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ANTIBACTERIAL ACTIVITY OF ENDOPHYTIC FUNGI ISOLATED FROM MEDICINAL PLANTS: *OCIMUM GRATISSIMUM* AND *JATROPHA TANJORENSIS*

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ABSTRACT: *Endophytes are gaining worldwide recognition because of their potential use in biotechnology and as sources of novel bioactive compounds. A total of 27 fungal endophytes were isolated from the root, stem and leaves of two medicinal plants: Ocimum gratissimum (13) and Jatropha tanjorensis (14). Occurrence of endophyte species of the plants were Alternaria alternata and Aspergillus flavus (22.22% each), Nigrosa oryzae and Penicillium chryseogenum (14.81% each) and Penicillium oxalicum (11.11%). Cladosporium sphaerospernum (7.41%) was isolated from Ocimum gratissimum only and Rhizoctonia solani (7.41%) from Jatropha tanjorensis only. More endophytes were present in the roots and leaves than stems of the plants. Antibacterial screening of 10 mg/ml of fungal extracts on five test organisms (Staphylococcus aureus, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae), using the Agar Well Diffusion Method showed Alternaria alternata with inhibitory zones 20.5±0.15 mm – 32.7±0.58 mm; Penicillium chryseogenum (14.5±0.20 mm – 28.79±0.26 mm); Aspergillus flavus (18.7±0.10 mm – 24.7±0.10 mm) and Nigrosa oryzae (18.6±0.15 mm – 24.7±0.06 mm each), with their highest antibacterial activity on Staphylococcus aureus. Cladosporium sphaerospernum (20.9±0.20 mm – 30.0±0.06 mm) and Penicillium oxalicum (11.3±0.01 mm – 22.8±0.15 mm) were most inhibitory to Bacillus cereus. Rhizoctonia solani had the least zones of inhibition (8.20±0.20mm – 14.7±0.06 mm) and most inhibitory to E. coli and Pseudomonas aeruginosa. The antibacterial activities of the extracts were comparable to that of the standard drug, chloramphenicol (28.4±0.40mm – 36.0±0.12mm). With further research and standardization, these extracts could serve as alternatives to synthesized antibiotics.*

KEYWORDS: Antibacterial activity, endophytic fungi, Jatropha tanjorensis, Ocimum gratissimum.

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INTRODUCTION

Endophytes are non-pathogenic microorganisms which occur intracellularly or are intracellularly in plant tissues. The association between the endophytes and hosts are normally symbiotic where nutrients for microbial growth are provided by the plant while the endophytes are beneficial to the plants themselves by stimulating their growth and suppressing pathogens, and also beneficial in stress tolerance (Schulz *et al*., 2002; Strobel *et al.*, 2004). Bioactive compounds produced by endophytes are noted to have antitumor, antioxidant, anti-inflammatory and antibiotic properties (Strobel *et al.*, 2004; Liu, 2011; Ezeobiora *et al*., 2021). Research interests in endophytic fungi are on the increase in recent years as they are considered possible alternatives for controlling diseases of plants and humans by the production of antibacterial, antifungal, antiviral, antitubercular, antimalarial, insecticidal and anticancer agents (Manganyi & Ateba, 2020; Toghueo, 2020; Adeleke & Babalola, 2021, Rai *et al*., 2021; Muhammad *et al.*, 2024). A lot of reports occur on the characterization of endophytic fungal metabolites of medicinal importance, isolated from medicinal plants (Meng *et al*., 2011; Rathod *et al*., 2014; Vijayalakshmi *et al*., 2016).

Herbal medicine is widely practiced, and with its growing popularity, concerns are raised on its safety, quality, availability, preservation and development. Though this type of health care is promising, there are limitations to its applicability because few are actually tested and monitored (Ekor, 2014; Awodele *et al.*, 2018). That notwithstanding, plants utilized in traditional medicine are considered lower risk compared to synthetic drugs because of their history of use in disease treatment (Monteiro *et al*., 2014; Zhang *et al*., 2014). The World Health Organization reported that herbal or traditional medicine is not only effective in the treatment of diseases (including chronic ones), and mental health but improves quality of life in the ageing population.

Medicinal plant, *Ocimum gratissimum* L. is widely distributed in Africa and Asia. Every part of the plant is important in traditional medicine. The plant is used as an antimalarial, anticonvulsant, antiseptic, antibacterial, antifungal, in the treatment of mental disorders among other ailments (Ezekwesili *et al*., 2004; Imosemi, 2020). *Jatropha tanjorensis* is used in Nigeria as a source of leafy vegetables. It is used in traditional medicine to cure malaria, anaemia, diabetes and cardiovascular diseases, properties which have been verified (Orhue *et al*., 2008; Omoregie and Sisodia, 2011; Omoregie and Osagie, 2012). The plant extracts showed antibacterial activities (Oboh and Masodje, 2009).

In low and middle income countries, an estimated 30 % of the 70 million annual deaths are caused by increasing drug resistance of microorganisms (WHO, 2005), and an estimated 4.59 million deaths related to bacterial antimicrobial resistance occurred in 2019 (Murray *et al*., 2021). The search for new and effective antimicrobial agents has become a necessity due to the increase in super resistant strains of microorganisms to antibiotics. This problem can be tackled by using bioactive metabolites of plant and microbe origin. Medicinal plants and their endophytes are important for the discovery of natural products.

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MATERIALS AND METHODS

Sample Collection

Fresh and healthy plants of *Ocimum gratissimum* and *Jatropha tanjorensis* were picked randomly from Otuoke in Bayelsa State, Nigeria; Latitude 4.8019^{o}N and Longitude 6.3189^{o}E. The plants were collected in clean plastic zip lock bags, transported to the laboratory and processed within 24 hours of collection.

Sample Processing and Isolation of Endophytic Fungi

The plants were processed using methods of Chen *et al*. (2012), and Pondei *et al*. (2018), with slight modifications. Plants were first rinsed under running tap water to remove all debris. Surface sterilization was done by immersing the whole plant (roots, stem and leaves) in 70 % ethanol for one minute, followed by 1 % sodium hypochlorite for 3 minutes. After this, plant tissues were rinsed three times in sterile distilled water and blotted dry with sterile filter paper before cutting into 0.5 x 0.5 cm pieces using sterile blades. The roots, stems and leaves of the plant were separated to give three subsamples per plant before cutting. Each subsample was then placed on Potato Dextrose Agar (PDA) plates and incubated at 25^{o} C ± 2^{o} C for 5–7 days. The efficacy of surface sterilization was confirmed by inoculating the surface sterilized water collected from the last wash of the sample in nutrient agar medium. The absence of growth on the media confirms the efficient surface sterilization of the segments. Pure cultures were obtained by subculturing distinct fungal growth on fresh PDA plates.

Identification of Endophytic Fungi

Emerging fungal growth was identified based on their morphological and cultural characteristics. Growth appearances were observed from the top and bottom sides of the culture plates. The form (the basic shape of the colony, e.g., circular, filamentous, etc.), elevation, size, margin/border, surface and colour aided in the identification of the fungi. The isolates were grouped according to colony appearance and pigmentation (James & Natali, 2013).

Extraction of Secondary Metabolites

To test the antimicrobial activity of fungal endophytes, all the isolates were cultivated to produce crude metabolites according to the protocols of Phongpaichit *et al*. (2007) with slight modifications. Endophytic fungal isolates were grown in a 500 ml Erlenmeyer flask containing 250 ml potato dextrose broth and incubated at 25^{o}C ± 2^{o}C for 14 days under a stationary condition. The crude fermentation broth was filtered using sterilized Whatman filter paper No. 1 and the filtrate was centrifuged at 4000 rpm for 5 minutes. The liquid supernatant was extracted three times with equal volumes of ethyl acetate. The organic solvent extract was then evaporated under reduced pressure, and the resulting extract of each isolate dissolved in Dimethyl Sulfoxide (DMSO) at a concentration of 10 mg/ml. This was used to assay for antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* obtained from the Microbiological Laboratory Unit of the Federal Medical Center (FMC), Yenagoa, Bayelsa State. The authenticity of the test organisms was confirmed by further subculturing and identification using standard protocols.

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Preparation of Inocular

Colonies from overnight cultures of the test organisms were picked and suspended in 5 mls normal saline. This was mixed and the absorbance was measured using a spectrophotometer (λ=600 nm); then it was adjusted to 0.5 McFarland standard turbidity containing ≈10^8 cfu/ml of the test organism.

Assay for Antibacterial Activity

The Agar Diffusion Method was employed for the assay. 100 µl of each test organism was inoculated onto Petri dishes containing 20 ml Mueller Hinton Agar using the spread plate method. This was allowed to diffuse for 15–20 minutes and wells of 6 mm diameter made in the medium using a sterile cork borer. The wells were then filled with 10 µl of each fungal endophytic extract. The standard antibiotic (chloramphenicol) was dissolved in DMSO to give 10 mg/ml and 10 µl used for the assay; this represented the positive control. Undiluted DMSO was employed as negative control. All plates were incubated at 37^{o}C for 24 hours. To determine the antibacterial activity of fungal endophytic secondary extracts, the diameters of inhibition zones around the wells were measured horizontally and vertically and the average was taken. All antimicrobial assays were performed in triplicate.

Statistical Analyses

Antibacterial activity of the various extracts of endophytic fungi on the test organisms were expressed as Mean ± Standard Deviation (SD) (Microsoft Excel, 2010). Analysis of variance (ANOVA) and post-hoc analysis of sources of variations using Duncan's multiple range (DMR) were determined using SPSS (version 20.0).

RESULTS AND DISCUSSION

Six different fungal species were isolated from each test plant: *Alternaria alternata, Aspergillus flavus, Cladosporium sphaerospermum, Nigrospora oryzae, Penicillium chrysogenum* and *Penicillium oxalicum* were isolated from *Ocimum gratissimum* (Table 1), while *Alternaria alternata, Aspergillus flavus, Nigrospora oryzae, Penicillium chrysogenum, Penicillium oxalicum* and *Rhizoctonia solani* were isolated from *Jatropha tanjorensis* (Table 2). The fungal endophytes were represented in the different parts of the plants with a total number of thirteen (13) isolates from *Ocimum gratissimum* and fourteen (14) from *Jatropha tanjorensis*. Higher and equal numbers of endophytes were present in the leaf and root of both plants, and less numbers from the stem.

The antibacterial activities of the fungal extracts on the test organisms, measured as their zones of inhibition, are given in Table 3. Extracts of *Alternaria alternata* exhibited the highest antibacterial activity (20.5±0.15 mm – 32.7±0.58 mm) and most inhibitory to *Staphylococcus aureus*, with the least activity on *Klebsiella pneumoniae*. Values of inhibition obtained for all organisms were statistically significant on comparison ($P<0.05$). *Cladosporium sphaerospermum* activity ranged from 20.9±0.20 mm to 30.0±0.06 mm with the highest activity on *Bacillus cereus* and the least on *Kl. pneumoniae*. For *Penicillium chrysogenum*, antibacterial activity was between 14.5±0.20mm and 28.9±0.36 mm, also showing the highest activity on *Staph. aureus* and the least on *Kl. pneumoniae*. *Aspergillus flavus* and *Nigrospora oryzae* extracts had similar ranges of inhibition zones (18.7±0.10 mm – 24.7±0.10 mm and 18.6±0.15 mm – 24.7±0.06 mm), and were most inhibitory to *Staph. aureus*. The extracts of

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Penicillium oxalicum showed an inhibitory range of 11.3±0.10 mm to 22.8±0.15 mm with the highest activity on *B. cereus. Rhizoctonia solani* extracts showed the least antibacterial activity (8.20±0.20 mm – 14.7±0.06 mm), having the highest zones of inhibition on *E. coli* and the least on *Staph. aureus*. The control antibiotic (chloramphenicol) had inhibition zones of 28.4±0.40 mm to 36.1±0.12 mm and was most inhibitory to *Staph. aureus*.

Endophytic microorganisms occurring in association with plants confer characteristics beneficial for the continuous existence of the association. These endophytes have been found in different compartments of plant tissue where they exert their beneficial properties. Such properties include stimulating plant growth by hormone production, suppressing pathogens and improving plant stress tolerance. Endophyte numbers are reported to differ in various plant compartments, with the root parts having higher numbers than both the stem and the leaves. The higher numbers in the root parts of plants have been attributed to their proximity to the rhizosphere (Oliveira *et al.*, 2014; Afzal *et al.*, 2014). In this study, equal and more fungal species occurred in both the roots and leaves of both plants than the stem.

Fungal endophytes are synthesizers of bioactive metabolites in plants and several studies on these are in existence. These include pigments, antioxidants, a range of antimicrobials and pesticides (Chutulo & Chalannavar, 2018; Tang *et al*., 2020). The crude extracts of the seven endophytic fungal species isolated from the studied plants exhibited some degree of antibacterial activity against the test organisms as compared with the positive control (chloramphenicol).

Alternaria alternata has often been isolated as an endophyte (Romero *et al*., 2001). The crude extract of *Alternaria alternata* showed the strongest antibacterial activity compared to other isolates. *Alternaria* is a well-known saprophyte and phytopathogen causing occasional medical problems or allergies in humans. Species of *Alternaria* are known to produce various mycotoxins and are considered as candidates of choice for biological pest management. The entomopathogenic nature of *Alternaria alternata* is the most commonly reported (Singh *et al*., 2012).

Many species of *Cladosporium* produce secondary metabolites, such as antibiotics (*Gallo et al*., 2004). The most isolated species are *Cladosporium sphaerospermum*, *Cladosporium cladosporioides, Cladosporium herbarum* and *Cladosporium elatum* (De Hoog *et al*., 1995; Masclaux *et al*., 1995). *Cladosporium sphaerospermum* isolated from only *Ocimum gratissimum* exhibited the second most antimicrobial property. Tânia *et al.* (2000) also found the fungus showed antibacterial activity against *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Pseudomonas aeruginosa*. *Cladosporium sphaerospermum* has been studied as a plant growth-promoting endophyte (Hamayun *et al*., 2009) and *Cladosporium* species are efficient biological insecticides, particularly against insects that have developed resistance to chemical insecticides (Abdel-Baky & Abdel-Salam 2003).

Penicillium chrysogenum and *Penicillium oxalicum* were isolated from both plants. *Penicillium* species are of major importance in the environment as well as in food and drug production. Secondary metabolites of *P. chrysogenum* include various penicillins, PR-toxin, among others, novel polyketides and hypocrellins from endophytic strains of *P. chrysogenum*, sorbicillin and sorbicillin-derived compounds, etc. (Hoog *et al*., 2000; Liyuan *et al*., 2011). The role of *Penicillium oxalicum* in the production of the extracellular enzyme cellulase has been reported by Li *et al*. (2016).

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Aspergillus flavus is an endophyte of both study plants. *Aspergillus* species are known decomposers, toxin producers and that cause plant diseases (Park *et al*., 2017). Endophytic fungi (*Aspergillus* spp.) of medicinal plant *Gloriosa superba* exhibited antibacterial activity against a variety of test organisms as well as novel metabolites which also had cytotoxic effect (Budhiraja *et al*., 2013).

In addition, *Penicillium* and *Aspergillus* are important biotechnologically as producers of enzymes and other macromolecules including organic acids (Kirk *et al*., 2008). Endophytic *Penicillium* species have shown some potential for use in bioremediation, as biocatalysts, plant growth promoters and with various potential applications in the pharmaceutical industry (Toghueo & Boyom, 2020).

Nigrospora oryzae is a fast-growing fungus, an endophyte, saprophyte and also a weak pathogen depending on the host and environmental conditions. This fungus occurs in various plants with potential use as a biocontrol agent, and the antimicrobial activity of griseofulvin from the fungus is documented (Rathod *et al*., 2014; Thanabalasingam *et al*., 2015). The crude extract from N. *oryzae* showed strong antibacterial activity against all the test organisms.

Rhizoctonia solani was isolated from the root and stem of *Jatropha tanjorensis* only. Its extract showed the weakest antibacterial activity. *Rhizoctonia solani* is a well-known economically important plant pathogen causing damage to a variety of agricultural crops (Ajayi-Oyetunde & Bradley, 2017).

With the increase in drug resistance in bacteria globally, attention has been drawn to the search for new antibacterial agents. The extracts of the endophytes studied exhibited antibacterial properties against both Gram positive and Gram-negative bacteria. *Cladosporium* sp., *Nigrospora* sp. and both species of *Penicillium* were more effective on Gram positive bacteria while *Alternaria* sp. and *Aspergillus* sp. showed no preference for a particular group. On the other hand, *Rhizoctonia* was more effective on Gram negative bacteria. The extracts, when standardized, could be used in the treatment of specific diseases or as gross spectrum antibiotics.

Endophytes are reservoirs of useful, unexplored bioactive metabolites, with increasing numbers of novel compounds being discovered in endophytic fungi. These natural products are of unique structures and bioactivities, offering enormous potentials for exploitation in medicinal, agricultural and industrial applications (Tan & Zou 2001). Secondary metabolites of fungal endophytes of medicinal plants are sources of discovery and production of drugs against many diseases.

CONCLUSION

Endophytes have proven to be rich sources of novel natural compounds with a wide spectrum of biological activities. A vast array of medicinal plants is under investigation as a source of these novel secondary metabolites against bacterial and fungal pathogens. The present study revealed that the crude extracts of endophytic fungi from medicinal plants, *Ocimum gratissimum* and *Jatropha tanjorensis* inhibited both Gram positive and Gram negative bacteria. Bioactive compounds produced by endophytes have shown to have promising potential and usefulness in safety and human health concerns. The findings of this research

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points out the potentials of endophytic fungi with emphasis on antibacterial activity which could be of relevance to the pharmaceutical industry.

FUTURE RESEARCH

Further research would entail the identification, characterization and standardization of the fungal metabolites to determine their effect on bacterial inoculated and diseased test animals.

REFERENCES

Abdel-Baky, N. F. & Abdel-Salam, A. H. (2003). Natural incidence of *Cladosporium* spp. as a bio-control agent against white flies and aphids in Egypt. *Journal of Applied Entomology*, 127: 228 – 235.

Adeleke, B. S. & Babalola, O. O. (2021). Pharmacological Potential of Fungal Endophytes Associated with Medicinal Plants: A Review. *Journal of Fungi*, 7: 147. https://doi.org/10.3390/jof7020147

Afzal, M., Khan, Q. M. & Sessitsch, A. (2014). Endophytic bacteria: Prospects an applications for the phytoremediation of organic pollutants. *Chemosphere*, 117: 232 – 24.

Ajayi-Oyetunde, O. O. & Bradley, C. A. (2018). *Rhizoctonia solani*: taxonomy, population biology and management of *Rhizoctonia* seedling disease of soybean. *Plant Pathology*, 67: 3 – 17.

Awodele, O., Fajemirokun , O., Oreagba, I., Samuel, T. A., Popoola, T. D., Coulidiaty, A. V. & Emeka, P. M. (2018). Herbal Medicines Use: Remedies or Risks. *West African Journal of Pharmacy*, 29(2): 1 – 18.

Budhiraja, A., Nepali, K., Sapra, S., Gupta, S., Kumar, S. & Dhar, K. L. (2013). Bioactive metabolites from an endophytic fungus of *Aspergillus* species isolated from seeds of *Gloriosa superba* Linn. *Medicinal Chemistry Research*, 22:323 – 329 DOI 10.1007/s00044-012-0032-z

Chen, W-M., Tang, Y-Q., Mori, K. M. & Wu, X-L. (2012). Distribution of culturable endophytic bacteria in aquatic plants and their potential for bioremediation in polluted waters. *Aquatic Biology*, 15: 99 – 110.

Chutulo, E. C. & Chalannavar, R. K. (2018). Endophytic mycoflora and their bioactive compounds from *Azadirachta indica*: A Comprehensive Review. *Journal of Fungi*, 4: 42; doi:10.3390/jof4020042

De Hoog, G. S., Gueho, E., Masclaux, F., Gerrits van den Ende, A. H., Kwon-Chung, K. J. & McGinnis, M. R. (1995). Nutritional physiology and taxonomy of human pathogenic *Cladosporium-Xylohypha* species. *Medical Mycology*, 33: 339 – 347.

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, (4): 177 doi:10.3389/fphar.2013.00177

Ezeobiora, C. E., Igbokwe, N. H., Amin, D. H. & Mendie, U. E. (2021). Endophytic microbes from Nigerian ethnomedicinal plants: a potential source for bioactive secondary metabolites—a review *Bulletin of the Natural Research Centre*, 45:103 https://doi.org/10.1186/s42269-021-00561-7

Ezekwesili, C. N., Obiora, K. A. & Ugwu, O. P. (2004). Evaluation of anti-diarrhoeal property of crude aqueous extract of *Ocimum gratissimum* L. (Labiatae) in rats. *Biokemistri*, 16(2):122 – 131.

Article DOI: 10.52589/RJBLS-SSS4H4UE
DOI URL: https://doi.org/10.52589/RJBLS-SSS4H4UE

Gallo, M. L., Seldes, A. M. & Cabrera, G. M. (2004). Antibiotic long-chain and a,b-unsaturated aldehydes from the culture of the marine fungus *Cladosporium* sp. *Biochemical Systematics and Ecology*, 32: 545 – 551.

Hamayun, M., Khan, S. A., Ahmad, N., Tang, D-S., Kang, S-M., Na, C-I., Sohn, E-Y., Hwang, Y-H., Shin, D-H., Lee, B-H., Kim, J-G. & Lee, I-J. (2009). *Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max* (L.) Merr. *World Journal of Microbiology and Biotechnology*, 25:627 – 632 doi 10.1007/s11274-009-9982-9

Hoog, G. S., de Guarro, J., Gené, J. & Figueras, M. J. (2000). Atlas of Clinical Fungi

Imosemi, I. O. (2020). A review of the medicinal values, pharmacological actions, morphological effects and toxicity of *Ocimum gratissimum* Linn. *European Journal of Pharmaceutical and Medical Research*, 7(7): 29 – 40.

James, G, C. & Natalie, S. (2013). Microbiology: A Laboratory Manual (10th ed.). Benjamin Cummings, New York. 560pp.

Kirk, P. M., Cannon, P. F., David, J. C. & Stalpers, J. A. (2008) Ainsworth & Bisby's dictionary of the fungi (10th ed.). CAB International, Wallingford. 771pp.

Li,Y., Zheng, X., Zhang, X., Bao, L., Zhu, Y., Qu, Y., Zhao, J. & Qin, Y. (2016). The different roles of *Penicillium oxalicum* LaeA in the production of extracellular cellulase and β-xylosidase. *Frontiers in Microbiology*, 7:2091. doi: 10.3389/fmicb.2016.02091

Liu, L. (2011). Bioactive metabolites from the plant endophyte *Pestalotiopsis fici*. *Mycology*, 2(1): 37 – 45, doi: 10.1080/21501203.2011.562248.

Manganyi, M. C. & Ateba, C. N. (2020). Untapped potentials of endophytic fungi: A Review of novel bioactive compounds with biological applications. *Microorganisms*, 8: 1934; doi:10.3390/microorganisms8121934

Masclaux, F., Gueho, E., De Hoog, G. & Christen, R. (1995). Phylogenetic relationships of human pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. *Medical Mycology*, 33: 327 – 338.

Meng, L., Sun, P.,Tang, H., Li, L., Draeger S., Schulz, B., Krohn, K., Hussain, H., Zhang, W. & Yi, Y. (2011). Endophytic fungus *Penicillium chrysogenum*, a new source of hypocrellins. *Biochemical Systematics and Ecology*, 39: 163 – 165.

Moreira, D. L., Teixeira, S. S., Monteiro, M. D., De-Oliveira, A. A. X. & Francisco, J. R. (2014). *Paumgartten Revista Brasileira de Farmacognosia*; 24: 248 – 257.

Muhammad, M., Basit, A., Ali, K., Ahmad, H., Li, W. J., Khan, A. & Mohammed, H. I. (2024). A review on endophytic fungi: a potent reservoir of bioactive metabolites with special emphasis on blight disease management. *Archives of Microbiology*, 206(3):129, doi:10.1007/s00203-023-03828-x. PMID: 38416214.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L. *et al*…. (2021). Global burden of bacterial antimicrobial resistance in 2019: a systemic analysis. *The Lancet*, 399(10325): 629 – 655.

Oboh, F. O. J. & Masodje, H. I. (2009). Nutritional and antimicrobial properties of *Jatropha tanjorensis* leaves. *American-Eurasian Journal of Scientific Research*, 4 (10): 7 – 10.

Oliveira, V., Gomes, N. C. M., Almeida, A., Silva, A. M. S., Simões, M. M. Q., Smalla, K. % Cunha, A. (2014). Hydrocarbon contamination and plant species determine the phylogenetic and functional diversity of endophytic degrading bacteria. *Molecular Ecology*, 23: 1392 – 1404. doi: 10.1111/mec.12559.

Omoregie, E. S. & Osagie A. U. (2012). Antioxidant properties of methanolic extracts of some Nigerian plants on nutritionally-stressed rats. *Nigerian Journal of Basic and Applied Science*, 20(1): 7 – 20.

Article DOI: 10.52589/RJBLS-SSS4H4UE
DOI URL: https://doi.org/10.52589/RJBLS-SSS4H4UE

Omoregie, E. S. & Sisodia, B. S. (2011). Invitro antiplasmodial activity and cytotoxicity of leaf extracts of *Jatropha tanjorensis*. *Pharmacology Online*, 2: 656 – 673.

Orhue, E. S., Idu, M., Atamen, J. E. & Ebite, L. E. (2008). Haematological and Histopathological studies of *Jatropha tanjorensis* (J. L. Ellis and Soroja) leaves in rabbits. *Asian Journal of Biological Science*, 1: 84 – 89.

Park, H-S., Jun, S-C., Han, K-H., Hong, S-B. & Yu, J-H. (2017). Diversity, Application, and Synthetic Biology of Industrially Important *Aspergillus* Fungi. *Advances in Applied Microbiology*, 100: 161 – 202.

Phongpaichit, S., Nikom, J., Rungjindamai, N., Sakayaroj, J., Hutadilok-Towatana, N., Rukachaisirikul, V & Kirtikara, K. (2007). Biological activities of extracts from endophytic fungi isolated from *Garcinia* plants. *FEMS Immunology and Medical Microbiology*, 51: 517 – 525.

Pondei, J. O., Ogugbue, C. J. & Okpokwasili, G. C. (2018). Distribution, Biochemical Properties and Genetic Relatedness of Endophytic Bacteria of Wetland Plants from Petroleum-Contaminated Sites of the Niger Delta, Nigeria. *International Journal of Horticulture, Agriculture and Food Science*, 2(5): 155 – 173.

Rai, N., Keshri, P. K., Verma, A., Kamble, S. C., Mishra, P., Barik, S., Singh, S. K. & Gautam, V.N. (2021). Plant associated fungal endophytes as a source of natural bioactive compounds. *Mycology*, 12(3): 139 – 159. https://doi.org/10.1080/21501203.2020.1870579.

Rathod, D. P., Dar, M. A., Gade, A. K. & Rai, M. K. (2014). Griseofulvin-producing endophytic *Nigrospora oryzae* from Indian *Emblica officinalis* Gaertn: a new report. *Austin Journal of Biotechnology and Bioengineering*, 1(3): 5.

Romero, A., Carrión, G. & Rico-Gray, V. (2001). Fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* fAsteraceae). *Fungal Diversity*, 7: 81 – 87.

Schulz, B. J. & Boyle, C. (2006). What are Endophytes? In Microbial Root Endophytes.

Schulz, B., Boyle, C., Draeger, S., Rommert, A-K. & Krohn, K. (2002). Endophytic pp i-13 doi: 10.1007/3-540-33526-9 1 fungi: a source of biologically active secondary metabolites. *Mycological Research*, 106; 996 – 1004.

Singh, B., Thakur, A., Kaur, S., Chadha, B. S. & Kaur, A. (2012). Acetylcholinesterase inhibitory potential and insecticidal activity of an endophytic *Alternaria* sp. from *Ricinus communis*. *Applied Biochemistry and Biotechnology*, 168: 991 – 1002.

Strobel, G. A., Daisy, B., Castillo, U. & Harper, J. (2004). Natural products from endophytic microorganisms. *Journal of Natural Products*, 67: 257 – 268.

Tan, R. X. & Zou, W. X. (2001). Endophytes: a rich source of functional metabolites. *Natural Product Report*, 18: 448 – 459.

Tang, Z., Wang, Y., Yang, J., Xiao, Y., Cai, Y., Wan, Y., Chen, H., Yao, H., Shan, Z., Li, C. & Wang, G. (2020). Isolation and identification of flavonoid-producing endophytic fungi form medicinal plant *Conyza blini* H, Lev that exhibit higher antioxidant and antibacterial activities. *Peer-reviewed Journal*, 15;8: e8978. doi:10.7717/peeri, 8978

Tânia, M. A. A., Andria, F. S., Mitzi, B., Telma, S. M. G., Elza de Fatima, A. S., Artur, S. J. & Carlos, L. Z. (2000). Biological Screening of Brazilian Medicinal Plants. *Memorias do Instituto Oswaldo Cruz, Rio de Janeiro*, 95(3): 367 – 373.

Thanabalasingam, D., Kumar, N. S., Jayasinghe, L. & Fujimoto, Y. (2015). Endophytic fungus *Nigrospora oryzae* from a medicinal plant *Coccinia grandis* a high yielding new source of phenazine-1-carboxamide. *Natural Product Communications*, 10(10): 1659 – 60.

Toghueo, R. M. K. (2020). Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. *Mycology*, 11: 1 – 21.

Article DOI: 10.52589/RJBLS-SSS4H4UE
DOI URL: https://doi.org/10.52589/RJBLS-SSS4H4UE

Toghueo, R. M. K. & Boyom, F. F. (2020). Endophytic *Penicillium* species and their agricultural, biotechnological, and pharmaceutical applications, *3 Biotech*, 10: 107 https://doi.org/10.1007/s13205-020-2081-1

Vijayalakshmi, R., Kairunnisa, K., Sivvaswamy, S. N., Dharan, S. S. & Natarajan, S. (2016). Enzyme production and antimicrobial activity of endophytic bacteria Isolated from medicinal plants. *Indian Journal of Science and Technology*, 9(14), doi: 10.17485/ijst/2016/v9i14/83143

Zhang, J., Onakpoya, I. J., Posadzki, P. & Eddouks, M. (2015). The safety of herbal medicine: from prejudice to evidence. Evidence-Based Complementary and Alternative Medicine Volume 2015, Article ID 316706, 3 pages http://dx.doi.org/10.1155/2015/316706

WHO (2005). World malaria report. World Health Organisation, Geneva Switzerland

Article DOI: 10.52589/RJBLS-SSS4H4UE
DOI URL: https://doi.org/10.52589/RJBLS-SSS4H4UE

Table 1: Endophytic fungal isolates of different parts of *Ocimum gratissimum*

Endophytic fungal isolate	Plant Compartment			Frequency
	Root	Stem	Leaf	
Alternaria alternata	+	+	+	3
Aspergillus flavus	+	+	+	3
Cladosporium sphaerospermum	+	-	+	2
Nigrospora oryzae	+	-	+	2
Penicillium chrysogenum	-	+	+	2
Penicillium oxalicum	+	-	-	1
Total	5	3	5	13

Key: + present - absent

Table 2: Endophytic fungal isolates of different parts of *Jathropha tanjorensis*

Endophytic fungal isolate	Plant Compartment			Frequency
	Root	Stem	Leaf	
Alternaria alternate	+	+	+	3
Aspergillus flavus	+	+	+	3
Nigrospora oryzae	-	+	+	2
Penicillium chrysogenum	+	-	+	2
Penicillium oxalicum	+	-	+	2
Rhizoctonia solani	+	+	-	2
Total	5	4	5	14

Key: + present - absent

Table 3: Antibacterial activities of the crude extracts of endophytic fungi

Endophytic Fungi	Antibacterial activity measured as zones of inhibition (mm)				
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Alternaria alternate	32.7 ± 0.58^{e}	24.1 ± 0.10^{c}	30.1 ± 0.10^{d}	22.7 ± 0.15^{b}	20.5 ± 0.15^{a}
Aspergillus flavus	24.7 ± 0.10^{D}	18.7 ± 0.10^{A}	23.4 ± 0.12^{C}	22.7 ± 0.10^{B}	24.6 ± 0.06^{D}
Cladosporium sphaerospermum	25.3 ± 0.15^{h}	30.0 ± 0.06^{i}	21.3 ± 0.15^{g}	21.5 ± 0.21^{g}	20.9 ± 0.20^{f}
Nigrospora oryzae	24.7 ± 0.06^{I}	24.2 ± 0.21^{H}	19.5 ± 0.20^{G}	18.6 ± 0.15^{F}	18.7 ± 0.15^{F}
Penicillium chrysogenum	28.9 ± 0.36^{m}	28.3 ± 0.21^{m}	16.4 ± 0.30^{l}	16.5 ± 0.25^{l}	14.5 ± 0.20^{k}
Penicillium oxalicum	20.2 ± 0.15^{M}	22.8 ± 0.15^{N}	11.3 ± 0.10^{K}	12.2 ± 0.20^{L}	11.5 ± 0.20^{K}
Rhizoctonia solani	8.2 ± 0.20^{p}	11.1 ± 0.10^{q}	14.7 ± 0.06^{s}	14.6 ± 0.20^{s}	12.3 ± 0.31^{r}
Chloramphenicol	36.1 ± 0.12^{T}	34.1 ± 0.12^{R}	34.1 ± 0.15^{R}	30.3 ± 0.25^{Q}	28.4 ± 0.40^{P}

Mean values on the same row with different alphabet represents statistical significant difference ($P<0.05$) using Duncan Multiple Range (DMR) post-hoc analysis of variance of comparisons of means.

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