# Molecular Characterization of Fungi Associated with *Senecio biafrae* (Oliv. & Hiern) in Ibadan Metropolis

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## Certification

This is to certify that Helen Kehinde, ADEMUYIWA with Matric No. LCU/PG/006596 carried out this research work titled ‘‘Molecular Characterization of Fungi Associated with *Senecio biafrae* in Ibadan Metropolis” in the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master Degree (M.Sc.) in Molecular Biology and Genomics and that this has not been previously submitted.



**Prof. Bukola A Bamkefa** **Date**

**Promoter**

**Dr. Omonike C Bakare** **Date**

**Head of Department**

**Dedication**

This research work is dedicated to Almighty God.

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Although the institutions and people named above helped with this seminar work, I alone am responsible for any error that are discovered.

## Abstract

*Senecio biafrae*, an indigenous green leafy vegetable notable for its nutritional, medicinal and economic potentials. Molecular characterization of fungi associated with *Senecio biafrae* in Ibadan metropolis was carried out using fresh samples collected from retailers in four markets. Portions from the samples were cut and surface sterilized in 1% Sodium hydrochloride for 1 minute, rinsed in sterile distilled water, and dried on sterile filter paper. The samples were plated on Potato Dextrose Agar and observed for fungal growth. Pure cultures were obtained by repeated subculturing, while identification was done macroscopically and microscopically. Pathogenicity tests of the fungal isolates were carried out on healthy leaves of *Senecio biafrae*. DNA was extracted from the unidentified fungal pure cultures using Quick-DNA Fungal/Bacterial MiniPrep$^{TM}$ Kit, PCR was done using universal primer pair; ITS1 and ITS4, while Sequencing was carried out using National Centre for Biotechnology Information database. Furthermore, Aflatoxin content of the *Aspergillus spp* isolated from the vegetable was carried out using ELISA method. Molecular identification and characterization through DNA sequencing validated the morphological identifications of the six fungal isolates (*Aspergillus niger*, *Aspergillus brunneoviolaceus*, *Penicillium oxalicum*, *Aspergillus fumigatus*, *Aspergillus tamarii*, and *Mucor irregularis). Aspergillus niger* had highest percentage of occurrence 13(31.71%), followed by *Aspergillus brunneoviolaceus* 11(26.83%), then, *Penicillium oxalicum* 8(19.51.%), and the least occurring were *Aspergillus fumigatus* 3(7.32%), *Aspergillus tamarii* 3(7.32%), and *Mucor irregularis* 3(7.32%). Pathogenicity test revealed that the fungal isolates were pathogenic because diseases were reproduced on healthy vegetable samples. The Aflatoxin content in the samples was lesser than the acceptable limit of 10µg/kg ranging from 6.8 ppb (Oje market) to 0.2ppb (Mapo market). The presence of fungi and positive aflatoxin contamination on the vegetable indicate accumulation of nutrient that enhance fungal growth, suggesting the need for awareness campaigns that encourages safe handling and hygienic storage practices to reduce health hazards.



**Keywords:** *Senecio biafrae*, Molecular characterization, Aflatoxin, Pathogenicity, Health hazard

**Word Count:** 300

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## List of Acronyms

| Abbreviation | Meaning |
|---|---|
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| PCR | Polymerase Chain Reaction |
| ITS | Internal Transcribed Spacer |
| NO | Nitric Oxide |
| CaCO3 | Calcium Carbonate |
| NASBA | Nucleic Acid Sequence-Based Amplification |
| LAMP | Loop-mediated isothermal amplification |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeats |
| RT-PCR | Real-Time Polymerase Chain Reaction |
| NGS | Next-Generation Sequencing |
| NCBI | Centre for Biotechnology Information |
| EBI | European Bioinformatics Institute |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| DNTPs | Deoxynucleoside Triphosphates |
| TEF | Translation Elongation Factor |
| PACBIO | Oxford Nanopore Technologies and Pacific Biosciences |
| AF | Aflatoxin |
| FUM | Fumonisins |
| TCT | Trichothecenes |
| ZEA | Zearalenone |
| DON | Deoxynivalenol |
| PDA | Potato Dextrose Agar |

| | |
|---|---|
| UPGMA | Unweighted Pair Group Method with Arithmetic Mean |
| BLAST | Basic Local Alignment Search Tool |
| NAFDAC | National Agency for Food and Drug Administration and Control |
| ppb | Part Per Billion |
| MEGA | Molecular Evolutionary Genetics Analysis |