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Biotic Threats in *Centella asiatica* (L.) Urban and its Impact on Pharmacological Potential

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Abstract

Centella asiatica (L.) Urban, widely known as gotu kola or Asiatic pennywort, is a small yet highly valued medicinal herb of the family Apiaceae. Native to the India, Sri Lanka, Bangladesh, Nepal, Southeast Asia, China, and some tropical islands. The plant is enriched with diverse bioactive constituents, particularly triterpenoid saponins—including asiaticoside, asiatic acid, madecassoside, and madecassic acid—along with flavonoids, phenolic acids, tannins, alkaloids, volatile oils, and essential minerals. Traditionally recognized as a "brain tonic," it is now supported by modern research for its neuroprotective, anxiolytic, and memory-enhancing properties. Additionally, its extracts promote microcirculation and strengthen the vasculature, making it beneficial for chronic venous insufficiency, varicose veins, and related disorders. Its antimicrobial, anti-ulcer, anti-stress, and mild sedative properties further support its application in gastrointestinal disturbances, fatigue, epilepsy, and general wellness. However, several biotic stresses, like fungal, bacterial, and viral pathogens, cause leaf spots, wilt, blight, mosaic symptoms, and tissue deterioration. Insect infestation further exacerbates yield losses and compromises phytochemical quality. In this study, we have done preliminary anatomical observations of infected versus healthy tissues, revealing significant discoloration, blackened lesions, and stomatal abnormalities, indicating pathogen-induced structural damage. These biotic constraints are crucial for developing integrated disease management strategies and ensuring the sustainable cultivation and pharmaceutical quality of Centella asiatica.

Keywords: *Centella asiatica*, Anatomy, Biotic stresses, Pharmaceuticals, Environmentally friendly.

Introduction

Centella asiatica L. Urban, also known as Asiatic pennywort, Indian pennywort, gotu kola, and pegaga, is a small creeping plant of the family Apiaceae (umbellifereae). It grows and develops throughout the year and throughout the worlds, in several tropical and subtropical regions such as Asia, especially in India, Sri Lanka, and China, and in several regions of Africa[1]. This plant has been employed in various traditional practices such as Ayurveda, Unani, Siddha, and Traditional Chinese Medicine for its healing properties related to wounds, cognitive function, skin disorders, and other conditions[2]. *Centella asiatica* has a longest history of use in traditional medicine. In Ayurvedic practices, it is appreciated for its restorative (or "rasayana") effects. It is commonlyused in traditional Chinese medicine to boost circulation, reduce stress, and eliminate heat, particularly concerning the skin and brain[3]. *Centella asiatica* has garnered attention in recent phytochemical and pharmacological research due to its extensive use in ethno medicine[4].

The pentacyclic triterpenoid saponins present in *Centella asiatica* are termed centellaoids and recognized as one of its essential characteristics. This includes compounds such as asiaticoside and madecassoside, along with their aglycones, asiatic acid and madecassic acid[5]. In the plant, these compounds typically found in the form of glycosides, whereas, they can also occur as aglycones[6]. As well as the main triterpenoid saponins, *Centella asiatica* contains a variety of secondary metabolites including flavonoids, phenolic compounds, glycosides, and tannins, which contribute to its pharmacological properties[7,8].

The array of phytochemicals in plants provides the chemical foundation for their extensive pharmacological effects. Modern analytical techniques, such as high-performance liquid chromatography (HPLC), UPLC-MS, NMR, and metabolomic profiling, have enabled the precise characterization and quantification of these bioactive compounds. This capability supports standardization and quality control in both research and commercial extracts[9].

Recent genomic studies have significantly enhanced our comprehension of the mechanisms by which *Centella asiatica* synthesizes its distinctive triterpenoids. A chromosome-level genome assembly of *Centella asiatica*, approximately 455 Mb in size, has revealed an expansion of gene families associated with triterpenoid biosynthesis, such as squalene synthase (SS) and farnesyl diphosphate synthase (FPS)[10]. Functional analysis has highlighted essential enzymes; for example, CaOSC4, an oxidosqualenecyclase, is known to generate ursane-type triterpenoids in *Centella asiatica*[11].

The recent discovery of tandemly duplicated glycoside glycosyltransferases (GGTs) has revealed how these enzymes incorporate sugar units like glucose and rhamnose into the triterpenoid structure. This finding has shed light on how glycosylated saponins, such as asiaticoside and madecassoside, are formed[12]. The expression of these GGTs in *Centella asiatica* leaves connects to the build-up of asiaticoside and madecassoside. This suggests their roles in metabolism and possibly in defence mechanisms[12].

The pharmacological profile of *Centella asiatica* is broad and backed by both lab and real-world studies. It aids in wound healing and skin repair. Asiaticoside and madecassoside stimulate collagen production, promote blood vessel growth, and accelerate wound healing[6].

i. *Neuroprotection and cognitive enhancement*- Various studies in animal models, along with some early human results, suggest that there are benefits in improving memory, reducing anxiety, and protecting nerve cells[13]. ii. *Antioxidant activity*-Such as tri-terpenes (like asiatic acid), asiaticoside, madecassoside, and madecassic acid have strong antioxidant properties. They reduce harmful reactive oxygen species (ROS) in cells that are under oxidative stress[14]. Anti-inflammatory effects. Compounds from *Centella asiatica* can regulate pathways such as NF-κB and cytokine production by lowering pro-inflammatory agents[15]. iii. *Safety and toxicity*- It is usually consider as a safe at therapeutic doses. However, some side effects, such as skin irritation and digestive issues, have reported[16]. The main triterpenoid saponins play animportant role in the therapeutic benefits of *Centella asiatica*. While *Centella asiatica* is valued for its health advantages, it can suffer from biological stress, such as infections from pathogens. It is important to understand how pathogens affect *Centella asiatica* to improve cultivation and maintain the quality of raw materials. iv. *Fungal wilt disease*- In Bangladesh, *Fusarium equiseti* has identified as the cause of wilt disease in *Centella asiatica*[17]. The pathogen thrives under specific pH and temperature conditions, which can severely harm the plant. v. *Leaf spot disease*- In some areas, leaf spot in *Centella asiatica* is caused by *Alternaria* spp[18]. vi. *White rot*- A study from West Bengal, India, reported that *Sclerotinia sclerotiorum* causes white rot in *Centella asiatica*[19]. vii. *Bacterial blight*- In Vietnam, a bacterial blight affecting *Centella asiatica* was linked to *Pseudomonas marginalis*[20]. viii. *Centella asiatica* has a variety of endophytic microorganisms, including bacteria and fungi.

These microorganisms can influence the plant's health, disease resistance, and metabolite production. A study found 31 endophytic bacteria in healthy *Centella asiatica* leaves. It showed that *Bacillus subtilis* and *Pseudomonas fluorescens* effectively inhibited the growth of the fungal pathogen *Colletotrichum higginsianum*. When these bacteria were applied to the plants before they encountered the pathogen, both the number of diseases and their severity decreased[21].

Surveys of fungal endophytes in *Centella asiatica* leaves from Madagascar showed high colonization rates of 78%[22]. Another interesting study investigated *Paradendryphiella arenariae*, an endophytic fungus from *Centella asiatica* stems. Its extracts displayed strong antibacterial activity against fish pathogens. This highlights the potential of *Centella asiatica*'s endophytes in biotechnology[23].

Endophytes defend against pathogens and influence the plant's secondary metabolism. In *Centella asiatica*, researchers have used endophytic elicitors to increase centelloid production. Techniques like callus culture, hairy root culture, and elicitation have used to raise the levels of asiaticoside and madecassoside[24].

The microbial ecology of *Centella asiatica*, including both harmful and beneficial aspects, is vital for the plant's health and changes in its metabolites. When a pathogen invades, whether it is fungal, bacterial, or viral, it likely disrupts the epidermal layer. This invasion damages the cell walls and causes necrosis in the underlying mesophyll. Such damage can impair photosynthesis, which reduces the energy available for metabolite production.

Pathogen stress may also affect stomatal behaviour. Changes in stomatal density and openings can occur, due to signals either from the pathogen or from physical disruption. Changes in stomatal function could affect transpiration, gas exchange, and internal water conditions, all of which influence secondary metabolism. Pathogen effects on *Centella asiatica* have direct implications for its use as a medicinal plant.

Infected plants may produce lower amounts of asiaticoside, madecassoside, and other related triterpenoids, potentially diminishing the effectiveness of extracts used for therapeutic or cosmetic purposes. Structural damage, like necrosis and tissue breakdown, could affect not only metabolite levels but also purity.

This can lead to contamination if not properly addressed. The presence of disease may cause variability in metabolite profiles from batch to batch, making it harder to standardize commercial extracts. Quality control must include health assessments of the source plants. It may also require microscopic or microbiological analysis before extraction.

Anatomical characterization involves preparing leaf sections from both healthy and diseased plants. This allows for the documentation of morphological changes, such as epidermal damage, mesophyll collapse, vascular injury, and stomatal changes, through microscopic observation.

Stomatal analysis focuses on stomata in healthy and infected leaves. This can reveal how pathogen stress affects gas exchange and, indirectly, overall plant function.

Materials and Methods

Plant Material: Fresh samples of *Centella asiatica* (Gotu Kola) collected from the Deen Dayal Upadhyaya Gorakhpur University. Both healthy and infected plants selected to ensure accurate comparative analysis. Healthy plants identified based on uniform green coloration, patches, and absence of visible lesions. Infected plants were chosen based on recognizable disease symptoms (Figure-1). All plant material collected in clean sample bags and processed immediately to prevent degradation.

Methods: ***Sectioning of Leaves:*** To study internal tissue structure, thin transverse sections (T.S.) of leaves from both healthy and infected samples were prepared. Fresh leaves washed gently to remove dust and contaminants. Using a sharp blade, very thin sections cut from the region of the leaf lamina to ensure uniformity. The sections then placed on a clean microscope slide containing a drop of distilled water. A coverslip applied carefully to avoid air bubbles, ensuring a clear field of view during microscopic observation.

Microscopic Analysis: Microscopic examination conducted using a compound light microscope under different magnifications. Observations focused on the epidermis, mesophyll tissues, vascular bundles, and stomatal structure. *Comparisons made between healthy and infected samples to identify*: i. Changes in tissue arrangement or cellular integrity, ii. Alterations in stomata, iii. Presence of pathogen like structures, iv. Structural deformities in mesophyll layers due to infection.

This analysis helped determine the extent of damage caused by the pathogen and its impact on leaf physiology.

Documentation: Photographic documentation carried out visually compare healthy and diseased conditions. Images taken covering: i. Whole plant appearance (healthy vs infected), ii. Close-up views of symptomatic leaves, iii. Microscopic images of healthy and infected transverse sections, iv. Any pathogen-specific structures observed during microscopy.

All images systematically labelled and archived for further analysis, presentation, and report preparation.

Results and Discussion

Results: Microscopic examination of healthy leaves showed a clearly defined epidermis with compact, intact epidermal cells. The palisade and spongy mesophyll tissues well organized, showing uniform cell arrangement and proper intercellular spaces. Stomatal openings were regular in shape and evenly distributed, indicating normal physiological functioning (Figure-2).

In contrast, infected leaf sections exhibited noticeable anatomical disruptions. Tissue Disintegration observed in the mesophyll region, with collapsed or irregularly shaped cells. The epidermal layer appeared damaged or distorted, and stomatal structure was disturbed, showing irregular openings, enlargement, or partial collapse. Some sections also showed darkened or necrotic areas, indicating pathogen invasion.

Overall, the visual comparison between healthy and infected samples clearly highlighted the extent of structural deterioration caused by the pathogen. The loss of cellular integrity, deformation of tissues, and alteration in stomatal architecture all indicated significant stress and damage to the plant (Figure-2).

Figure-1: a. Infected leaves of Centella asiatica. b. healthy leaves of Centella asiatica.

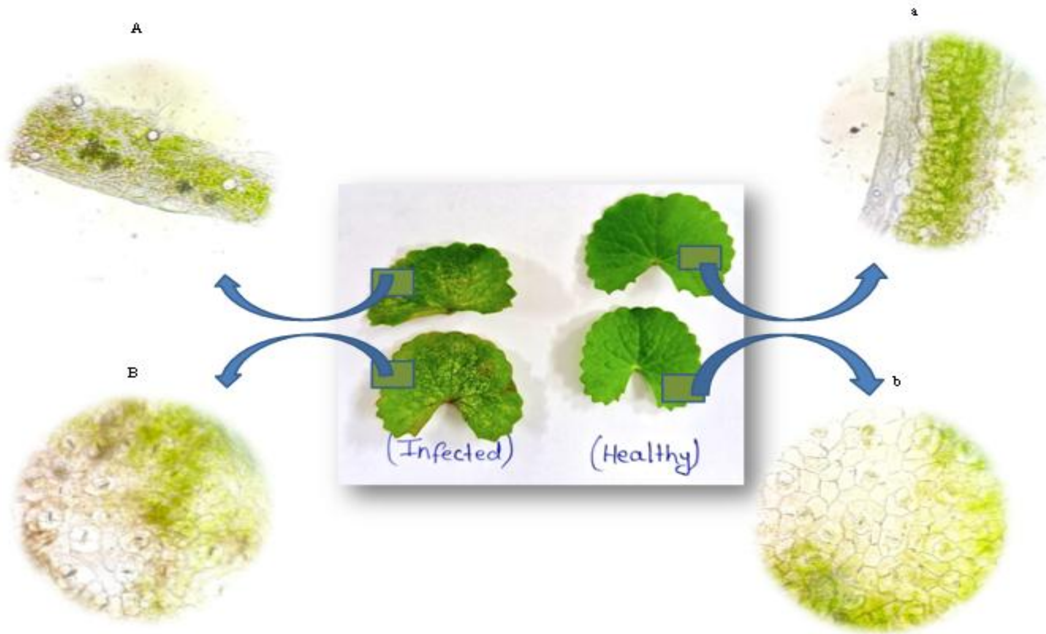

Figure-2: A. Tissue disintegration B**.** Disturbed stomatal balance structure. a. Tissue integration b. Stomatal balanced structure.

Discussion: The observations indicate that infection in *Centella asiatica* leads to substantial anatomical modifications, which directly reflect the plant's defensive response and the severity of pathogen activity. These infections frequently alter the normal leaf structure, resulting in collapsed mesophyll tissues, irregular or malformed stomata, disrupted epidermis, and reduced cellular organization.

Such structural deformities negatively influence essential physiological processes, including photosynthesis, transpiration, and gas exchange. Reduction in chlorophyll content, along with distortion of palisade cells, limits the plant's ability to capture light efficiently. Disturbed stomatal function alters water regulation and increases stress levels.

Importantly, these anatomical and physiological changes also affect the biosynthesis and accumulation of key medicinal metabolites such as asiaticoside, madecassoside, and triterpenoids. When the plant experiences infection-induced stress, metabolic pathways may shift; leading to either reduced metabolite production or altered composition.

Therefore, the microscopic and visual differences observed between healthy and infected leaves emphasize the broader impact of pathogen attack on the structural health, metabolic stability, and overall medicinal value of *Centella asiatica*.

Conclusion

The current research clearly illustrates that pathogenic infections in *Centella asiatica* significantly disrupt its anatomical structure, physiological functions, and overall medicinal properties. Structural anomalies such as disrupted tissue organization, collapsed cells, and irregular stomatal patterns consistently observed in infected samples, indicating a direct impact on plant health and metabolic efficiency. Conversely, healthy specimens exhibited well-organized tissues and effective stomatal function, which supports their enhanced phytochemical profile and therapeutic capabilities.

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