

SUMMARY OF FINAL REPORT OF MINOR RESEARCH PROJECT

Entitled

**Isolation and evaluation of bioactive compounds from the efficient
endophytic isolates of *Limonia acidissima***

Submitted To



University Grant Commission,

Western Regional Office

Ganeshkhind,

PUNE

Submitted By

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Annexure – VII

**PROFORMA FOR SUBMISSION OF INFORMATION AT
THE TIME OF SENDING THE FINAL REPORT
OF THE WORK DONE ON THE PROJECT**

Annexure – VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Title of the Project : "Isolation and evaluation of bioactive compounds
from the efficient endophytic isolates of
Limonia acidissima"
2. Name and Address of the Principal Investigator: **Dr. Suvarna P. Patil**
3. Name and Address of the Institution: **Taywade College, Koradi.**
4. UGC Approval Letter No. and Date: **File no.47-1254/14(General/77/WRO)
XII Plan 22nd March 2017**
5. Date of Implementation: **1st May 2017**
6. Tenure of the Project: **Two years**
7. Total Grant Allocated: **Rs. 1,90,000/-**
8. Total Grant Received: **Rs. 1,45,000/-**
9. Final Expenditure: **Rs. 1,91,415/-**
10. Title of The Project: "Isolation and evaluation of bioactive compounds
from the efficient endophytic isolates of *Limonia
acidissima*
11. Objectives of The Project:
 - i. Brief objective of the project :Objectives of research project are as follows:
 1. Isolation & Identification of endophytic microorganisms from *Limonia
acidissima*.
 2. Study of bioactive Compounds produced by Endophytic microorganisms

3. Study of bioactive Compounds present in various parts of *Limonia acidissima*.
4. Separations of bioactive compounds & study their biological activity.
5. Molecular characterization of effective endophytic microorganisms.

12. WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS): Yes, all the objectives were achieved. The present study comprises of following

1. successful isolation of endophytes (bacteria and fungi)
2. Identification of endophytes (bacteria and fungi)
3. Qualitative analysis of secondary metabolites present in plant part extracts and produced by endophytic isolates.
4. GCMS analysis of plant part extracts and fermented broth of endophytic isolates.
5. Molecular study i.e. plasmid isolation and characterization of one efficient isolate.

The study carried out is in line with the objectives of the project.

13. ACHIEVEMENTS FROM THE PROJECT: One abstract in the form of poster was published.

14. SUMMARY OF THE FINDINGS (IN 500 WORDS):

In the present study plant *Limonia acidissima* has been investigated in detail for their bioactive compounds present in root, stem bark and leaves, along with that presence of endophytic fungi and bacteria are also been investigated successfully in relation to the plant growth promoting features and antibacterial as well as antifungal activity.

In the first phase, roots stem bark and leaves extract (10g/100ml) of *L. acidissima* in the solvents such as petroleum ether, chloroform, acetone, methanol and water have been prepared.

In the study, plant extract were found to contain alkaloids, carbohydrates, glycosides, proteins and amino acids, saponins and phytosterols, fats, phenolic compounds and flavonoids, gum and mucilage when tested by biochemical analysis.

In the present study ability of endophytes of *L. acidissima* has been investigated successfully providing beneficial features to the host plant. Here bacterial endophytes total fifteen in number were isolated from root, stem, bark and leaves. Similarly, total fifteen fungal isolates were isolated from root, stem bark and leaves.

Ability of endophytic isolates to produce plant growth promoting biomolecules have been evidenced.

In the present study, Indole acetic acid producing bacterial isolates successfully detected and those isolates (ERb5, ESb3, ESb5 and ELb3) were isolated prominently from the roots, stem bark and leaves of *L. acidissima* having value 0.13, 0.28, 0.24 and 0.23 respectively.

Here all bacterial species have not demonstrated phosphate solubilisation features but ammonia production recorded to be present in all bacterial species. Hence studied bacterial species having PGPA and reported as the isolates with agricultural potential.

Along with the bacterial species, fungal isolates also reported to be promising PGPA when one fungal (ERf6) isolate found to be IAA producer prominently isolated from root; one isolate (ESf2) from stem bark and two isolate (Elf1 and Elf4) from leaves part having IAA value 0.06, 0.02, 0.06 and 0.08 respectively.

Further all fungal isolates reported to be negative for phosphate solubilisation and positive for ammonia production and hence it has been summarized that *L. acidissima* posses both bacterial and fungal species as PGPA and few of them reported to be promising isolates as shown in the study.

Among these PGPA attributed bacterial species number of isolates were identified successfully with VITEK and 16S rRNA gene sequencing and isolates were identified as *Aeromonas sobria*, *Bifidobacterium thermacidophilum* and *Pantoea Spp.*

In a similar manner, fungal species were identified as *Curvularia lunata*, *Aspergillus flavus*, *Bipolaris tetramer*, *Aspergillus nidulans* confirmed with prominent PGPA features.

Once the identity of the isolates (Bacteria and fungi) has been confirmed with promising PGPA features: these bacterial as well as fungal isolates were grown successfully on M9 minimal media and Czapek dox broth, respectively to produce bioactive compound in a respective medium.

In the present study, bacterial isolates such as *Aeromonas sobria*, *Bifidobacterium thermacidophilum*, *Pantoea Spp.* found to be prominent in producing bioactive compounds when detected in a M9 minimal media of each isolates. They were majorly recorded with alkaloids, protein and amino acids, saponins, phytosterols, fats, phenolic compounds, flavonoids, gum and mucilages.

In a similar manner, fungi broth medium found to be prominent with presence of bioactive compounds produces alkaloids, carbohydrates, glycosides, protein and amino acids, saponines, phytosterols, fats, phenolic compounds, flavonoids, gum and mucilages. This data summarises that both bacterial and fungal able to show plant growth promoting features and which are getting liberated in a medium as it was traceable by the number of tests.

Ability of these bacterial and fungal bioactive molecules to inhibit *E. coli*, *B. subtilis*, *S. aureus*, *S. boydii*, *P. aeruginosa* and *S. abony* as well as *A. brasiliensis* and *C. albicans* found to be successful. Similarly plant pathogens like bacteria *X. compestris 1*, *X. compestris 2* and fungi *Rhizoctonia bataticola* and *Sclerotium rolfsii* also been able to get inhibited by these bacterial broths with a better zone of inhibition. Overall study summarises that both bacterial and fungal population present as an endophyte in *L. acidissima* able to produce compounds which can inhibit bacterial as well as fungal pathogens successfully.

In the present study, after confirming the presence of bioactive compounds in the plant extract, along with endophytic bacterial and fungal broth, exact nomenclature of the secondary metabolites formed by each isolate or plant has been determined by gas chromatography mass spectroscopy and it has been put forward that bioactive compounds belonged to phytosterol, fatty acids, alkaloids and other type and were prominently present. In a few cases, common secondary metabolites also been evident to be present in bacterial broth, fungal broth and plant extracts simultaneously which are related with plants defence system as these bacterial and fungal isolates sampled as endophytes and probably those features could be given by the endosymbiosis of plants, bacteria and fungi to the host plant. For example, 3, 3, 5-Trimethylheptane recorded in

Bifidobacterium thermacidophilum ESb3 and *Aeromonas sobria* ELb3 along with acetone extract of stem bark indicated the promising sharing of the common compound by endophytes and plant. Study also confirmed that plant growth promoting features of bacterial species can be linked with plasmid.

Overall study based on all these above points indicated that plant *L. acidissima* governs many bioactive features by expressing bioactive compounds capable of controlling bacterial and fungal growth which were capable of infecting human and plant pathogens.

Study also put forward that plant *L. acidissima* posses many endophytes which are also potent in producing plant growth promoting biomolecules as well as those were capable of controlling bacterial as well as fungal growth similar to the plant *L. acidissima*.

Symbiotic association between bacteria, fungi and plant *L. acidissima* beneficial for the growth of each other and it can be concluded from the present study that they are sharing many biomolecules for their survival and provide an opportunity to isolate and purify many biomolecules capable of plant growth as well as to control bacterial and fungal growth. Present investigation is also very important with respect to exploitation of endophytes for producing many useful biomolecules.

15. Contribution to the Society (Give Details): Certainly present study is very beneficial to society in various means. Instruments purchased under project are asset to college and are being used by the students. The data generated in the present study would definitely add knowledge to the subject and would pave the way for further study. Present investigation is also very important with respect to exploitation of endophytes for producing many useful biomolecules on commercial basis.

16. Whether Any Ph.D. Enrolled/Produced Out Of the Project: - - -

17. No. of Publications out Of the Project: One abstract is published- **Annexure 1**



(PRINCIPAL INVESTIGATOR)



(PRINCIPAL)
Principal

Taywade College

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