International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.12 No.03, pp 280-286, 2019

Screening Potential Endophytic Fungi of *Fusarium oxysporum* Isolated from *Andrographis paniculata* for its Antibacterial Activity

Chandran Masi[1]*, Eazhilnambi. A[2], Gokul Krishna V. R[2], Yuvaraj.D[2], Shanmuga Sundhar. D[3]

[1]Department of Biotechnology, College of Biological and Chemical engg., Addis Ababa Science and Technology University, Addis ababa, Ethipia.,
[2]Department of Biotechnology, Vel Tech High Tech Dr.Rangarajan Dr.Sakunthala Engineering College, Chennai, India
[3]Biozone Research Technologies, Teynampet, Chennai, India

Abstract : The frequent use of antimicrobial agents and antibiotics worldwide has lead to the development of Antibiotic resistance and mutation in Bacterial genes, which leads to a number of infections and diseases. On the contrary, the drugs which were once used to treat the infections are no longer effective against the particular organism since it gains resistance overtime. Numerous studies are made on isolating new and effective antimicrobial agents that could sustain as novel agents. Endophytic fungi produce a number of active biomolecules that has emerged as a recent trend in research. The current study involves the isolation of endophytic fungi from *Andrographis paniculata* and studying the potential uses of their secondary metabolites. Antibacterial well diffusion assay confirms that one among the five selected fungi secretes secondary metabolites that shows significant antibacterial properties. This could pave a way for new therapeutic agents that could be used as potential drugs against the selected bacteria or its infections. The endophytic fungal species with the most activity was characterized as *Fusarium oxisporum* by DNA barcoding.
Keywords : Endophytic fungi, metabolites, antimicrobial, DNA barcoding.

Introduction

World health problems caused by drug-resistant bacteria and fungi are increasing. An intensive search for newer and effective antimicrobial agents is needed. Endophytic fungi have been recognized as useful sources of bioactive secondary metabolites. A recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown. Many endophytic fungi have the ability to produce antimicrobial substance (Schulz et al., 2002 ; Phongpaichit, *et al*., 2006).

DOI= http://dx.doi.org/10.20902/IJCTR.2019.120336

Endophytes are microorganisms that reside inside the internal tissues of living plants without any harm to the host plants (Kannan & Vasanthi, 2001; YiingYng Chow & Adeline S.Y. Ting, 2015). *Andrographis paniculata* is an erect annual herb extremely bitter in taste in each and every part of the plant body. *A. paniculata* is used in traditional Siddha and Ayurvedic systems of medicine as well as in tribal medicine in India and some other countries for multiple clinical applications like enzyme induction, antihepatotoxic, hepatoprotective, antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic effects (C.Arunachalam & P.Gayathri, 2010). Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like cancer and HIV infections (Shahid Akbar, 2011; N. Karmegam, *et al*.,2015).

Materials and Methods

Sample Collection:

Fresh, disease free leaf samples were collected from Thirumullaivoyal during the months of January. They were washed and transferred in a sterile bag to the laboratory and stored at -20^{o}C until use. Healthy parts from *plant* are taken from the surrounding area. Rinse the leaves with distilled water until the surface is cleaned thoroughly. Keep the leaves over the tissue paper for drying. After the leaves are dried, the leaves are cut over the midrib region. The alternative midrib pieces are taken to avoid colonization of same organism.

Surface sterilization:

The leaves are sterilized before placing on the media, using the chemicals aseptically. The leaves are sterilized with 70% ethanol for 1 minute. Then it is sterilized with sodium hypochlorite for 4 minutes. Next it is washed with 0.1% Mercuric chloride for 4 minutes. Finally it is washed with distilled water to remove traces of $HgCl_2$ for 5 minutes. The leaves were trimmed using sterile blade and inoculated in PDA. The plates were incubated at 28°C for 7 days and observed for growth of fungus from leaves.

Isolation of endophytic fungi:

The leaf along with grown mycelia is sub cultured into the plate containing potato dextrose agar.

Secondary metabolites:

The fungi were inoculated in PDB media as per the following composition and incubated for 21 days. The crude extract were filtered, condensed and stored at 4^{o}C for further use.

Antibacterial study:

The secondary metabolites of the endophytic fungi were tested against pathogenic bacterial strains and fungal strains by agar well diffusion method.

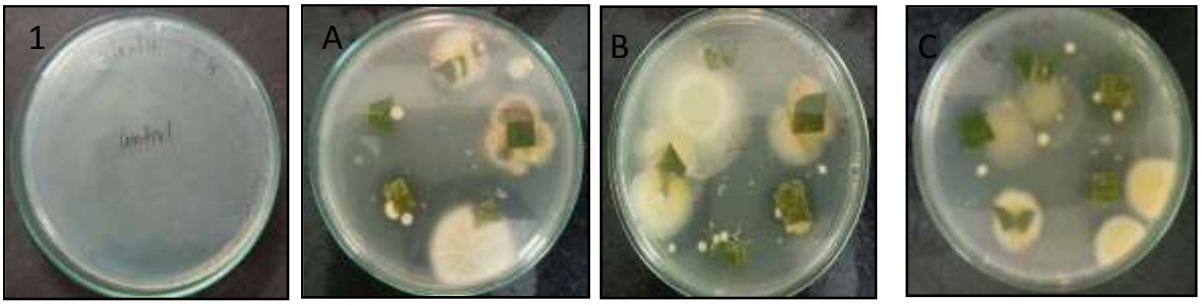
DNA barcoding:

The fungal genomic DNA was isolated and ITS in the genome was amplified using PCR. The PCR product was then sequenced and phylogenetic tree was constructed . The fungi was found to be *Fusarium oxysporum* with 98% similarity with accession number KR047060.1 already present in NCBI database (Felsenstein J, 1985 ; Tamura.K & Nei.M, 1993)

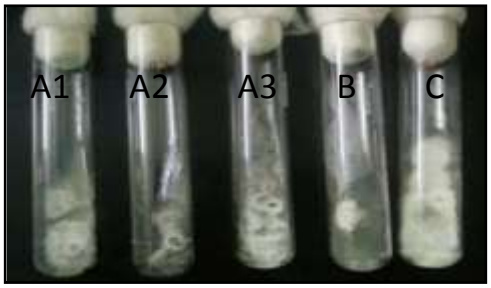
Results & Discussion

Antibacterial activity was done against bacterial strains *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia,* and *Escherichia coli* by well diffusion method**.** Fungal extract used as various concentration in well diffusion method against bacteria result**.** Fungal extract of five strains can perform different concentration in well diffusion method**.**

Screening and Isolation of Endophytic Fungi



1- Control **A- Young leaves** **B- Mature leaves** **C- Mature leaves**



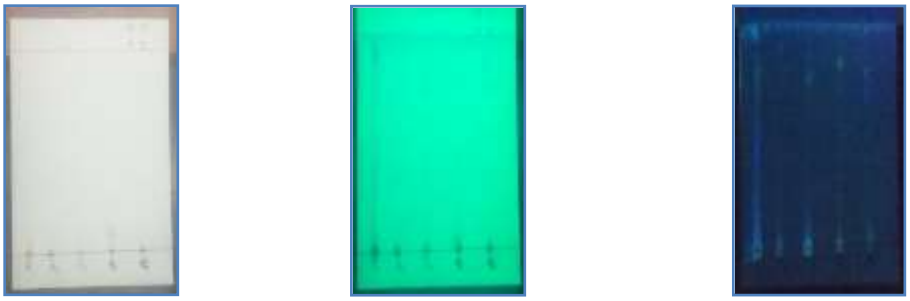
A1, A2 & A3- Young leaves strains

B & C- Mature leaves

LPCB Staining

Figure shows the fungal stained by Lacto phenol Cotton Blue Method

Thin Layer Chromatography



Visible light image **Short UV image (254 nm)** **Long UV image (365 nm)**

Antibacterial Activity

Bacillus subtilis

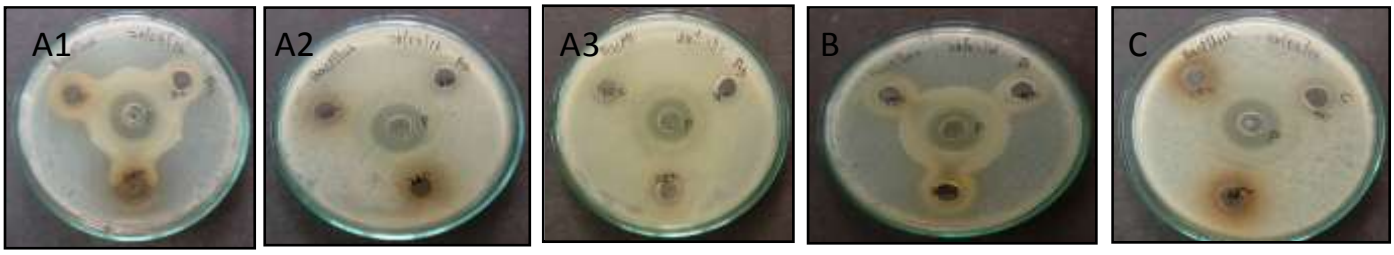


Figure shows the zone of inhibition against fungal extracts and positive control

Salmonella typhi

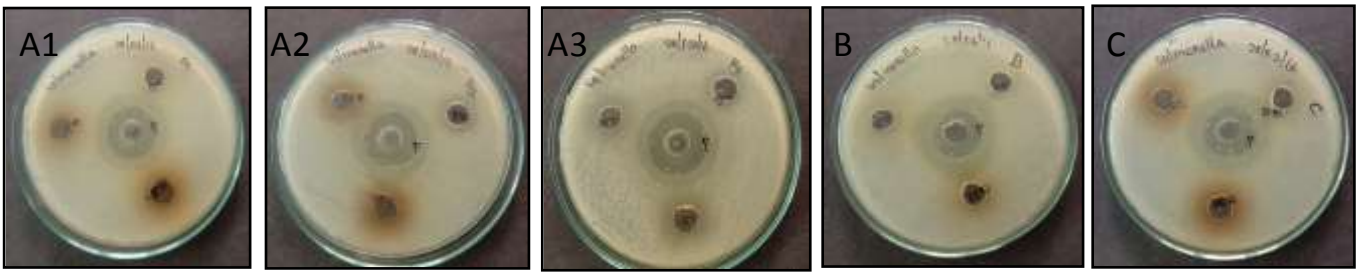
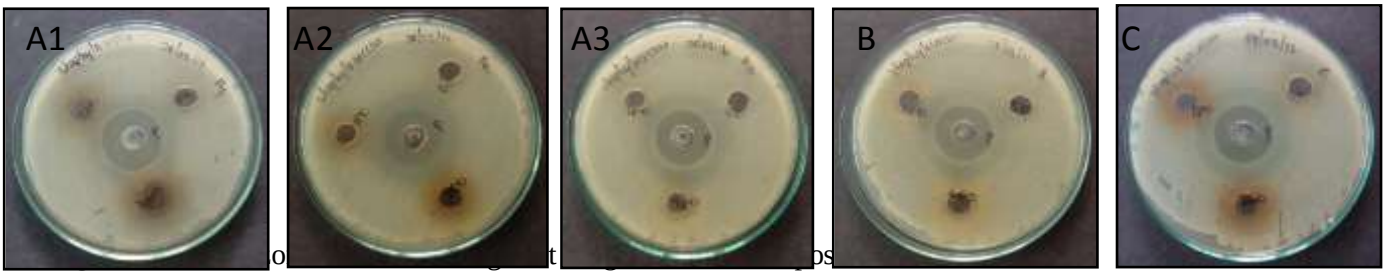


Figure shows the zone of inhibition against fungal extracts and positive control.

Staphylococcus aureus



Klebsiella pneumonia

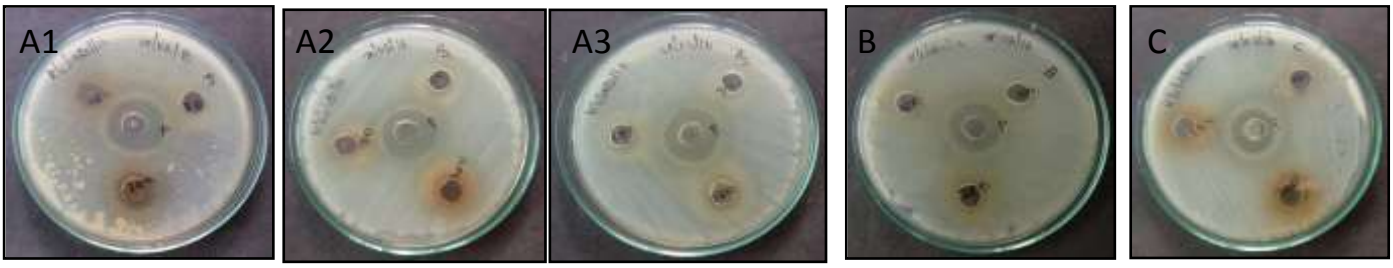


Figure shows the zone of inhibition against fungal extracts and positive control.

Escherichia coli

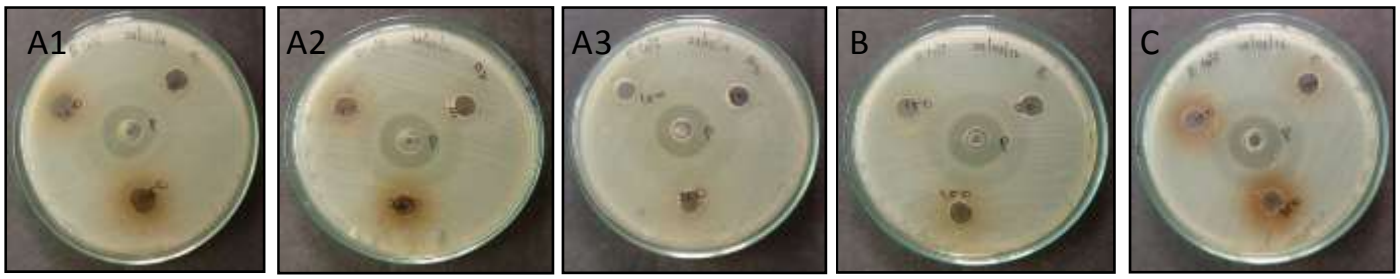


Figure shows the zone of inhibition against fungal extracts and positive control.

Antibacterial Activity

Bacillus subtilis ***Salmonella typhi***

	A1	A2	A3	B	C		A1	A2	A3	B	C
500(conc/µg)	12	-	-	15	-	500(conc/µg)	-	14	16	-	18
1500(conc/µg)	15	-	13	18	-	1500(conc/µg)	-	17	19	16	20
2500(conc/µg)	17	-	16	19	-	2500(conc/µg)	-	18	21	19	21
Positivecontrol	21	32	31	21	33	Positivecontrol	31	30	32	30	35

Staphylococcus aureus ***Klebsiella pneumoniae***

	A1	A2	A3	B	C		A1	A2	A3	B	C
500(conc/µg)	-	-	-	-	-	500(conc/µg)	-	20	19	-	-
1500(conc/µg)	16	-	-	-	-	1500(conc/µg)	-	21	21	-	-
2500(conc/µg)	19	-	-	-	-	2500(conc/µg)	-	23	22	-	-
Positivecontrol	37	37	38	38	36	Positivecontrol	21	34	37	20	23

Escherichia coli

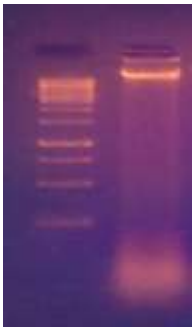
	A1	A2	A3	B	C
500(conc/µg)	-	-	-	-	-
1500(conc/µg)	-	-	-	-	-
2500(conc/µg)	-	-	-	-	-
Positivecontrol	35	38	41	37	37

Table shows zone of inhibition against fungal extracts and positive control.

Of the five secondary metabolites of fungal extract C showed no significant activity against any of the organism while A3 showed greater zone of inhibition against *Bacillus subtilis, Salmonella typhi*, and *Klebsiella pneumonia*.

DNA Barcoding:

Genomic DNA isolation ***PCR amplification of ITS regions***



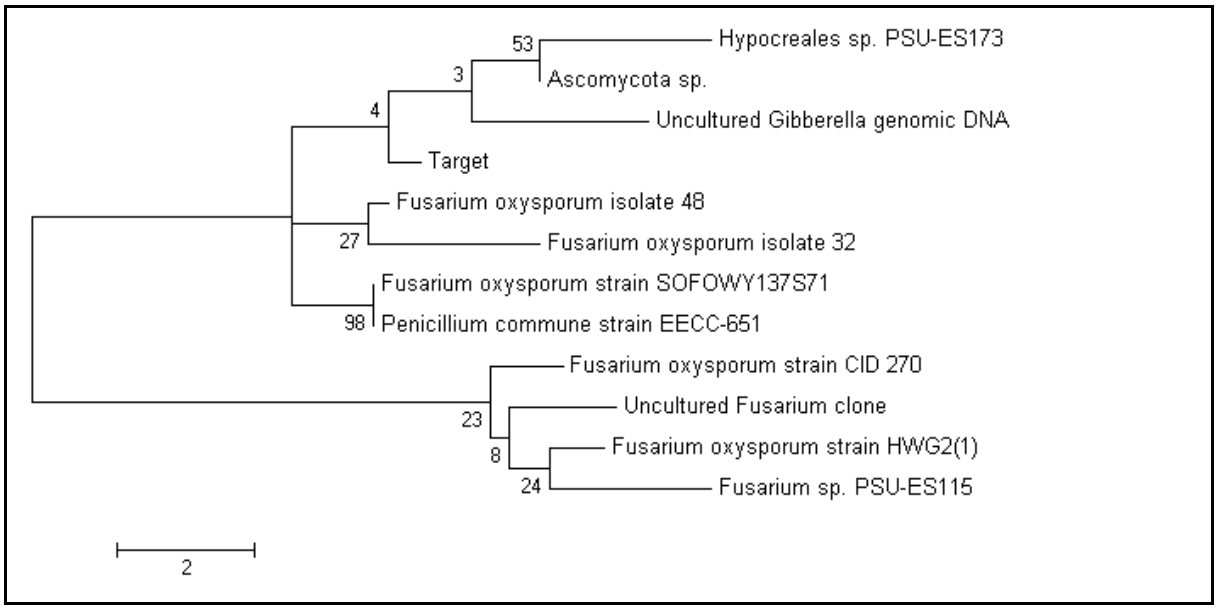
Lane 1: 1 Kb Ladder **Lane 1: 1 Kb Ladder**

Lane 2: Genomic DNA of the fungi **Lane 2: PCR Amplicon R3:~ 600 bp**

Sequence:

AAAACACACCTGAATTTGATAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGT
TTACAACTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAA
AACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCAT
AAATAAATCAAAACTTTCAACAACGGATCTCTTGATTCTGGCATCGATGAAGAACGCAGCAAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCG
CCAGTATTCTGGCGGGCATGCCTTTTCGAGCGTCATTTCAGCCCTCAAGCACAGCTTGGTGTTGGG
GCTCGCGTTAATTCGCGTTCCCCAAATTGATTGGCGGTCACGTCGGGCTTCCATAGCGTAGTAGTA
AAACCCCCGTTACTGGTAATCGTCGCGGCCACGCCGTTTAACCCCAAATTCTGAATGTTGACCTCG
GATCAGGTAGGAATGCCCACTGAACTTAAGCATACTATGCCCGCCAAA

Phylogenetic Analysis:



The previous study of endophytic fungi of *Andrographis paniculata* using different leaves such as young, mature, yellow, dry and infected leaves and performed two method for isolated endophytic fungi by agar plate and moist chamber method (Bijaya Kumar Nayak, 2015). A crude aqueous extract of leaves exhibit significant antimicrobial activity against grampositive *S. aureus, methicillin-resistant S. aureus* and gram-negative *Pseudomonas aeruginosa*, but had no activity against *Escherichia coli or Klebsiella pneumonia* (Anil Kumar et al., 2012).Evolutionary analyses were conducted in MEGA6 (Tamura.K, *et al*.,2013)

Conclusion

The emerging need for new novel drugs that can be used as effective antimicrobial agents has lead to the search of new sources of potential drugs. Numerous components of plants, fungi, actinomycetes and marine organisms are being studied for their potential biomolecules. Endophytic fungi synthesize a number of compounds that are necessary for the defense of organism inside the host plant. The study of five endophytic fungi of the plant *Andrographis paniculata* revealed that the secondary metabolites of such fungi have active antibacterial activity. One among the five selected fungi which was found to be *Fusarium oxysporum* by DNA barcoding showed significantly higher antibacterial activity in comparison with the others. The compounds responsible for inhibiting bacterial growth in the secondary metabolites of the fungus can be used as an antibacterial agent.

References

1. Anil Kumar, Jyotsna Dora, Anup Singh and Rishikant Tripathi, 'A review on king of bitter (Kalmegh)', IJRPC, ISSN: 2231-2781, Vol. 2(1), 2012.
2. Bijaya Kumar Nayak, 'Comparative assessment of two methods for isolation of endophytic fungi from varied leaves of *Andrographis paniculata*', IJCRGG, ISSN: 0974-4290, Vol.7, No.4: pp 2085-2089, 2015.
3. C. Arunachalam and P.Gayathri, 'Studies on bioprospecting of endophytic bacteria from the medicinal plant of Andrographis paniculata for their antimicrobial activity and antibiotic susceptibility pattern', Int J Curr Pharm Res, ISSN- 0975-1491, Vol 2(4): pp 63-68, 2010**.**
4. Felsenstein.B, 'Confidence limits on phylogenies: An approach using the bootstrap',Evolution, Vol. 39: pp 783-791, 1985.
5. Kannan and Vasanthi, 'Extraction, Purification and characterisation of chitosan from Endophytic fungi isolated from medicinal plants', World Journal of Science and Technology, Vol. 1(4): pp 43-48; 2001.
6. N. Karmegam, R. Nagaraj, S. Karuppusamy and M. Prakash, 'Biological and Pharmacological Activities of Andrographis spp. (Acanthaceae) Distributed in Southern Eastern Ghats, India', Int. J. Curr. Res. Biosci. Plant Biol., ISSN: 2349-8080, Vol. 2(9): pp 140-153, 2015.
7. Shahid Akbar, 'Andrographis paniculata: A Review of Pharmacological Activities and Clinical Effects', Alternative Medicine Review, Vol. 16(1), 2011.
8. Schulz B, Boyle C, Draeger S, Rommert A-K & Krohn K, 'Endophytic fungi: a source of novel biologically active secondary metabolites', Mycol Res Vol.106:pp 996–1004, 2002.
9. Souwalak Phongpaichit, Nattawut Rungjindamai, Vatcharin Rukachaisirikul & Jariya Sakayaroj, 'Antimicrobial activity in cultures of endophytic fungi isolated from Garcinia species', FEMS Immunol Med Microbiol,Vol. 48: pp 367–372, 2006.
10. Tamura K and Nei M, 'Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees', Molecular Biology and Evolution Vol.10: pp 512-526, 1993.
11. Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S, 'MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0', Molecular Biology and Evolution, Vol.30: pp 2725-2729, 2013.
12. YiingYng Chow, Adeline S.Y. Ting, 'Endophytic L-asparaginase-producing fungi from plants associated with anticancer properties', Journal of Advanced Research, Vol. 6: pp 869–876, 2015.

*****