging [7]. The most prevalent and extensively studied species is *Centella asiatica* (Linn.). Urban exhibits a prostrate, creeping runner growth habit and exudes a faint aromatic quality. Indigenous to Southeast Asian countries, this plant has a pan-tropical distribution and is known by various vernacular names such as Indian pennywort and water pennywort. While the genus Centella encompasses more than 50 species, *Centella asiatica* reigns supreme as the most recognized and studied. It is found in tropical and subtropical regions, including India, Sri Lanka, Bangladesh, Africa, Indonesia, and various parts of Asia [7].

Pertinent discussions in Vinolina and Sigalingging's works [7] shed light on the expected presence of *Centella asiatica* species in Indonesia, emphasizing the plant's significance due to its medicinal properties and bioactive compounds. Endeavors are underway to

domesticate the wild plant, locally known as Pegagan in Indonesia. Concurrent studies are being conducted to examine its growth and bioactive compounds under commercial field conditions. Furthermore, research conducted in Samosir, North Sumatra, highlights the adaptability of *Centella asiatica* to acidic soil conditions, emphasizing its potential for growth in various environments [7].

Collectively, these detailed insights provide a comprehensive overview of *Centella asiatica* and its common species, establishing the foundation for further exploration of rare species in subsequent sections of this literature review. The nuanced details presented in these scholarly works not only contribute to the scientific understanding of *Centella asiatica* but also provide a strong foundation for identifying and examining rare species in the broader botanical landscape.

Significance of identifying rare species

The significance of identifying rare *Centella asiatica* species, as discussed in scholarly works by Sakthipriya., *et al*. [8], extends across a diverse range, playing a crucial role in the fields of conservation, genetic improvement, and medical advancement. An inherent aspect of this significance lies in the presence of distinct secondary metabolites, particularly rare varieties' exclusive triterpenoids, which elucidate the critical nature of identifying them. Highlighting the importance of conservation and genetic diversity analysis in *Centella asiatica*, Sakthipriya., *et al*. [8] emphasize the potential medicinal benefits that rare species may possess [8]. Reinforcing this notion, the analysis of genetic diversity in the study conducted by Khotimah., *et al*. [9] suggests that *Centella asiatica* possesses distinct genetic traits that play a significant role in its medicinal properties. This emphasizes the importance of identifying and conserving these rare species [9].

Beyond their genetic uniqueness, the distinct secondary metabolites found in rare varieties of *Centella asiatica*, notably triterpenoids, exhibit formidable antibacterial activity, presenting potential avenues for the development of novel antibacterial drugs. This highlights the therapeutic potential of rare species in addressing the growing problem of antimicrobial resistance, which is a major concern in the healthcare industry. Furthermore, the discourse presented in scholarly works by Khotimah., *et al*. [9] emphasizes the use of unique compounds from rare species of *Centella asiatica* for specific pharmaceutical treatments, particularly in areas such as wound healing and cognitive enhancement. This highlights the wider implications of identifying these rare varieties [9].

The identification of rare species extends its impact to the discovery of novel compounds and multitarget inhibitors, thereby expanding the reservoir of potential drug targets and enriching drug discovery pipelines [10]. The implications of these findings go beyond basic taxonomy and have significant repercussions for pharmaceutical research and development. The multifaceted significance underscored across these scholarly contributions emphasizes the critical role of identifying rare species of *Centella asiatica* in advancing both conservation efforts and pharmaceutical applications. In essence, this intricate exploration not only contributes to the scientific understanding of *Centella asiatica* but also advances translational research with significant implications for healthcare and biodiversity conservation.

DNA barcoding and sequencing methods

DNA barcoding, an influential molecular technique for species identification, has been systematically applied to the study of *Centella asiatica*, as elucidated in scholarly works by [11]. The barcoding process is intricately orchestrated, leveraging specific genomic regions, such as rbcL or matK. However, the precise primer sets are not explicitly mentioned in the provided sources [11]. It is have proven instrumental in providing essential insights into the foundational DNA barcode regions, including rbcL and matK, which are designed for the precise differentiation of *Centella asiatica* from other botanical species. Notably, the study suggests that matK is the main region for identification within the Indonesian context, while ITS2 and rbcL are proposed as alternative or complementary regions [11]. This highlights the need for ongoing exploration and research to determine the most effective primer sets for DNA barcoding in the complex taxonomy of *Centella asiatica*.

While specific case studies related to *Centella asiatica* are not explicitly delineated in [11], the success of DNA barcoding in identifying rare plant species, including orchids and medicinal herbs in Indonesia, is unequivocally demonstrated. The study serves as evidence of the effectiveness of DNA barcoding in accurately identifying Indonesian medicinal plant species [11]. Highlight the gaps in DNA barcoding research in the Indonesian context. They emphasize the need for comprehensive surveys and studies focused on discovering new species, including those that may be relevant to *Centella asiatica*. This insightful observation not only highlights an opportunity for further investigation but also suggests that DNA barcoding is a powerful method to enhance our understanding of

the genetic diversity and distribution of *Centella asiatica* within the region.

The integration of DNA barcoding in the analysis of *Centella asiatica* is essential, as ongoing research requires the improvement of primer sets for optimal effectiveness. The success stories of DNA barcoding in identifying rare plant species in Indonesia, as exemplified in [10], have potential applications in the nuanced taxonomy of *Centella asiatica*. The acknowledged gaps in research, as highlighted , not only underscore the need for comprehensive surveys but also call for a concerted effort to unravel the genetic makeup of *Centella asiatica* in the Indonesian botanical landscape using DNA barcoding.

Bioinformatics software for species identification

Within the intricate domain of species identification through DNA sequences, the key to progress lies in the utilization of bioinformatics tools, meticulously described in scholarly works by [12]. These bioinformatics tools, namely BLAST, MEGAN, and DADA2, are considered essential for accurately identifying species using DNA data. BLAST, a widely adopted tool, facilitates sequence similarity searches, enabling researchers to calculate genetic distances, align sequences, and cluster them through pairwise comparisons [12,13]. MEGAN, positioned as metagenomic analysis software, proves instrumental in taxonomic classification and species identification through DNA sequences [13]. DADA2, a bioinformatics pipeline dedicated to amplicon sequence variant analysis, enhances species identification by accurately detecting and correcting errors in DNA sequences, providing a high-resolution method [12].

However, it is paramount to acknowledge that despite the potency of these tools, the specific methodologies for sequence comparison and taxonomy assignment within the intricate framework of *Centella asiatica* are not explicitly explained in the provided sources [14]. In practical application, the insightful findings presented in the works of [16] shed light on the use of BLAST, MEGAN, and DADA2 for species identification in *Centella asiatica* DNA data. BLAST, in this context, is used for conducting sequence similarity searches against the comprehensive NCBI database. MEGAN is employed for analyzing taxonomic data to identify species. DADA2 is considered the linchpin for achieving precision through meticulous error correction [16].

Further enriching our understanding, the insightful findings from [15] emphasize the nuanced factors that are crucial for accurately identifying the species of *Centella asiatica* DNA data using bioinformatics tools. The careful selection of classification methods and reference databases emerges as a crucial factor that significantly impacts the accuracy and dependability of species-level identification [15]. This discussion also highlights the impact of limitations present in short-read metagenomic data and incomplete reference databases, emphasizing the complexities that affect identification accuracy. In their response, [15] emphasize the crucial significance of collaborative efforts among scientists who employ various methodologies and drive the advancement of bioinformatic tools designed for practical applications. This collaborative ethos emerges as an essential aspect in advancing the practical application of bioinformatics tools for the detailed exploration of *Centella asiatica*'s genetic diversity and taxonomy.

Genetic diversity of rare Centella asiatica species

Importance of genetic diversity

The intricate relationship between genetic diversity and *Centella asiatica*'s adaptability to various climates holds significant importance, although specific correlations remain unexplored in the provided literature [16]. *Centella asiatica* demonstrates botanical resilience through phenotypic plasticity, thriving in diverse environmental conditions such as different elevations, habitat types, and physiography [16]. However, further research is needed to elucidate the genetic determinants underlying this adaptability [16]. Studies have revealed that genetic makeup significantly influences the production of unique secondary metabolites in *Centella asiatica*, contributing to its distinct pharmacological properties [17-19]. For instance, metabolomics studies suggest that genetic variance accounts for a substantial portion of the total phenotypic differences in metabolite levels [18]. The genetic richness of *Centella asiatica* thus plays a crucial role in determining the composition and concentration of bioactive compounds, including asiaticoside, with implications for pharmaceutical and cosmetic applications [17-19].

Previous research on genetic diversity

Explorations into *Centella asiatica*'s genetic diversity have been conducted worldwide, utilizing various methodologies to analyze its complex gene pool. Studies have utilized genomic microsatellite markers and SSR markers to examine the genetic diversity within *Centella asiatica* populations [20]. For example, genetic diversity analyses in southern Indian populations revealed significant genetic differentiation and variation within the species. Similarly, investigations in China and India utilized inter-simple sequence repeat (ISSR) markers and SSR markers, respectively, to unravel the genetic makeup of *Centella asiatica* populations [20]. These studies

collectively contribute to a comprehensive understanding of *Centella asiatica*'s genetic variations and structure, guiding conservation efforts and genetic improvement initiatives [20]. Additionally, next-generation sequencing technology has emerged as a transformative tool for genetic diversity analysis in *Centella asiatica*, offering new insights into its genetic landscape [21].

Metrics for quantifying genetic diversity

In the realm of population genetics, metrics such as nucleotide diversity (π) and FST values are essential for quantifying genetic diversity and differentiation among populations. Nucleotide diversity reflects the average number of nucleotide differences per site between two sequences within a population and provides insights into genetic variation. On the other hand, FST values quantify genetic differentiation between populations, with higher values indicating increased differentiation [22]. Thresholds such as FST > 0.25 are considered significant indicators of population divergence, suggesting limited gene flow and potential barriers to gene exchange [22]. These metrics offer quantitative insights into *Centella asiatica*'s genetic composition and population dynamics, enhancing our understanding of its evolutionary history [22].

Implications of unique genetic characteristics

The exploration of genetic traits within rare *Centella asiatica* species has significant implications for medicinal applications, particularly in enhancing asiaticoside production. Promising research involves the use of biotic elicitors to promote plant biomass and asiaticoside content, offering opportunities for optimizing *Centella asiatica*'s therapeutic properties. Additionally, the identification of genetic markers associated with rare *Centella asiatica* alleles opens avenues for precise manipulation and selective breeding, facilitating the cultivation of varieties with improved medicinal properties. The strategic integration of genetic markers into breeding programs holds promise for advancing *Centella asiatica* research and enhancing its pharmaceutical potential [23].

Antimicrobal potential against MRSA

MRSA and antimicrobal resistance

The scholarly literature provides invaluable insights into the prevalence and implications of Methicillin-resistant Staphylococcus aureus (MRSA) infections, a global concern with varying prevalence rates worldwide [24]. For instance, Sweden reports a prevalence rate of 0.4%, while hospitals in various regions, including Asia, Malta, North and South America, face rates surpassing 50% [24]. In Nigeria, MRSA prevalence ranges between 33-43%, indicating a significant public health burden. In the United States, over 2 million illnesses and 23,000 deaths annually are attributed to antimicrobial-resistant pathogens, with MRSA being prominent [24]. Despite comprehensive insights into MRSA prevalence and genetic resistance mechanisms, recent infection rates and mortality data remain inadequately examined, highlighting critical gaps in the literature [24].

In the Italian context, MRSA significantly contributes to healthcare-associated infections, although precise percentages are not explicitly stated. Similarly, specific MRSA percentages in intensive care unit infections are not detailed, indicating notable information gaps [25]. Nevertheless, the urgent need to address MRSA infections due to their extensive public health impact is emphasized [25].

Previous studies on the antimicrobial properties of *Centella asiatica*

Transitioning to the antimicrobial properties of *Centella asiatica*, the literature underscores its targeted efficacy against MRSA. Triterpenoids like asiatic acid and madecassic acid exhibit robust antibacterial activity against MRSA strains by disrupting bacterial cell membranes and inhibiting biofilm formation [26]. These compounds present potential alternatives or adjuncts to conventional antimicrobials for treating MRSA infections [26].

Besides triterpenoids, *Centella asiatica* contains other antibacterial compounds such as triterpene saponins, notably madecassoside, which demonstrate renin inhibitor activity [27]. However, studies focusing on the MRSA-inhibiting properties of other triterpene saponins in *Centella asiatica* are limited [27]. Additionally, *Centella asiatica* extracts exhibit broad-spectrum antimicrobial activity against various bacterial strains, including those relevant to MRSA infections, with minimum inhibitory concentration (MIC) values indicating their efficacy [28].

Specific compounds within *Centella asiatica*, namely asiatic acid and madecassic acid, exhibit inhibitory effects on MRSA, showcasing potential as anti-MRSA agents. Madecassic acid, for instance, demonstrates multifaceted mechanisms of action, including cell membrane and wall destruction, DNA-related process interference, and enzymatic activity inhibition, suggesting its candidacy as an anti-MRSA drug [29].

Methods for testing antimicrobial activity

Methods for evaluating the antimicrobial effectiveness of *Centella asiatica* extracts against MRSA include standard in vitro techniques like determining minimum inhibitory concentration (MIC) and measuring the zone of inhibition through agar disc diffusion assays [30]. The use of guidelines set by the Clinical and Laboratory Standards Institute (CLSI) aids in determining MIC and minimum bactericidal concentration (MBC) through serial dilution tests. Complementary approaches like the checkerboard assay evaluate synergistic effects of *Centella asiatica* extracts with antimicrobials against drug-resistant bacteria [31].

Comparing antimicrobal activity to known antimicrobals

A thorough investigation into the antimicrobial activity of *Centella asiatica* extracts, compared to established antimicrobials like vancomycin, reveals intriguing findings regarding its potential effectiveness. The Minimum Inhibitory Concentration (MIC) values for *Centella asiatica* extracts were documented by Wong and Ramli (2021). The MIC values ranged from 7.81 mg/mL for Candida albicans to 125 mg/mL for various strains, including Staphylococcus aureus [32]. Regrettably, the lack of MIC values for vancomycin in the sources hinders a direct quantitative comparison of its antibacterial efficacy. Nonetheless, the discernible outcome that *Centella asiatica* outperformed in agar disc diffusion assays suggests its promising role as a potent antibacterial agent [32].

The clinical implications of variations in antibacterial effectiveness require careful consideration. Insights gleaned from randomized controlled trials (RCTs), as outlined by Wald-Dickler., *et al*. (2018), challenge the prevailing belief that bactericidal agents are inherently superior to bacteriostatic agents in the treatment of bacterial infections [33]. Factors such as optimal dosing, pharmacokinetics, and tissue penetration emerge as pivotal determinants that influence efficacy. Unraveling these nuanced facets assumes paramount significance in evaluating the potential of *Centella asiatica* as an alternative treatment. Notably, *Centella asiatica* exhibits its discernible antibacterial properties, particularly in the field of wound dressings, where it demonstrates superior efficacy compared to Polyvinyl Alcohol (PVA) fibers. This highlights the need to explore its clinical applications.

Despite the extensive discussions in the sources elucidating anticipated outcomes and rationales for observed effects in antimicrobial activity concerning known antimicrobials [34,35], there is a notable gap in directly correlating these insights to the antimicrobial activity of *Centella asiatica*. Consequently, a call to action resonates - experimentation becomes imperative to ascertain whether *Centella asiatica* genuinely manifests antibacterial properties. This could be achieved through meticulous MIC assays conducted against well-defined bacterial strains. This experimental validation stands as a cornerstone, indispensable in establishing the credibility of *Centella asiatica* as a genuine antibacterial agent.

Large-scale cultivation of rare Centella asiatica Species

Importance of large-scale cultivation

The large-scale cultivation of the rare *Centella asiatica* species emerges as a pivotal strategy to meet the escalating demand for pharmaceutical products derived from this botanical marvel [36]. The literature strongly emphasizes the importance of cultivating *Centella asiatica* to meet the growing demand for pharmaceutical products derived from it. These products range from creams and supplements to traditional medicines. The inherent value of *Centella asiatica* lies in its abundant triterpenoid centellosides constituents, which are extensively utilized in the formulation of pharmaceutical and cosmetic products. In the contemporary healthcare landscape, characterized by a growing preference for plant-based formulations and an increasing awareness of the adverse effects associated with synthetic drugs, large-scale cultivation assumes a role of paramount importance. This approach not only caters to the soaring demand but also serves as a stronghold against the over-exploitation of medicinal plant resources from their natural habitats, demonstrating an unwavering commitment to sustainable practices [37].

The temporal aspect of cultivation ages emerges as a significant factor that influences the chemical composition of *Centella asiatica*. It has discernible effects on pivotal compounds such as madecassoside and asiaticoside. The concept of large-scale cultivation offers a solution to this dilemma by ensuring a reliable and abundant supply, thereby facilitating a consistent production of pharmaceutical products. The scale of cultivation facilitates the implementation of metabolomics analysis methodologies, including, but not limited to, Fourier Transform Infrared (FTIR) spectra and High-Performance Liquid Chromatography (HPLC). Such large-scale analyses enable a comprehensive evaluation of the chemical composition, thereby ensuring the quality and efficacy of *Centella asiatica*. In essence, large-scale cultivation is not only a logistical necessity but also a crucial element in meeting the increasing demand for *Centella asiatica* pharmaceuticals. This highlights the commitment to ensuring a stable supply of plants that possess optimal chemical composition and medicinal properties [38].

The wide range of products driving the demand for large-scale cultivation of *Centella asiatica* spans across the pharmaceutical, cosmetic, and herbal industries. *Centella asiatica* plays a crucial role as a key ingredient in a variety of formulations, including skincare and anti-aging creams, as well as cognitive-enhancing supplements. The magnitude of demand is underscored by India alone, which traded an impressive 500-1000 metric tons annually in 2016. The significance of large-scale cultivation extends beyond market dynamics; it becomes a catalyst for socio-economic upliftment in local communities by creating employment opportunities. This dual impact encapsulates the critical role that large-scale cultivation plays, not only as a logistical necessity but also as a linchpin in ensuring the sustainable availability of high-quality *Centella asiatica* for various industries [38].

Existing methods for cultivating *Centella asiatica*

The various methods used in cultivating *Centella asiatica* are crucial in meeting the increasing demand for this botanical resource. These methods ensure optimal plant growth and help achieve the desired phytochemical content. Among these approaches, hydroponic cultivation emerges as a highly effective method for enhancing phytochemical content, particularly the crucial centelloside. This is accomplished by carefully supplementing elicitors such as sucrose, auxins, cytokinins, kinetin, and methyl jasmonate. The controlled nutrient delivery in hydroponics results in increased phytochemical content and potentially higher biomass output. The meticulous standardization of production practices ensures the consistent availability of high-quality raw materials rich in centellosides, making hydroponic cultivation a promising method for *Centella asiatica* production [39].

Controlled environment farming, distinguished by its precise control over environmental factors, emerges as an ideal method for year-round production of *Centella asiatica*. This approach ensures optimal conditions for growth and development, maintaining a consistent and uniform growth and yield throughout the year. The integration of advanced technologies, such as hyperspectral imaging and unmanned aerial vehicles, plays a pivotal role in monitoring and diagnosing the nutritional needs of *Centella asiatica* plants. This facilitates the precise application of fertilizers, contributing to desirable production outcomes. Moreover, controlled environment farming serves as a proactive response to the challenges posed by climate change, including elevated CO2 levels, salinity, and drought stresses. This method ensures that the nutritional composition of plants remains unaffected. In essence, controlled environment farming represents a promising paradigm for consistent and high-quality *Centella asiatica* production, offering tangible solutions to the multifaceted challenges introduced by climate change [40].

Shade-net cultivation emerges as a strategically beneficial approach, particularly in specific geographical regions where the provision of necessary shade conditions mimics *Centella asiatica*'s natural habitat in forests. This method effectively moderates light conditions, shielding the plant from excessive sunlight and heat stress, which could hinder its growth and yield. Techniques such as shade netting contribute significantly to improving crop productivity and quality. Ongoing research is dedicated to determining the optimal intensity and duration of shading, tailored to the diverse geographical nuances. This highlights the importance of taking region-specific factors into account when implementing shade-net cultivation for *Centella asiatica* [41].

Mitigating disease susceptibility on a large scale in *Centella asiatica* cultivation involves a detailed examination of mechanisms that involve antagonistic interactions and the implementation of biological control methods. The introduction of coinfections with other pathogens, such as parasites, viruses, and bacteria, can induce antagonistic interactions that reduce the susceptibility of hosts to diseases. Furthermore, the inclusion of organic sulphides, dietary fibers, and plant sterols obtained from cruciferous and Allium vegetables shows promise in disease prevention strategies. Plant-based diets, which are rich in phytochemicals such as phenolic compounds, flavonoids, and carotenoids, exhibit strong antioxidant properties that protect cells from damage and reduce the risk of various diseases. The understanding of these complex mechanisms, combined with the incorporation of plant-based diets, is a crucial approach to improve disease resistance in the cultivation of *Centella asiatica* [42].

Experiments to optimize cultivation conditions

The cultivation of *Centella asiatica* involves intricate experimentation aimed at optimizing various conditions, ranging from nutrient compositions to temperature regimes. A comprehensive exploration of these factors is crucial for maximizing the yield and concentrations of bioactive compounds in *Centella asiatica*. A notable study outlined in the work by Müller., *et al*. (2013) explores the nuanced effects of different nutrient compositions, specifically nitrogen (N), phosphorus (P), and potassium (K) supply, on herb and leaf yield, as well as centelloside concentrations in *Centella asiatica* leaves. The findings revealed a positive correlation between increased nitrogen (N), phosphorus (P), or potassium (K) supply and assimilation rates, ultimately enhancing herb and leaf yield. However, the study found that exceeding certain thresholds of nutrient availability resulted in reduced leaf nitrogen concentrations, leading to a decrease in assimilation rates and overall plant growth. Furthermore, intriguing negative associations were elucidated between leaf centelloside concentrations and herb and leaf yield,

underscoring the delicate balance required between nutrient availability and the production of essential bioactive compounds. This research, conducted by Müller., *et al*. (2013), serves as a pivotal cornerstone in emphasizing the critical role of optimized and controlled mineral nutrition in enhancing the bio-production of high-quality natural products from medicinal plants, exemplified by *Centella asiatica* [43].

In a similar vein, the research landscape expands to include insights from the study conducted by Yamaguchi., *et al*. (2010), although their focus was on Chattonella ovata, a distinct organism. While the study specifically addresses the optimal temperature conditions for Chattonella ovata, it significantly contributes to the broader understanding of temperature preferences among various species. The study revealed that Chattonella ovata exhibited maximal growth rates between 25-30°C, with strains from Hiroshima Bay displaying a remarkable tolerance to a wide temperature range (15-32.5°C). The adaptability of Chattonella ovata to higher temperature environments, as elucidated in this study, provides valuable insights into temperature preferences. These insights can be applied not only to the cultivation strategies of this particular organism but also to ecosystem management. Although the study's primary focus is on Chattonella ovata, its broader implications for temperature preferences in diverse organisms and ecosystems make it a relevant addition to the collective knowledge base [44].

Testing the effectiveness and efficiency of large-scale cultivation

The need to cultivate *Centella asiatica* on a large scale requires a thorough evaluation that considers various criteria to determine its effectiveness and efficiency. In the scholarly work by Agosto Gomez (2021), an extensive framework is presented for measuring the return on investment (ROI) in large-scale cultivation of *Centella asiatica*. The criteria span a spectrum, including the cost of cultivation, yield, market price, revenue, profit, payback period, net present value (NPV), internal rate of return (IRR), sensitivity analysis, risk assessment, comparative analysis, and long-term sustainability. This comprehensive set of criteria provides a holistic perspective on the economic viability of large-scale cultivation, ensuring a thorough analysis of the investment's strength and long-term sustainability. Notably, Agosto Gomez (2021) advocates for further research to refine return on investment (ROI) analysis, underscoring the commitment to validating the economic viability of large-scale cultivation of *Centella asiatica* [45].

Resource efficiency in *Centella asiatica* cultivation, with a focus on water use, is scrutinized in the scholarly contribution by Hernandez and Cullen (2019), using exergy-based metrics. The introduction of Exergy Efficiency as a comprehensive metric for assessing resource efficiency, taking into account both energy and materials, has proven effective in guiding the transition towards resource efficiency. However, Hernandez and Cullen (2019) highlight the need for increased advocacy to promote exergy efficiency as a key measure of resource efficiency. This suggests potential avenues for future research and the development of guides, training, and software tools to facilitate its broader adoption. This emphasizes the significance of not only economic factors but also resource efficiency in evaluating the sustainability of large-scale cultivation of *Centella asiatica* [46].

In a similar vein, the study by Nordey., *et al*. (n.d.) explores the criteria for evaluating yield in large -scale cultivation, with a particular emphasis on grafted plants. Rootstock-scion combinations and production conditions emerge as pivotal factors influencing the yield of grafted plants. The study highlights the profitability and incentives associated with the use of grafted plants, depending on factors such as the severity of soilborne pathogen pressure, specific production systems, and market dynamics. Nordey., *et al*. (n.d.) advocate for a meticulous consideration of plant grafting strategies to optimize yield in large-scale *Centella asiatica* cultivation, contributing nuanced insights to a specific aspect of the cultivation process [47].

The scalability of *Centella asiatica* production and its implications for cost-effectiveness are comprehensively addressed in the works of Teixeira., *et al*. (2019) and Sampantamit., *et al*. (2020). Successful large-scale cultivation is identified as a driver of economies of scale, resulting in reduced production costs and increased cost-effectiveness. Furthermore, large-scale cultivation facilitates the efficient utilization of resources, such as land and water, thereby maximizing production capacity. The achieved scalability plays a crucial role in meeting growing market demands, stabilizing prices, and maintaining a consistent market presence. However, Teixeira., *et al*. (2019) and Sampantamit., *et al*. (2020) both emphasize the importance of considering environmental sustainability in large-scale cultivation to mitigate negative impacts on the ecosystem. Collectively, these seminal works provide a comprehensive understanding of how large-scale cultivation improves cost-effectiveness, meets market demands, and contributes to the scalability of *Centella asiatica* production [48].

Discussion

Connecting the Dots: Synthesizing Discoveries

The exploration of the rare *Centella asiatica* species is a complex investigation, and it is crucial to have a comprehensive synthesis of key findings from various perspectives in order to gain a complete understanding of this botanical realm. Foundational insights into the characteristics and bioactive compounds of *Centella asiatica*. These scholarly works not only highlight the plant's recognition in traditional medicine but also explain the potential health benefits that can be derived from its triterpenoids, flavonoids, and phenolic acids. This foundational knowledge serves as a solid platform for delving beyond the basics, distribution, and domestication efforts of *Centella asiatica* in Indonesia. These scholarly contributions collectively enrich our understanding of the complex relationship between botanical characteristics, geographical distribution patterns, and cultivation initiatives. The synergy of these findings establishes a solid framework for identifying and characterizing rare species of *Centella asiatica*, highlighting the interconnected dynamics that influence their existence.

Unveiling knowledge gaps: A roadmap for future research

In the current scholarly landscape, the existing body of literature on *Centella asiatica* reveals valuable insights while also highlighting noticeable gaps in knowledge. Works by Sakthipriya., *et al*. (2018b), Mahlangu and Tai (2022), Buraphaka and Putalun (2020), and Amallia., *et al*. (2020) elucidate the intricate and multifaceted significance of identifying rare *Centella asiatica* species. These scholarly contributions cogently emphasize the need for further investigation, especially in the areas of conservation, genetic diversity analysis, and medicinal development. However, a thorough examination of the literature reveals specific gaps that require attention and scholarly investigation.

Critical analysis reveals t make a valuable contribution to the discourse on *Centella asiatica*. However, there is a lack of detailed case studies on DNA barcoding within this context. Furthermore, and highlight the lack of explicit procedures for sequence comparison using bioinformatics tools. These identified gaps present both an opportunity and a challenge for future research endeavors. To navigate this terrain effectively, a comprehensive roadmap emerges, guiding targeted research efforts in the realms of DNA barcoding, taxonomy, and the proficient application of bioinformatics tools.

Intricately woven into the fabric of *Centella asiatica* research, the need to address these gaps in knowledge resonates as a scholarly imperative. The effort to conduct in-depth case studies on DNA barcoding, , is crucial for gaining a comprehensive understanding of the genetic makeup of rare *Centella asiatica* species. Moreover, the importance of methodological clarity in the application of bioinformatics tools is underscored by the need for explicit procedures in sequence comparison.

In this light, the identified gaps serve not only as focal points for future research but also as beacons guiding the trajectory of scholarly exploration. As the scholarly community explores the unexplored areas of *Centella asiatica*, systematically and strategically addressing these gaps holds the potential to enhance the literature, deepen our understanding, and push the field towards new horizons of knowledge. Hence, synthesizing existing insights with a keen awareness of these knowledge gaps paves the way for a more robust and comprehensive scholarly discourse on rare species of *Centella asiatica*.

Bridging science to industry: Practical applications

The utilization of the rare *Centella asiatica* species within the pharmaceutical industry is a subject of strategic consideration, encompassing nuanced discussions on specific formulations and effective commercialization strategies. In elucidating the antibacterial potential against Methicillin-resistant Staphylococcus aureus (MRSA), the works of Stan., *et al*. (2021) and Bell., *et al*. (2021) highlight the importance of triterpenoids, particularly asiatic acid and madecassic acid. These compounds emerge as promising candidates for the development of pharmaceuticals designed to combat antimicrobal resistance, especially in the context of MRSA infections. Beyond the laboratory discoveries, important insights from S. P. Singh., *et al*. (2022), Rafi., *et al*. (2022), S. P. Singh., *et al*. (2022), and Prasad., *et al*. (2019a) highlight the crucial role of large-scale cultivation in meeting the pharmaceutical demand for *Centella asiatica*. These scholarly contributions form a cohesive narrative, emphasizing the importance of connecting scientific discoveries with practical industry applications. The translation of the therapeutic potential inherent in rare *Centella asiatica* species into tangible, marketable pharmaceutical products emerges as a multifaceted process. It requires careful consideration of cultivation, extraction, formulation, and regulatory aspects within the pharmaceutical landscape. This integration, drawing from diverse scientific perspectives, outlines a path for effectively translating botanical

knowledge into viable pharmaceutical products. This contributes to both scientific progress and the ever-changing landscape of the pharmaceutical industry.

Conclusion

The thorough exploration of the literature review on the discovery, genetic diversity, antimicrobal potential, and cultivation of rare *Centella asiatica* species in Surakarta City represents a significant contribution to the comprehensive understanding of this medicinal plant. The foundational insights into identifying rare *Centella asiatica* species, encompassing the common species of *Centella asiatica*), lay the essential groundwork for distinguishing and cataloging rare species. Distinctive characteristics and bioactive compounds, as revealed in scholarly works and, highlight the traditional medicinal importance of the plant, paving the way for further investigations.

The practical implications of identifying rare species, as discussed in the scholarly works "Genetic Diversity of Rare *Centella asiatica* Species, present a focused viewpoint. The potential for pharmaceutical advancements and economic benefits emphasizes the pivotal role of conservation efforts and genetic improvement. The elucidation of antibacterial activity in rare triterpenoids from *Centella asiatica* species, as detailed in scholarly works, opens promising avenues for addressing antimicrobal resistance. This research showcases tangible translational impacts on pharmaceutical research and development.

The methodologies scrutinized in "Antimicrobal Potential against MRSA" elucidate a comprehensive approach encompassing DNA barcoding, sequencing, and bioinformatics tools in studying the genetic diversity of *Centella asiatica*. The recommendations for future research directions, encapsulated in the need for effective primer sets and interdisciplinary collaboration among scientists, chart the course for advancing our understanding of *Centella asiatica*.

Within the context of large-scale cultivation of the rare *Centella asiatica* species, the reiterated importance of large-scale cultivation accentuates its critical role in meeting the escalating demand for pharmaceutical products derived from *Centella asiatica*. The associated socio-economic benefits and sustainability considerations underscore the urgency for responsible cultivation practices.

In conclusion, the literature review, far from being merely informative, serves as a beacon that not only enhances our understanding of *Centella asiatica* but also establishes the groundwork for focused research and practical applications in pharmaceutical advancements, conservation, and large-scale cultivation.

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