The anti-pseudomonal potentials of metabolites from some endophytic fungi isolated from *Garcinia kola* leaves

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Abstract

The morbidity and mortality rates from *Pseudomonas aeruginosa* infections are increasing, due to the development of drug-resistant strains. This study aimed to explore the secondary metabolites of endophytic fungi of *Garcinia kola* for their antibacterial activities against *P. aeruginosa*. The endophytic fungi associated with healthy leaves of *G. kola* were isolated using the standard methods. These fungi were subjected to solid-state fermentation on rice media at 28°C for 21 d. The fungal secondary metabolites were extracted using ethyl acetate, and then concentrated under vacuum. The fungal crude extracts were screened for their antibacterial activities against clinical and laboratory strains of *P. aeruginosa*, using the agar diffusion method. The bioactive components of the fungal extracts were identified using High-Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) analysis. Three endophytic fungi mainly; *Aspergillus* sp., *Fusarium* sp. and *Colletotrichum* sp. were isolated. At concentration of 1 mg/ml, extracts of the three fungi displayed anti-pseudomonal activities against all the isolates, except for a *P. aeruginosa* isolate recovered from urine. Results of the HPLC-DAD analysis revealed the presence of several active compounds such as; indole-3-acetic acid, p-hydroxybenzoic acid, and protocatechuic acid, among others in the fungal extracts. These compounds have been previously reported to have significant antimicrobial properties. This study reveals that endophytic fungi associated with *G. kola* leaves possess promising anti-pseudomonal potential.

Keywords: *Pseudomonas aeruginosa*, *Garcinia kola*, Endophytic fungi, Anti-pseudomonal, Secondary metabolites
1. Introduction

*Pseudomonas aeruginosa* is a major etiological agent of healthcare-associated bacterial infections and is responsible for 11% of the hospital-acquired infections, which results in high mortality and morbidity rates (Haque et al., 2018). Unfortunately, *P. aeruginosa* is susceptible to a limited number of antibacterial agents, suggesting that the high mortality rate associated with this bacterium could be attributed not only to its virulence, but also to the administration of ineffective empirical antibacterial therapy. The morbidity and mortality from *P. aeruginosa* infections are increasing, due to these drug-resistant strains. Several previous studies of Ozer et al. (2009); Marilyn et al., (2012); Egbujor et al., (2020) reported that resistance development in *P. aeruginosa* is multifactorial, with mutations in several genes contributing for resistance to β-lactams, carbapenems, aminoglycosides, fluoroquinolones and sulphonamides. Accordingly, it has become of utmost importance to develop new antibacterial agents to inhibit or completely kill this bacterium, thus saving our society from its deleterious effect. A previous study of Petrini, (1991) defined endophytes and endophytic fungi as microorganisms that grow intercellularly and asymptotically within the plant living tissues, thus establishing a mutual relationship with this host plant. Yan et al., (2011); Okoye et al., (2013); Nwobodo et al., (2017) highlighted that the endophytic fungal species are considered as exciting novel sources of new bioactive compounds for drug discovery. A large number of the world’s populations especially from developing countries rely mainly on traditional medicines derived from plants for their primary health care. A recent study conducted by Lawrence and Vandecar, (2015) revealed that as the search for more effective and safer bioactive compounds from natural sources continues, products derived from plant sources require large scale harvesting and probably destruction of such plants, which in turn may lead to climatic imbalance and environmental disruption. The study of plant-associated endophytes could provide an alternative way of discovering novel active metabolites with anti-pseudomonal properties. Several previous studies of Tende et al., (2011); Aljabry et al., (2017) demonstrated that *G. kola* (Heckel) plant is reputed to exhibit several potent pharmacological activities such as; antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties. This plant belongs to the family Guttiferae that is popular in African traditional medicine (Indabawa and Arzai, 2011). Considering the numerous therapeutic potentials of this plant, as well as the eco-friendly properties of the endophytic fungi drug discovery approach, the objective of this study was to screen for the in-vitro anti-pseudomonal potency of the endophytic fungal extracts of *G. kola*, against the clinical and laboratory strains of *P. aeruginosa*.

2. Material and methods

2.1. Isolation and purification of the endophytic fungi

Samples of fresh leaves of mature healthy *G. kola* plants were collected from Nsukka, Enugu State, South-Eastern Nigeria. Isolation of endophytic fungi from the plant leaves was carried out as described by Eze et al., (2018). The leaves were washed thoroughly in running tap water, and then cut into small fragments (about 1 cm²). The leaf fragments were surface-sterilized by immersion in 2% sodium hypochlorite solution for 2 min., 70% ethanol for nearly 2 min., before a final rinse in sterile water for 5 min. These leaf fragments were transferred into malt extract agar (MEA) plates, supplemented with chloramphenicol (500 mg/ l). The Petri plates were then incubated at 27°C for 7 d. Hyphal tips of fungal colonies emerging from the leaf segments were sub-cultured on fresh MEA plates, and then purified using single spore technique.

2.2. Solid state fermentation and extraction of the fungal secondary metabolites

Massive production of the isolated endophytic fungi through solid-state fermentation, and extraction of the fungal metabolites were carried out as described.
Rice medium was prepared in 1000 ml Erlenmeyer flasks as follows: approximately 200 ml of dist. water was added to 100 g of rice, and then autoclaved at 121°C for 30 min. The flasks were inoculated individually with 4 agar blocks (3 mm diameter), cut from each pure endophytic fungal culture using a sterile cork borer, and then incubated at 28°C for 21 d. After incubation, the culture media and the growing mycelia were extracted using ethyl acetate, and then separated by filtration. The organic phase was vacuum-concentrated at 40°C under reduced pressure, using a rotary vacuum evaporator to obtain the crude extracts.

2.3. In vitro anti-pseudomonal activity assay

2.3.1. Test bacteria

Various clinical isolates of *P. aeruginosa* were obtained from orthopedic wound infections; urine, sputum, and a vaginal swab, provided by the National Orthopedic Hospital Enugu, Nigeria, and a laboratory strain were used for the study. The identities of the bacterial isolates were confirmed at the Pharmaceutical Microbiology Laboratory, Nnamdi Azikiwe University, Agulu, using the standard morphological and biochemical characteristics of the bacteria, according to *Bergey and Holt, (2000)*.

2.3.2. The in vitro bioassay

The preliminary screening of the endophytic fungal extracts for their anti-pseudomonal activity was carried out using the agar well diffusion assay of *Onyegbule et al., (2014)*. Stock concentrations (1 mg/ml) of the fungal extracts were prepared by dissolving the extracts in dimethyl sulphoxide (DMSO 100% v/v). About 0.1 ml (1× 10^5 cells/ ml) of test bacterial isolates (*P. aeruginosa*) was spread aseptically and individually onto the surface of Mueller Hinton Agar (MHA) plates. All plates were allowed to dry for about 5 min., and then agar wells were made by using a sterile cork-borer (6 mm in diameter). These wells were individually inoculated with 20 μl of each of the fungal extracts and the controls. The plates were then kept at room temperature for 1 h, and then incubated at 37°C for 24 h. Gentamicin antibiotic disc (10 μg/ml) and DMSO (100% v/v) were used as the positive and negative controls, respectively. The inhibition zones diameters (IZDs) were measured using a calibrating ruler. The assay was conducted in triplicates, and repeated twice.

2.4. High performance liquid chromatography (HPLC) analysis

The HPLC analysis was carried out on the fungal extracts as described by *Eze et al., (2018)*. A Dionex P580 HPLC system coupled to a photodiode array detector (UVD340S, Dionex Softron GmbH, Germering, Germany) was used during the analysis. The separation column (125 × 4 mm; length × internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), a linear gradient of nano-pure water (adjusted to pH 2 by addition of formic acid), and methanol was used as eluent. A weight of 2 mg of each fungal extract was reconstituted with 2 ml of HPLC grade methanol, the mixture was sonicated for 10 min., and thereafter centrifuged at 3000 rpm for 5 min. A volume of 100 μl of the dissolved sample was transferred to a vial containing 500 μl of HPLC grade methanol, and then the vial was put in the HPLC machine for analysis. Detection was carried out at 235 nm. The absorption peaks of the fungal extracts were analyzed by comparing with those in the HPLC-UV/Vis database.

2.5. Statistical analysis

Data obtained were presented as means of experiments that were carried out in triplicates. The mean inhibition zones diameter of the fungal extracts against the various *P. aeruginosa* isolates were compared using one way ANOVA. Statistical significance was considered at *p* ≤ 0.05. Analysis of data and graph were made using Microsoft ExceIs 2013 software and SPSS version 20.

3. Results

3.1. Isolation and identification of the endophytic fungi
A total of three endophytic fungi were isolated from the leaf segments of *G. kola* plant, and labeled as: Gc1-3. The three isolates exhibited different colony morphologies on MEA on (Fig. 1). These isolates were identified according to their morphological and microscopic characters as; Gc1: *Aspergillus* sp., Gc2: *Fusarium* sp. and Gc3: *Colletotrichum* sp. (Table 1).

![Figure 1](image_url) **Fig 1**: Macroscopic colony morphologies of the three endophytic isolates. Gc1: *Aspergillus* sp., Gc2: *Fusarium* sp., and Gc3: *Colletotrichum* sp.

**Table 1.** Cultural morphological and microscopic features of the endophytic fungi isolated from *G. kola* leaves

| Isolates | Color                    | Reverse     | Growth Rate | Texture       | Hyphae   | Spores                           | Identified fungus                                      |
|----------|--------------------------|-------------|-------------|---------------|----------|----------------------------------|--------------------------------------------------------|
| Gc1      | Brownish Green, with a white border | Hyaline     | Moderate    | Dry and Hard  | Septated | Micro conidia                    | *Aspergillus* sp. (Afzal et al., 2013). |
| Gc2      | White                    | Colorless   | Rapid       | Cottony Smooth| Septated | Micro and Macro conidia          | *Fusarium* sp. (Tayung et al., 2011).                |
| Gc3      | White/orange Whitish grey | Moderate    | Cottony Rough| Septated     |          | Macro conidia with ascus/ascospores | *Colletotrichum* sp. (Arivudainambi et al., 2011). |
3.2. *In vitro* anti-pseudomonal activity of the fungal extracts

The *in vitro* antibacterial potential of the fungal extracts against the clinical isolates of *P. aeruginosa* are demonstrated in Fig. (2). The laboratory isolate (*P. A*) and the vaginal isolates (*P. E*) are susceptible to 100 % of the tested fungal extracts at a concentration of 1 mg/ ml. While the *P. aeruginosa* isolate from urine is resistant to all fungal extracts. The orthopedic wound isolate (*P. B*) is susceptible only to the extracts of *Colletotrichum* sp., recording an IZD of 2± 0 at 1 mg/ ml. All the tested fungal extracts inhibited at least 60 % of the *P. aeruginosa* isolates under study. The mean values of the zones of inhibition obtained for the three fungi are statistically not significant as *p* > 0.05. However, when compared to Gentamicin, *p* < 0.001.

3.3. Separation and identification of the compounds present in the fungal extracts

Results present in Table (2) demonstrate the active compounds present in the individual fungal extracts identified through the HPLC, and their previously reported biological activities. About six known compounds were identified as; Enniatin A, Protocatechuic acid, P-hydroxybenzoic acid, P-methoxycumarin, Indolyl-3-acetic acid, and 4-hydroxyphenyl acetic acid. The chromatogram and structures of *Fusarium* sp. and *Colletotrichum* sp. extracts are represented in Fig 3(a, b), respectively. They reveal the peaks and structures for P-methoxycumarin and indole-3-acetic acid, respectively.

**Fig. 2:** Inhibition zones diameters (mm) of the different clinical *Pseudomonas* isolates, caused by treatments with the three fungal extracts and the antibiotic (Gentamicin). Where; *P. A* = Laboratory isolate, *P. B* = Orthopedic wound infection isolate, *P. C* = Urine isolate, *P. D* = Sputum isolate, *P. E* = Vaginal swab isolate. Values above the columns represent diameters of the inhibition zones (mm). The mean values of the inhibition zones are statistically not significant as *p* > 0.05 for the fungal extracts; however, for Gentamicin, *p* < 0.001.
Table 2. The compounds detected in the fungal extracts and their antimicrobial properties

| Fungi Extract | Identified compounds          | Reported biological activities                  | References                        |
|---------------|-------------------------------|-------------------------------------------------|-----------------------------------|
| *Aspergillus* sp. | Enniatin A                   | Inhibition of drug efflux pump,                 | Firáková *et al.*, (2007)         |
|                | Protocatechuic acid           | Antimicrobial                                   | Kakkar and Bais, (2014)           |
| *Fusarium* sp. | Enniatin A                    | Inhibition of drug efflux pump,                 | Firáková *et al.*, (2007)         |
|                | *P*-hydroxybenzoic acid       | Antimicrobial                                   | Manuja *et al.*, (2013)           |
|                | *P*-methoxycumarin            | Antimicrobial                                   | Malhotra *et al.*, (2008)         |
| *Colletotrichum* sp. | Indole-3-acetic acid     | Antimicrobial                                   | Trinagaraju *et al.*, (2015);     |
|                | 4-hydroxyphenyl acetic acid   | Antimicrobial                                   | Arnao *et al.*, (1996)            |

![HPLC chromatogram of *Fusarium* sp. extract, showing UV spectrum and structure of *P*-methoxycumarin](image-url)

**Fig. 3a:** HPLC chromatogram of *Fusarium* sp. extract, showing UV spectrum and structure of *P*-methoxycumarin
4. Discussion

In accordance with our results, the fungal endophytes isolated from G. kola in this study have been previously isolated as endophytes in several previous studies; Aspergillus sp., Fusarium sp. (Phongpaichit et al., 2006; Zhao et al., 2010) and Colletotrichum sp. (Lu et al., 2000). The majority of the compounds identified in the endophytic fungal extracts in this study, have been previously reported to be produced by fungi of endophytic origins. Lu et al. (2000) reported the production of indole acetic acid and hydroxyphenyl derivatives by Colletotrichum sp.; in addition, two new indole alkaloids were isolated from Aspergillus sp. by Hasan et al. (2015). Likewise, in other different independent and separate studies, Fusarium sp. was reported to produce Enniatins A (Firáková et al., 2007; Lucasz and Waskiewicz, 2013).

The recorded antibacterial activity displayed by the endophytic fungal extracts in this study could be attributed to the presence of antimicrobial compounds in their metabolic extracts. Several previous studies of Anthony, (2009); Carolina et al. (2010); Oksana et al. (2012), reported that 4-hydroxy benzoic acid is an organic chemical that exhibits antimicrobial activity against a number of microorganisms such as Gram positive as well as Gram negative bacteria, including P. aeruginosa. Moreover, Manuja et al., (2013); Eze et al., (2019) added that Protocatechuic acid (3, 4-dihydroxybenzoic acid) is a natural phenolic acid found in most edible and medicinal plants. This compound has also been reported to be produced by several species of bacteria and fungi (Nguyen et al., 2015; Eze et al., 2018). Previous studies conducted by Kakkar and Bais, (2014); Nwobodo et al., (2017) revealed that protocatechuic acid exhibited varying biological activities including; antibacterial, antioxidant, anticancer, and anti-inflammatory potentials. On the other hand, indole-3-acetic acid is a plant hormone, which regulates various aspects of plant growth and development (Fu et al., 2015). There are many reports on the production of indole-3-acetic acid by certain bacterial and fungal species (Kakkar and Bais, 2014; Fu et al., 2015; Nwobodo et al., 2017). A previous study of Trinagaraju et al., (2015) described the metal complexes of 3-indole acetic acid as having good inhibitory activity against the Bacillus subtilis as well as Candida sp. The other compounds recorded in this study including Enniatins A, P-methoxycumarin, P-hydroxybenzoic acid and 4-hydroxyphenyl acetic acid, have all been reported to possess antimicrobial activities (Table 2.).

Fig. 3b: HPLC chromatogram of Colletotrichum sp. extract, showing UV spectrum and structure of indole-3-acetic acid.
Extracts of the three fungal spp. isolated in this study displayed varying anti-pseudomonal activities against all the bacterial isolates, except \textit{P. aeruginosa} isolate that was recovered from urine. The extract of \textit{Aspergillus} sp. exhibited the best inhibitory activity, with an average inhibition zone of 5 mm at 1 mg/ml. Brown and Izundu, (2004); Garba et al., (2012) reported that \textit{P. aeruginosa} wound isolates are highly resistant, when compared to the same isolates from other sources.

An emerging problem with \textit{P. aeruginosa} infection is that this pathogen exhibits a high degree of resistance to a broad spectrum of antibiotics. Interestingly, \textit{P. aeruginosa} isolate from wound regarded to be a very resistant strain, is currently observed to be susceptible to the extract of \textit{Colletotrichum} sp. This may be due attributed to the presence of 4-hydroxyphenyl acetic acid and indole-3-acetic acid identified in the fungal extract, as revealed by the HPLC results. Since only the extract of \textit{Colletotrichum} sp. reported to contain both 4-hydroxyphenyl acetic acid and indole-3-acetic acid, and had an activity against the wound isolate, thus the combination of both of these principal compounds could produce synergistic mechanisms, responsible for the inhibitory activity observed against the wound isolate. Moreover, 4-hydroxyphenyl acetic acid is a phenolic compound, and phenolics have been previously reported by Merkl et al., (2010); Bouarab-Chibane et al., (2019) to possess antimicrobial properties. This is an important observation, and suggests that the extract of \textit{Colletotrichum} sp. possesses potential as an antibacterial agent against the possible drug-resistant strains of \textit{P. aeruginosa}. A previous study of Webber, (1981) documented that the presence of bioactive compounds confer resistance to plants against the bacteria, fungi, and several other pests. This probably further explains the antibacterial potency demonstrated by the extracts of the plant endophytic fungi recorded in the current study. It is noteworthy that, the compounds responsible for the observed antibacterial activities may have a structural analogue to the previously established drugs, known to show such effective anti-pseudomonal activity. This can be ascertained through purification and further structural elucidation of the identified compounds, which may be considered as promising compounds for the development of anti-pseudomonal drugs.

Conclusions

The current recorded \textit{in vitro} anti-pseudomonal activity of the endophytic fungal extracts of \textit{G. kola} appears promising. This may be attributed to the presence of several active compounds identified in these fungal extracts. Thus they could be used as promising natural sources of anti-pseudomonal agents, for treatment of infections caused by the pathogenic strains of \textit{P. aeruginosa}.

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Conflict of interest

The authors declare that they have no any competing interests.

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Ethical approval

Non-applicable.

5. References

Afzal, H.; Shazad, S. and Nisa, S.Q. (2013). Morphological identification of \textit{Aspergillus} species from the soil of Larkana district (Sindh, Pakistan). Asian Journal of Agriculture and Biology. 1(3): 105-117.
Aljabry, A.S.; Hussein, N.M.; Ali, M.S.; Mohamed, L.A.; Saleh, M.M. and Alrashied, A.A. (2017). Antimicrobial and antifungal properties of *Garcinia kola* on some standard laboratory pathogens. International Journal of Medical and Health Sciences. 6(4): 201-205.

Anthony, C.D. (2009). The internal and external use of medicinal plants. Clinics in Dermatology. 27: 148-158.

Arivudainambi, U.S.E.; Anand, T.D.; Shanmugaiah, V.; Karunakaran, C. and Rajendran, A. (2011). Novel bioactive metabolites producing endophytic fungus *Colletotrichum gloeosporioides* against multidrug-resistant *Staphylococcus aureus*. FEMS Immunology and Medical Microbiology. 61: 340-345.

Arnao, M.B.; Sanchez-Bravo, J. and Acosta, M. (1996). Indole-3-carbinol as a scavenger of free radicals. Biochemistry and Molecular Biology International. 39(6): 1125-1134.

Bergey, D.H. and Holt, J.G. (2000). Bergey’s manual of determinative bacteriology. 9th ed. Philadelphia: Lippincott Williams and Wilkins.

Bouarab-Chibane, L.; Forquet, V.; Lanteri, P.; Clement, Y.; Leonard-Akkari, L.; Oulahal, N.; Degraeve, P. and Bordes, C. (2019). Antibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure-Activity Relationship) Models. Frontiers in Microbiology. 10: 829.

Brown, P.D. and Izundu, A. (2004). Antibiotic resistence in clinical isolates of *P. aeruginosa* in Jamaica. Revista Panamericana de Salud Publica. 16 (2): 125-130.

Carolina, C.; Arribas, V.M.; Martín, P.J.; Bills, G. and Vicente, M.F. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. Research in Microbiology. 161(5): 372-382.

Egbujor, M.C.; Nwobodo, D.C.; Egwuatu, P.I.; Abu, I.P. and Ezeagu, C.U. (2020). Sulphonamide drugs and *Pseudomonas aeruginosa* resistance: A review. International Journal of Modern Pharmaceutical Research. 4(1): 78-83.

Eze, P.M.; Nnanna, J.C.; Okezie, U.; Buzugbe, H.S.; Abba, C.C.; Chukwunwejim, C.R.; Okoye, F.B.C. and Esimone, C.O. (2019). Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities. The EuroBiotech Journal. 3(1): 10-18.

Eze, P.M.; Ojimba, N.K.; Abonyi, D.O.; Chukwunwejim, C.R.; Abba, C.C.; Okoye, F.B.C. and Esimone, C.O. (2018). Antimicrobial Activity of Metabolites of an Endophytic Fungus Isolated from the Leaves of *Citrus jamhiri* (Rutaceae). Tropical Journal of Natural Products Research. 2(3): 145-149.

Firáková, S.; Proksa, B. and S’turdı’kova, M. (2007). Biosynthesis and biological activity of enniatins. Pharmazie. 62: 563-568.

Fu, S.; Wei, J.; Chen, H.; Liu, Y.; Lu, H. and Chou, J. (2015). Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behavior. 10(8): e1048052.

Garba, I.; Lusa, Y.H.; Bawa, E.; Tijjani, M.B.; Aliyu, M.S.; Zango, U.U. and Raji, M.I. (2012). Antibiotics susceptibility pattern of *Pseudomonas aeruginosa* isolated from wounds in patients attending Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Nigerian Journal of Basic and Applied Science. 20(1): 32-34.

Haque, M.; Sartelli, M.; McKimm, J. and Bakar, M.B. (2018). Healthcare-associated infections- an overview. Infectious Drug Resistance. 11: 2321-2333.

Hasan, S.; Ansari, M.I.; Ahmad, A. and Mishra, M. (2015). Major bioactive metabolites from marine fungi: A review. Bioinformation. 11(4): 176-181.

Indabawa, I.I. and Arzai, A.H. (2011). Antibacterial Activity of *Garcinia kola* and *Cola nitida* Seed
Extracts. Bayero Journal of Pure and Applied Sciences. 4(1): 52-55.

Kakkar, S. and Bais, S. (2014). A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacology. 1-9. http://dx.doi.org/10.1155/2014/952943.

Lawrence, D. and Vandecar, K. (2015). Effects of tropical deforestation on climate and agriculture. Nature Climate Change. 5: 27-36.

Lu, H.; Zou, W.; Meng, J.C.; Hu, J. and Tan, R.X. (2000). New bioactive compound produced by Collectotrichum sp., an endophytic fungus of Artemisia annua. Plant Science. 151(1): 67-73.

Lucasz, S. and Waskiewicz, A. (2013). Sequence divergence of the Enniantin synthase gene in relation to production of beauvericin and enniatin in Fusarium species. Toxin (Basel). 5(3): 537-555.

Malhotra, S.; Shakya, G.; Kumar, A.; Vanhoecke, B.W.; Cholli, A.L.; Raj, H.G.; Saso, L.; Ghosh, B.; Bracke, M.E.; Prasad, A.K.; Biswal, S. and Parmar, V.S. (2008). Antioxidant, Anti-inflammatory and Anti-invasive Activities of Biopolyphenolics. ARKIVOC. (6) 119-139.

Manuja, R.; Sachdeva, S.; Jain, A. and Chaudhary, J. (2013). A Comprehensive Review on Biological Activities of P-Hydroxy Benzoic Acid and Its Derivatives. International Journal of Pharmaceutical Science Review Research. 22(2): 109-115.

Marilyn, P.G.; José, V.B. and Santiago, N.C. (2012). Overview of Multidrug-Resistant Pseudomonas aeruginosa and Novel Therapeutic Approaches. Journal of Biomaterials and Nanobiotechnology. 3: 519-527.

Merkl, R.; Hrádková, I.; Filip, V. and Šmi Drkal, J. (2010). Antimicrobial and Antioxidant Properties of Phenolic Acids Alkyl Esters. Czech Journal of Food Science. 28(4): 275-279.

Nguyen, X.H.; Naing, K.W.; Lee, Y.S.; Moon, J.H.; Lee, J.H. and Kim, K.Y. (2015). Isolation and characteristics of protocatechuic acid from Paenibacillus elgii HOA73 against Botrytis cinerea on strawberry fruits. Journal Basic Microbiology. 55(5): 625-34.

Nwobodo, D.C.; Ihekereme, C.P.; Ugwu, M.C. and Okoye, F.B.C. (2017). Screening of Endophytic Fungal Secondary Metabolites from Garcinia kola and Cola nitida for Antioxidant Properties. Open Access Journal of Pharmaceutical Research. 1(6): 000136.

Okoye, F.B.C.; Lu, S.; Nworu, C.S. and Abdessamad, D. (2013). Depsidone and diaryl ether derivatives from the fungus Corynespora cassiicola, an endophyte of Gongronema latifolium. Tetrahedron Letter. 54: 4210-4214.

Oksana, S.; Marian, B.; Mahendra, R. and Hong, B.S. (2012). Plant phenolic compounds for food, pharmaceutical and cosmetics production. Journal of Medicinal Plants Research. 6: 2526-2539.

Onyegbule, F.A.; Iloumo, I.O.; Eze, P.M.; Abba, C.C. and Chigozie, V.U. (2014). Evaluation of the Analgesic, Anti-Inflammatory and Antimicrobial Activities of Leaf Extracts of Breynia nivosa. Chemical Science Review Letter. 3(12): 1126-1134.

Ozer, B.; Tatman-Otkun, M.; Memis, D. and Otkun, M. (2009). Characteristics of Pseudomonas aeruginosa Isolates from Intensive Care Unit. Central European Journal of Medicine. 4(2): 156-163.

Petrini, O. (1991). Fungal endophyte of tree leaves. In Andrews, J.H. and Hirano, S.S. (eds.), Microbial Ecology of Leaves. Springer- Verlag, New York. USA. pp. 179-197.

Phonpapichit, S.; Rungjindamai, N.; Rukachaisirikul, V. and Sakayaroj, J. (2006). Antimicrobial activity in cultures of endophytic fungi isolated from Garcinia species. FEMS Immunology and Medical Microbiology. 48: 367-372.
Tayung, K.; Barik, B.P.; Jha, D.K. and Deka, D.C. (2011). Identification and characterization of antimicrobial metabolite from an endophytic fungus, *Fusarium solani* isolated from bark of *Himalayan yew*. Mycosphere. 2(3): 203-213.

Tende, J.A.; Ezekiel, I.; Dare, S.S.; Okpanachi, A.O.; Kemuma, S.O. and Goji, A.D. (2011). Study of the Effect of Aqueous Extract of Kolanut (*Cola nitida*) on Gastric Acid Secretion and Ulcer in White Wistar Rats. British Journal of Pharmacology and Toxicology. 2(3): 132-134.

Trinagaraju, K.; Prasad, A.V.; Venkateswara, R.P. and Prasad, P.S. (2015). Synthesis, Characterization and Biological activity of 3-Indole Acetic acid. International Journal of Novel Trends in Pharmaceutical Sciences. 5(1): 21-24.

Webber, J. (1981). A natural control of Dutch elm disease. Nature. 292: 449-451.

Yan, X.N.; Sikora, I.R. and Zheng, J.W. (2011). Potential use of cucumber (*Cucumis sativus* L.) endophytic fungi as seed treatment agents against root-knot nematode. Journal of Biomedicine and Biotechnology. 12(3): 219-225.

Zhao, J.; Mou, Y.; Shan, T.; Li, Y.; Zhou, L.; Wang, M. and Wang, J. (2010). Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla* var. yunnanensis. Molecules. 15: 7961-7970.

Zuo, W.J.; Jin, P.F.; Dong, W.H.; Dai, H.F. and Mei, W.L. (2014). Metabolites from the endophytic fungus HP-1 of Chinese eaglewood. Chinese Journal of Natural Medicines. 12(2): 151-153.