

Screening of Secondary metabolites produced from dry leaves of *Centella asiatica* and its isolated endophytic fungi

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Abstract

The new emerging investigation has challenged the scientific community to explore the efficacies of some medicinal plants and their associated microbial diversity. The fungal endophytes play a vital role in human life because these are the sources of drug for various deadly diseases like cancer, bacterial, viral fungal infections. Present work deals with biochemical screening for the presence of different types of secondary metabolites of *Centella asiatica* whole tissue extract as well as its isolated endophytic fungal extract. The endophytic fungi are present in almost all types of plants, which live as symbiont. Many studies revealed that most of the secondary metabolites are common in both endophytes as well as its host tissue *Centella asiatica*, which show a wide array of biological and pharmacological properties. Because of these properties the crude extract of many medicinal plants and their endophytes are investigated for their efficacies against different diseases. On my previous work the morphological and molecular characterization of isolated endophytic fungi from *Centella asiatica* was observed, which revealed the presence of *Penicillium*, *Aspergillus*, *Collectotricum*, *Cladosporium*, *Curvularia*, *Alternaria* species, during present investigation methanolic extract of *Centella asiatica* was prepared for screening of secondary metabolites. Similarly methanolic extract of endophytic fungi isolated from *Centella asiatica* was carried out to observe the presence of secondary metabolites. For isolation of endophytic fungi, a definite protocol was followed and cultured.

Further it was investigated that endophytic fungi originated from *Centella asiatica* have the capability to produce same secondary metabolites which are produced by the whole tissue of *Centella asiatica*. In future the products from endophytic fungi will be a cheap source for medical, agriculture and pharmaceutical industries. It is sure that the research on endophytic fungi will lead to isolate more novel compounds.

Keywords: Endophytes, secondary metabolites, novel drugs, pharmaceutical, symbiont. I.

Introduction:

In this study *Centella asiatica* (L.) was chosen as a host plant for endophytic fungi isolation which is abundant in the state Jharkhand. It belongs to Family-Apiaceae, it is commonly known as Gotu kola or Indian Pennywort, is a herbaceous plant with great medicinal value. Triterpenoid, saponins and sapogenins, particularly asiaticoside, madecassoside, asiatic acid and madecassic acid, are the chief bioactive molecules present in this plant (Mathur *et al.*, 2007; Randriamampionona *et al.*, 2007). Asiaticoside extracted from the aerial parts of the plant is known to exert strong diuretic, antispasmodic, circulation stimulatory and wound healing actions and is frequently used in the

treatment of leprosy, ulcer, asthma, bronchitis, elephantiasis, eczemas, anxiety, mental disorders and urethritis (Mathur *et al.*, 2007; Gohil *et al.*, 2010). Asiaticoside derivatives are also widely used in the treatment of Alzheimer's disease as they can protect cells against b-amyloid induced cell death (Mook-Jung *et al.*, 1999). Recently, studying the possible mode of action of asiaticoside in wound healing and suppression of proliferation of keloid-derived fibroblasts and collagen production, Tang *et al.*, (2011) have demonstrated that asiaticoside suppresses collagen expression and TGF- β /Smad signalling by inducing Smad7 and inhibiting TGF- β receptors RI and RII in keloid fibroblasts to

In vitro Root Induction of *Centella asiatica* (L.) in Different Concentrations of Indol 3-Butyric Acid (IBA)

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Abstract: *Centella asiatica* L. is an important herbal medicinal plant, abundant in Jharkhand. It is considered a brain tonic and also a good wound healer. The study was conducted to determine the most suitable explant and different concentrations of IBA for adventitious root induction. The detailed study of adventitious root induction has been performed by varying the concentration of IBA in the range of (0, 0.5, 1.0, 1.5 2.0) mg/l. The highest percentage of explants forming adventitious roots, number of roots per explant, and longest roots generated from leaf explant were observed in the concentration of 1.5 mg/l IBA, Tap roots were also observed in nodal explant.

Keywords: Explant, adventitious roots, IBA, *Centella asiatica*, tap roots etc

1. Introduction

In traditional medicine, the roots of many plants are used and harvested by local people for the production of herbal medicine. Roots are an abundant source of much-valued secondary metabolites, which can be beneficial to mankind for combating various ailments. The rootstock of *Centella asiatica* consists of rhizomes, a growing horizontal underground stem that puts out lateral shoots and adventitious roots at intervals. An adventitious root formation is a key step in the vegetative propagation of many crops and therefore of at most economic importance (Scheres 2000). They are pale yellow in color and covered with root hairs. They are capable of producing some economically important compounds such as asiaticoside and madecassoside which are active triterpene compounds produced in insignificant amounts in the root of *Centella asiatica* (L.) Urban. The asiaticoside has wound curative ability by increasing angiogenesis and collagen formation. It enhances the stretching strength of newly formed skin and promotes the wound's healing. Madecassoside is an active antioxidant that has the ability to scavenge free radicals. Therefore, the root cultures can display high biosynthetic capabilities that are often comparable to those of normal roots (Keveres et al 1999). The culture of adventitious roots is a potential source for the production of valuable plant secondary metabolites on a commercial scale (Min et al, 2007) Roots are the principal material for the preparation of drugs from the traditional system of medicine.

The present study is based on the regeneration of adventitious roots in response to different concentrations of IBA. Phytohormones play a significant role in the formation of adventitious roots. Adventitious roots are a special type of root system that arise from an organ other than the root, usually from non-root parts of plants such as leaves, petioles, nodes, and internodes, and help plants to survive in environmentally adverse conditions. Several complex molecular processes such as endogenous and exogenous

physiological factors like stress and wounding are responsible for the formation of adventitious roots. These roots are formed during normal growth and development or either in the reaction to wounding, nutrient deficiency, or various kinds of environmental stresses. Adventitious roots (AR) also facilitate gas and water transport and uptake of minerals and nutrients and ensure plant survival (Sauter, 2013). Well-organized root regeneration was observed on an MS medium containing different concentrations of IBA on different explants of *Centella asiatica*.

2. Materials and Methods

Sampling

Mature and healthy branches of *Centella asiatica* with no visible symptoms of the disease were carefully selected were collected from the herbal garden of Ranchi Women's College, brought to the laboratory in sterile bags, and processed within 24 hr after sampling. These plantlets were identified by Prof. Kunul Kandir, Taxonomist of the University Department of Botany, Ranchi University

Surface sterilization of the explant

The surface sterilization was done as per the procedure of Tejavathi et al. (1996). Initially, the plant samples were washed for 5-10 minutes thoroughly under running tap water to remove adhered dust and debris. It was then washed with teepol and was cut into small pieces with 1.5 mm segments which were surface sterilized with 70% ethyl alcohol for 1 min. The segments were soaked in 0.2% (w/v) mercuric chloride solution for 1 min and then rinsed with 70% ethyl alcohol for 1 min, and finally rinsed thrice with sterile distilled water.

Media preparation:

1 L of Murashige and Skoogs media were prepared by using various inorganic nutrients, it includes macro and micronutrients along with vitamins and carbon sources in 400 ml double distilled water. The pH was adjusted to 5.8

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Antibacterial Activities of Different Solvent Extracts of *Centella asiatica* (L.) Urb. and its Endophytic Fungi

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Abstract: Evaluation of the antibacterial properties of fungal endophytes associated with *Centella asiatica* (L.) Urb., the ethnomedicinal plant widely used in traditional medicine practices, is part of the current study. Considering both molecular characterization and morphological characteristics, five endophytic fungi were isolated and identified namely *Cladosporium* sp. (F1), *Penicillium* sp. (F2), *Aspergillus* sp. (F3), *Colletotrichum* sp. (F4) and *Curvularia* sp. (F5). *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus cereus* are the four pathogenic bacterial strains that were examined for the antibacterial activity of plant extract and its endophytic fungus extract. The lowest concentration of the extract which inhibits any visual growth was considered to be Minimum Inhibitory Concentration (MIC). The zone of inhibition that different extracts produced against selected strains was measured. The results showed that among all endophytic fungal extracts studied F4, *Colletotrichum* sp. exhibited the highest zone of inhibition against *E. coli*, while the hydroalcoholic and aqueous extracts of the *Centella asiatica* leaf sample showed the maximum zone of inhibition against *E. coli* and *S. aureus*, respectively.

Keywords: Endophytic fungi, *Centella asiatica* (L.) Urb., Antibacterial activities, Apiaceae, ethnomedicinal, etc.

1. Introduction

Centella asiatica (L.) Urb. is an herbaceous, perennial plant commonly known as Indian pennywort and Asiatic pennywort belongs to the family Apiaceae [1]. It is locally known as Jal Brahmi, Beng Saag, and grows in many temperate and tropical marshy places. It is native to Southeast Asia, the Indian subcontinent, and the wetland areas of the southeastern US [2, 3]. Due to the generation of various bioactive secondary metabolites, *Centella asiatica* is one of the most significant therapeutic herbs used in Indian Ayurvedic traditions. It is also known for its antibacterial, antifungal, antidiabetic, antidiuretic, and antioxidant [4] properties. Its diverse bioactive ingredients, which possess cytotoxic and anticancer [5], cardioprotective [6], anti-inflammatory [7], neuroprotective [8], and wound healing [9] properties, are essential for improving medical problems. *C. asiatica* prevents the oxidative damage that takes place in neuropathological disorders, including stroke, Parkinson's disease, and Alzheimer's disease, by improving the antioxidant neurological state related to aging.

Endophytic microorganisms are recognized as one of the most chemically promising groups of microorganisms in terms of diversity and pharmaceutical potential. These are microorganisms, primarily fungi and bacteria, that proliferate in the intercellular spaces of higher plants without evidently harming their hosts. Endophytic fungi form enduring associations with their host plants because

endophytes have been shown to mediate the development of secondary metabolites in some plant species. A few endophytic fungi, such as *Colletotrichum gloeosporioides*, have been documented in various existing literature to produce asiaticoside and madecassoside as secondary metabolites [10]. Several bioactive secondary metabolites, such as steroids, alkaloids, peptides, terpenoids, tannins, quinone, flavonoids, and phenolics, are characteristic of endophytic microorganisms, particularly fungi [11]. It is now known that ethno-medicinal plants include a wealth of endophytes that may yield new metabolites with significant therapeutic value [12, 13, 14]. Certain natural compounds that are associated with endophytic fungi may have antibacterial, antioxidant, anti-tumour, and anti-inflammatory properties [15].

The exploration of plants for endophytic fungi can be of immense value in screening for potential metabolites. So, the present investigation has been designed to evaluate the antibacterial and antioxidant activities of endophytic fungi associated with *Centella asiatica* (L.) Urb.

2. Materials and Methods

2.1 Sampling

Centella asiatica plants with no visible symptoms of the disease were carefully selected and collected from the local area of Ranchi. The plant was identified and authenticated



Estimation of total Phenolic, Flavonoid Content and Antioxidant Activity of Endophytic Fungi associated with *Centella asiatica* (L.) Urb.

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ABSTRACT

The current investigation aimed to elucidate the free radical scavenging activity of *Centella asiatica* (L.) Urb. and its endophytic fungi. It is a perennial herb, widely used in ayurvedic practices, and has increased over the years basically due to its beneficial functional properties. Its potential antioxidant effect is due to its bioactive constituents. The present study deals with total phenolic content, total flavonoid content, and free radical scavenging activity. The free radical scavenging activity of the plant and its five isolated endophytic fungal extracts were estimated using DPPH and FRAP assays. The total phenolic and total flavonoid content was found to be higher in the hydroalcoholic extract than the aqueous extract of *Centella asiatica* (L.) Urb. while in endophytic fungal extract, *Penicillium sp.* (F2) and *Aspergillus sp.* (F3) showed the highest amount of total phenolic and total flavonoid content respectively. The results of the antioxidant assay indicated that the hydroalcoholic extract of *Centella asiatica* (L.) Urb. exhibited greater free radical scavenging activity than the aqueous extract, while *Aspergillus sp.* (F3) exhibited the highest scavenging activity among the ethyl acetate endophytic fungal extract. During the present work, the highest FRAP value of hydroalcoholic extract was observed while among endophytic fungal extracts, *Aspergillus sp.* (F3) has the highest FRAP value. Thus, in the present study, it can be concluded that the major contributor for antioxidant activity in each assay was the total flavonoid content present in extracts. So, the metabolites from fungi and their host plants are an excellent source of natural antioxidants and efficient against diseases that pose a threat to human health. Hence, these can be used for novel drug formulations.

KEYWORDS- Antioxidant activities, DPPH assay, FRAP assay, Phenolics, Flavonoids

1.INTRODUCTION-

Centella asiatica (L.) Urb. commonly known as Indian Pennywort, belongs to the family Apiaceae. It is a creeping perennial herb that frequently grows in Indian environments and has been traditionally used to treat a variety of ailments and has several distinct biochemical components that are crucial to its use in medicine and nutraceuticals. It has been used to treat skin conditions and has anti-inflammatory, antioxidant, wound-healing, and memory-boosting

properties [1]. Also, it has been shown to have a protective effect against oxidative damage caused by neurotoxicity induced by lead acetate [2].

Medicinal plants are a reservoir of endophytes with novel metabolites of therapeutic significance. Research on natural commodities can greatly benefit from the endophytes of medicinal plants [3]. Endophytes comprise a significant amount of the diversity of microorganisms [4]. It is more likely that