

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:

Profiling of *Moringa oleifera* endophytes with antimicrobial potential

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR

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3. NAME AND ADDRESS OF THE INSTITUTION

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4. UGC APPROVAL LETTER NO. AND DATE

MRP-Major-MICR-2013-16570 dated 5.10.2015

5. DATE OF IMPLEMENTATION 01.07.15

6. TENURE OF THE PROJECT 3 Years (01.07.15 to 30.06.18)

7. TOTAL GRANT ALLOCATED Rs.10,70,000 /-

8. TOTAL GRANT RECEIVED Rs. 8,15,259 /-

9. FINAL EXPENDITURE Rs. 8,62,864 /-

10. TITLE OF THE PROJECT

Profiling of *Moringa oleifera* endophytes with antimicrobial potential

11. OBJECTIVES OF THE PROJECT

- To isolate endophytes from different plant parts of *Moringa oleifera*.
- To screen the isolated endophytes for their antimicrobial activity.

- To carry out various *in vitro* studies like MIC, VCC, post antibiotic effect etc. to establish their antimicrobial potential
- To work out the biosafety of the active antimicrobials by MTT assay and Ames test.
- To characterize the potential principles by qualitative and quantitative techniques such as chromatographic techniques, UV, IR, MS and NMR etc

12. WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS)

Yes, the above mentioned objectives were achieved as follows:

- Out of the twenty fungal isolates from bark, seven were bioactive, out of ten fungal isolates from seeds four isolates showed the antimicrobial potential
- The bioactive fungal strains were further narrowed down to two best fungi and their molecular identification was done from Agharkar Research Institute, Pune as *Aspergillus fumigatus* and *Chaetomium globosum*.
- Classical and statistical optimization was done which resulted in increase in antimicrobial activity upto 1.4 folds and 1.3 folds in case of *Aspergillus fumigatus* and *Chaetomium globosum* respectively.
- The chloroform was found to be best organic extractant to elute the bioactive components in both the fungi and their antimicrobial activity was comparable with standard antibiotics.
- The fungi have been further tested for their antimicrobial potential in terms of minimum inhibitory concentration, viable cell count studies and post antibiotic effect etc.
- These extracts were found to be biosafe as tested by Ames test and MTT assay.
- Two antimicrobial compounds were purified by using chromatography and semi prep HPLC.
- Further the antimicrobial activity has been upheld and validated against some resistant clinical isolates of MRSA and *Enterococcus* spp. obtained from the medical hospital thus providing clinical importance to the study.

13. ACHIEVEMENTS FROM THE PROJECT

The project was helpful in demonstrating the medicinal properties of magic plant *Moringa oleifera* and led to the isolation of important fungal isolates with antimicrobial potential. *Aspergillus fumigatus* and *Chaetomium globosum* were best endophytic fungi isolated which have been optimized for the production of antimicrobial metabolites. The fungal extracts gave a comparable activity with standard antibiotic and were found to be biosafe. Their

relevance to public health has been demonstrated as evident from their activity against multidrug resistant clinical isolates obtained from Government Medical College and Hospital Amritsar. One book chapter and one international publication (Impact factor 1.79) resulted from the project while other research papers are in process. The work was presented at an international conference. The project also helped in training the manpower.

14. SUMMARY OF THE FINDINGS (IN 500 WORDS)

Infectious diseases and the alarming rise in multidrug resistant pathogens have led the researchers to look for the novel sources of antimicrobial agents to control the drug resistant pathogens. Keeping this in mind the present study was designed to isolate and screen the endophytes from *Moringa oleifera*, an important medicinal magic plant, for their antimicrobial potential. Out of the twenty bacterial isolates, nine were bioactive, from bark seven out of twenty showed the antimicrobial potential and from the seeds, four out of ten were bioactive. From the fungal bioactive isolates, two isolates i.e. DSE 17 and DSE 72 were selected for further studies. The identification of the isolates was done by National Fungal Culture Collection of India (NFCCI), Agharkar Research Institute, Pune, India and they were identified as *A. fumigatus* (DSE 17) and *Chaetomium globosum* (DSE 72). Both the fungi showed a broad spectrum activity which encouraged the further optimization of various physiochemical parameters to enhance the production of antimicrobial agents. The statistical optimization using Plackett burman design and Response surface methodology was done which resulted in enhancement of antimicrobial activity up to 1.4 folds and 1.3 folds in case of *A. fumigatus* and *C. globosum* respectively. Further the extraction of secondary metabolites has been employed with different organic solvents where the chloroform was found to be the best extractant in case of both the fungi. The antimicrobial activity of the chloroformic extract was also found to be comparable with standard drugs. The fungi have been further tested for their antimicrobial potential in terms of minimum inhibitory concentration, viable cell count studies and post antibiotic effect etc. The MIC values for the chloroformic extract of both the fungi ranged from 0.05-5 mg/ml which revealed the quite good potency of both the extracts as compared to other studies. Further the viable cell count studies is a more powerful prediction tool than MIC for determining the antimicrobial action of extracts, as it depicts time related rate of bactericidal activity. Encouragingly, no re-growth was observed in any test organism against chloroformic extracts of both the fungi i.e., the extracts exhibited bactericidal nature, which will be quite useful when considered for drug development purpose. Further, before taking a natural compound to the level of commercialization and intending it for human use, it is necessary to make sure of its non

cytotoxic profile. The organic extract from both the fungi, along with their antimicrobial potential have been evaluated for their biosafety by Ames and MTT assay. The organic extracts of both the fungi were subjected to chromatography and further purification of the bioactive compound by HPLC, NMR and mass spectroscopy and the compounds were tested for antimicrobial activity by ADA. To add up to the value of the study, the organic extracts of both the endophytic fungi showed potent antimicrobial activity against some resistant clinical isolates of MRSA and *Enterococcus* spp. Thus metabolites harvested from both the fungi have potential to be taken up further for commercial use as the chloroformic extract of both the fungi were active not only against the reference strains but also the resistant strains. The study can substantially contribute to the battle against the prevailing antibiotic resistance.

15. CONTRIBUTION TO THE SOCIETY (GIVE DETAILS)

- The study has got the potential for market value to obtain the lead molecules of drug which may help in public health.
- Man power trained at M.Sc and Ph. D level.
- The work has also been presented at international conference AMI (2017) and will be presented at BRSI to be held at Hyderabad in November 2018.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT

Yes, one project fellow registered and completed her course work and thesis will be submitted shortly on the basis of project work.

17. NO. OF PUBLICATIONS OUT OF THE PROJECT (PLEASE ATTACH):

- Arora D. S. and Kaur, N. (2016). Bioactive *Moringa oleifera* and its Endophytes. In *Microorganisms: Tools of sustainability*. Edt. Rani, N., Joshi, M and Sagar, A. Pub. Bisen Singh Mahendra Pal Singh Dehra Dun (India). pp. 257-272.
- Arora, D.S. and Kaur, N. (2018). Antimicrobial potential of fungal endophytes from *Moringa oleifera*. *Applied Biochemistry and Biotechnology*. 1-21. (IF 1.79)
- Arora, D.S. and Kaur, N. Prospecting antimicrobial potential of *Chaetomium globosum* an endophyte from *Moringa oleifera* against reference strains of human pathogens and some resistant clinical isolates. *3 Biotech* (Communicated)

Prof. Daljit Singh Arora

(PRINCIPAL INVESTIGATOR)

REGISTRAR (Seal)