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## RESEARCH ARTICLE

Screening of *Clitoria ternatea* and *Cymbopogon citratus* for presence of endophytes

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## Abstract

The term 'endophytes' encompasses a suite of micro-organisms that thrive in association with plants without causing any apparent symptoms or damage to the host. The two medicinal plants selected for the current study, *Clitoria ternatea* L. and *Cymbopogon citratus* L. were successfully screened for endophytes. Bacterial endophytes were isolated and characterized. These isolates were further studied for any beneficial property with a view to develop a useful application. The endophytes were studied for production of phenolic compounds using test and total phenolic content was quantitated by performing a spectrophotometric assay employing Folin Ciocalteau reagent. The endophytic isolates were also screened for production of antioxidants and the percent antioxidant activity of the culture broth was determined.

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## 1. Introduction:-

Indian medicinal systems like Ayurveda, Siddha and Unani employ a number of plants in the treatments of various diseases. The therapeutic properties possessed by the plants are utilized to their fullest in many parts of the world. However, in many cases it was found that the medicinal compounds extracted from these plants were actually being produced by the micro-organisms residing in the tissues of the host plants. These microorganisms belong to diverse species of bacteria and fungi and are called as endophytes. Endophytes are the microorganisms that live inside the cells of the plants without causing any apparent symptoms or damage to the host.

Endophytes are found in a large number of plants in different types of associations ranging from mutual to mildly parasitic. The host plant provides the endophytes with a safe conducive habitat and the endophytes help the plant in return. The endophytes are known to provide a number of properties to the host plants viz., resistance to a disease, production of medicinal compounds, growth factors, etc. Endophytes have been discovered in most of the plants studied for their presence. They have been isolated from diverse plants like tomatoes, coffee, marigold, trees like Yews and pine, cereal plants like maize, wheat, rice, fodder plants like alfalfa, sorghum, tubers and roots like potatoes and sweet potatoes etc. (Rosenblueth et. al., 2006)

The most significant discovery was the anticancer compound- 'taxol' which was initially extracted from the tree *Taxus brevifolia*. Taxol (or paclitaxel) is very effective in treating advanced stages of cancer. However, the trees it is extracted from are rare and slow growing and produce very small quantity of taxol. Hence, the compound is very expensive when it is isolated from its natural source. Besides, using natural source leads to deforestation and therefore disrupts the ecological balance of environment. It is hence necessary to find an alternative approach in acquisition of these medicinal compounds. This was achieved when Taxol-producing fungal endophyte *Taxomyces andreae* was isolated from the source tree. It was later on found to be produced by a host of different fungi. (Pimentel M. R. et al., 2011) There have been similar instances when the medicinal compound thought to be produced by a particular plant was found to be actually produced by the endophytes of that plant. If the endophytes are isolated and directly used for producing the medicinal compounds in vitro, they would prove to be a beneficial, faster, cheap and eco friendly alternative.

Since, medicinal plants used in treatments are known to produce different compounds; they have higher prospects of harboring significant endophytes. The plants chosen for the current study are *Cymbopogon citratus* and *Clitoria ternatea*.

*Clitoria ternatea*, is a plant species belonging to the Fabaceae family and sub-family Papilionaceae. This plant is native to tropical equatorial Asia and is also called as butterfly pea, blue pea or Gokarna in Marathi. It is a perennial herbaceous vine or creeper, with elliptic, obtuse leaves. The most striking feature about this plant are its vivid deep blue flowers; solitary, with light yellow markings. They are about 4 cm long by 3 cm wide. There are some varieties that yield white flowers. The fruits are 5 – 7 cm long, flat pods with 6 to 10 seeds in each pod. It is a legume. (Zingare M.L. et.al, 2013)

In traditional Ayurvedic medicine, it has been used for centuries for treating different disorders and as a memory enhancer. In animal tests, the methanolic extract of *Clitoria ternatea* roots demonstrated nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. The phyto chemicals of this plant include pentacyclic triterpenoids like taraxerol and taraxerone, a nucleoprotein, delphinidin-3,3,5-triglucoside,  $\beta$ -sitosterol, hexacosanol and anthocyanin glucoside (in seeds), pentosan, essential amino acids, adenosine, an anthoxanthin glucoside, a phenol glycoside, 3,5,7,4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid, ethyl D-galactopyranoside, p-hydroxy cinnamic acid polypeptide, a highly basic protein-finotin, and a bitter acid resin, tannic acid. It also contains anti-fungal proteins. The plant seeds contain lectin designated as CTL (*Clitoria ternatea* lectin) which is a potential tool for cancer studies. Its blue flower extracts are used as natural colorant for food, cosmetic and pharmaceutical products as it is nontoxic and has antidotal and antioxidant properties. (Das N. et al., 2014)

*Cymbopogon citratus* is a tropical grass belonging to family Poaceae – sub family Gramineae. It is a plant from South Asia, commonly known as lemon grass. It is an aromatic perennial, tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters. Its leaves are traditionally used in cooking. *Cymbopogon citratus* contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol. It contains various phytochemicals like alkaloids, tannins, saponins, flavonoids, quinine and anthraquinone. (Hindumathy C. K., 2011) It has been used in folk medicine to treat cough, consumption, elephantiasis flu, gingivitis, headache leprosy, malaria, ophthalmia, pneumonia and vascular disorders. It is principally taken as tea as a remedy for digestive problems, diarrhoea and stomach ache (Carlin, et al., 1986). Studies on extracts from *Cymbopogon citratus* leaves have demonstrated anti-inflammatory, vasorelaxing, diuretic, antibacterial, carminative, insect repellent and valuable remedy in treating ringworm as a local application (Melo, et al., 2001; Runnie, 2004; Oloyede, O. I., 2009)

## 2. Materials and methods:-

### 2.1 Collection of plant material:

Healthy leaf samples of *Clitoria ternatea* which is a potted plant were procured from Dombivli (19.2184° N, 73.0867° E) in Maharashtra and *Cymbopogon citratus* was obtained from a nursery in Thane district, Maharashtra (19.1724° N, 72.9570° E). The fresh samples were collected in clean, sterile bags and brought to lab. Samples were processed within 2 hours of collection.

### 2.2 Isolation of endophytes:

The samples were initially washed and cleaned with tap water. Later they were processed according to the method described by Gayathri P. et al., 2013. The leaves were surface sterilized by washing with 70% ethanol for 1 minute followed by a wash with 3-5% sodium hypochlorite for 1 minute. Then finally three consecutive washes were given with sterile distilled water for 2 minutes.

The surface sterilized leaves were then placed on sterile nutrient agar plates, the lower side of the leaves facing down on the agar. These plates were then incubated overnight at 28°C. Growth obtained after incubation of 24 hours was isolated further on nutrient agar plates and studied. The plates were further incubated for 5 days to allow any additional growth. There was no additional growth obtained at the end of the incubation.

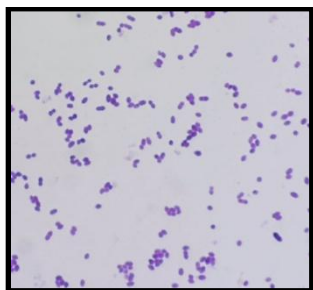
The initial selection of colonies was based on their phenotypic appearance. Colonies with visibly different characteristics were isolated and sub-cultured on nutrient agar plates to obtain pure strains. These endophytic strains were found to be bacterial strains on microscopic examination. They were further gram stained, characterized and studied for different properties.

The endophytic isolates were tested for production of phenolic compounds in liquid media by performing qualitative as well as quantitative tests. The qualitative test involved interaction of sample with lead acetate to give white precipitate if phenolic compounds are present. The estimation of phenolic compounds was done by spectrophotometric assay as per Jing Chung Chen method in triplicates. Average total phenolic content was calculated using the spectrophotometric readings.

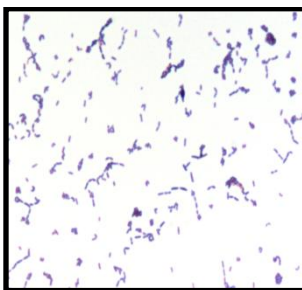
The cultures were grown in liquid media for 24 hours and then centrifuged. The supernatant was tested for antioxidant activity by performing free radical scavenging assay by Chang et.al. method (2001). Each sample was tested in triplicates and the average activity was calculated.

### 3. Results and discussion:-

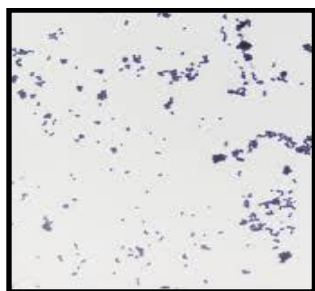
After initial incubation, the endophytic growth was obtained around the leaf along the periphery. This growth was further isolated and repeatedly sub-cultured to get 4 pure isolates (2 from each plant). The 4 bacterial endophytes were further gram stained and found to be gram positive. The isolates 1 and 2 were isolated from *Clitoria ternatea* and isolates 3 and 4 were isolated from *Cymbopogon citratus*. Isolate 1 (figure 1) was found to be gram positive cocci in clusters and Isolate 2 (figure 2) was observed as gram positive short rods in chains. Isolate 3 (figure 3) was observed to be gram positive cocci in singles and clusters and Isolate 4 (figure 4) was gram positive cocci in clusters. All the isolates showed production of phenolic compounds and a good antioxidant activity as indicated in the Table 1 below.



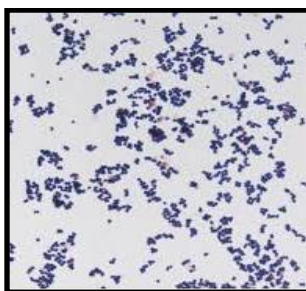
**Figure 1:** Isolate 1-  
gram positive cocci in  
clusters



**Figure 2:** Isolate 2-  
gram positive short  
rods in chains



**Figure 3:** Isolate 3-  
gram positive cocci in  
singles and clusters



**Figure 4:** Isolate 4-  
gram positive cocci in  
clusters

**Table 1: Total phenolic content and antioxidant activity produced by endophytic isolates**

Endophyte	Phenolic ( $\mu\text{g/ml}$ )	Antioxidant activity (%)
Isolate 1	11.583	38.725
Isolate 2	12.834	41.975
Isolate 3	13.08	42.615
Isolate 4	11.085	46.31

#### 4. Conclusion:-

The two medicinal plants under study, *Clitoria ternatea* and *Cymbopogon Citratus*, were successfully screened for endophytes and four bacterial endophytes were isolated. These endophytic strains were further studied for their properties and showed significant antioxidant activity and production of phenolic compounds. These results suggest that the endophytic isolates might actually be responsible for production of some bioactive compounds which need to be identified for further applications in the fields of pharmaceuticals, agriculture, etc.

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