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Antioxidant and cytotoxicity evaluation of the Endophytic Fungus, *Fusarium equiseti*, isolated from the leaves of *Ocimum gratissimum*

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Abstract

Antioxidants serve as defense mechanisms which protects against oxidative damage through preventing or retarding oxidation caused by free radicals. Free radicals are highly reactive molecules with an odd number of electrons generated from oxygen capable of damaging various cellular structures. Endophytes have shown capacity to create beneficial biologically active molecules with a wide range of pharmacological properties usually identical to those of the host plant. Their feature has continued to gain fame due to their ability to produce metabolites within the host system without causing harm to the host while sequestering reactive oxygen species within the host systems. Endophytic fungal isolation, fungal fermentation, extraction of secondary metabolites, antioxidant and cytotoxicity studies were carried out using standard laboratory methods. Therefore, this study evaluated the antioxidant and cytotoxicity activity of *fusarium equiseti* previously isolated from *Ocimum gratissimum***.**

The findings revealed that the crude extract of an endophyte associated with *O. gratissimum* called *F. equiseti* produced substances that have strong antioxidant and cytotoxic properties. The antioxidant and cytotoxic potential were due to presence of compounds previously reported for cytotoxic activity, such as Enniatins and Sarasinoside H1 and while the antioxidant activity was due to presence of Orientin a compound known to have shown antioxidant property.

Therefore, this endophyte may serve as a source of new substances with potential for use in pharmaceutical applications for diseases associated with reactive oxygen species.

Keywords: Cytotoxicity; Antioxidants; *Fusarium equiseti; Ocimum gratissimum*; Endophytes

1. Introduction

The world has experienced an increase in the prevalence of chronic diseases, and this has been a major source of concern to the health system. In developing countries, these chronic diseases constitute a high health burden so much so that

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mortality rate from chronic disease exceeds that from communicable disease. (1) Researchers have been working to find out the root causes of these diseases and to proffer solutions. So many factors have been identified as risk factors to the development of chronic diseases including environmental factors such as pollution and behavioral factors such as diet and exercise. (2) These factors can lead to significant increase in oxidative stress levels which in turn leads to the development of chronic diseases (3).

Oxidative stress has been implicated as the major cause of many diseases, including cardiovascular disease, diabetes, cancer, hypertension, neurogenerative diseases, aging, etc. (4) (5). Oxidative stress arises from excess of reactive oxygen and nitrogen species (ROS/RNS) in the body. Once there is an imbalance between the rate of production of ROS/RNS and the rate of counteraction by cellular antioxidants, the excess reactive species begins to interfere negatively with other body functions leading to damages in the body. This has led to an increase in the research and use of medicinal plants that possess antioxidant activity to prevent or treat diseases that are caused by free radicals. Several medicinal plants generate large quantities of antioxidants such as flavonoids and phenolics and the phytoconstituents of these plants are widely documented (6).

An important component of medicinal compounds that is of great interest to researchers is endophytes. Endophyte is defined as an organism colonized in the internal tissue of a plant throughout its life cycle irrespective of whether it was beneficial, detrimental or unbiased to its host (7). Endophytes synthesize a vast array of bioactive secondary metabolites that possess distinctive structures, including alkaloids, flavonoids, phenolic acids, quinones, steroids, terpenoids, and others which have found great application as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants and anticancer agents (8). Endophytes are classified base don he type of microbe and includes fungi, bacteria and actinomyces (9). Recent research according to several reports has shown that Nigerian medicinal plants are undeniably sources of novel endophytic fungus with huge potential to produce novel bioactive lead compounds yet untapped (10–13).

Recent studies isolated and identified an endophytic fungus called *Fusarium equiseti* from the leaf of *Ocimum gratissimum* (14). Numerous compounds of potent therapeutic and pharmacological importance has been isolated from O. gratissimum essential oil of the whole plant including thymol, thymol p-cymeme, γ-terpene,t-sabiene hydrate, β-phellandrene, limonene, eugenol, eugenol spathulenol, geraniol, eugenol γmuurolene, 1,8-cineole, gratissimol, germacrene D and β-caryophyllene and external flavones mainly xantomicrol and cirsimaritin (15–18).

Previous studies by (14) detected and reported compounds with antimicrobial activities which includes: equisetin, epi-equisetin, an episomer of equisetin, Naamine A, Carbonarone A, destruxin B, cytosporin G, and WLIP (-hydroxydecanoyl-Leu1-D GluDe-Thr-Dval-DLeu3 DSer-Leu-Dser-Ile). Also, recently in another research (19) reported numerous bioactive substances including: Enniatin A, Aureonitol, Serasinoside H1, Altenusin, Aplysinamisin, benzylnitril, Ruspolinone, and Orientin. These compounds detected in *fusarium equiseti* has been previously reported in studies for wide range of biological activity, including antiviral, antifungal, hepatoprotective, antibacterial, anticancer, cytotoxic, and antioxidant characteristics (19). It is important to take harness and advantage of the endophytes as the alternative sources of secondary metabolites for the synthesis and development of novels drugs for treatment of various diseases caused by reactive oxygen species.

The scavenging of ROS and the prevention of lipid peroxidation have purportedly been shown by phenol-containing phytochemicals and their derivatives (20). The surge in use of plant derived chemicals as sources of antioxidants to benefit health has sparked a resurgence of interest in its research and use. Therefore, consuming antioxidant plant substances like flavonoids and phenylpropanoids, among others, is regarded to be positively correlated with the prevention of diseases associated with oxidative stress including cancer (21). Therefore, this study evaluated the antioxidant and cytotoxicity activity of *fusarium equiseti* previously isolated from *Ocimum gratissimum*.

2. Material and methods

2.1. Isolation, identification and fermentation of endophytic fungus

The isolation, molecular identification and fermentation of the fungal endophyte, Fusarium equiseti, was previously described (14).

2.2. Vacuum Liquid Chromatography

This was carried out as discussed in our previous report (14). The collected fractions were used for other chromatographic and biological analyses.

2.3. Gel Permeation Chromatography

Fractions obtained from VLC were subjected to gel chromatography on sephadex LH-20 as previously described by (19).

3. Results

3.1. Cytotoxicity Effect of Crude extract *Fusarium equiseti* against ovarian cancer cell line

The result of the cytotoxicity of the crude extract of *Fusarium equiseti* against ovarian cancer cell line is presented in Figure 1. The result showed that the crude extract had a higher cytotoxic effect at a low dose. It showed that at 10 µg/ml absorbance is higher when compared with the negative control that had less than 400 absorbances and this is higher when compared to the negative control while 100 µg/ml had an absorbance of less than 100. There was no significant difference between the positive control and the crude extract at 10 µg/ml and this implied that at 10 µg/ml extracts of *F. equiseti* had the best cytotoxicity effect and therefore should be exploited for future and further studies.

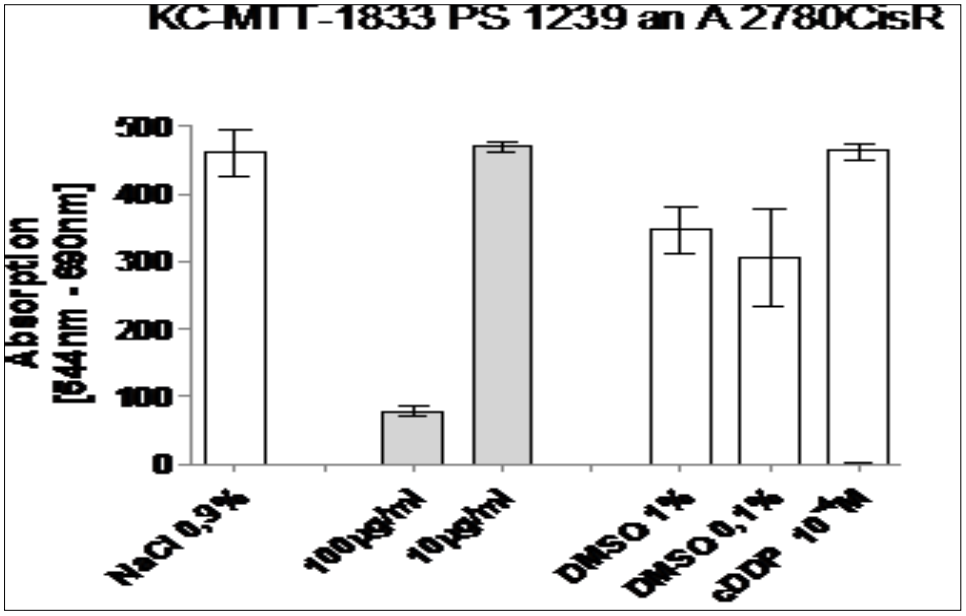
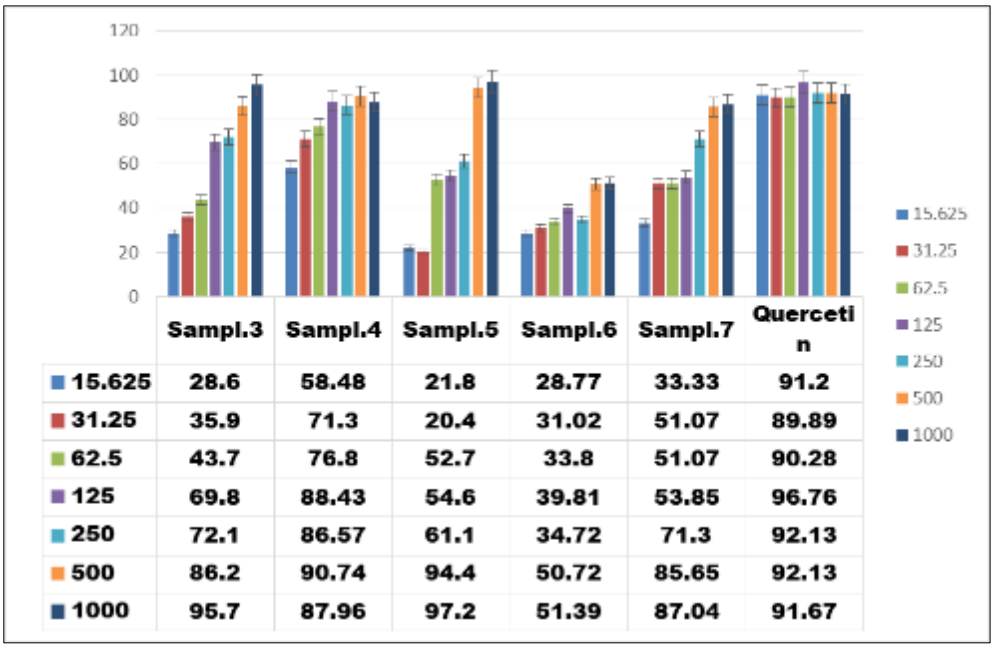


Figure 1 Cytotoxicity activity of crude extract of *Fusarium equiseti* against the cisplatin-resistant ovarian cancer cell line (A2780-CisR)

3.2. Antioxidant evaluation on fractions of *Fusarium equiseti* extract.

The result of the antioxidant activities of the fractions of the crude extract of *O. gratissimum* endophytic fungus *Fusarium equiseti* is presented in figure 2. The result showed a dose-dependent reduction of oxidation of all the fractions of *Fusarium equiseti*. The result showed that sample 3 (n-hexane /ethyl-acetate: 50:50) had over 70 % percent reduction of oxidation at 150, 250, and 500 and had over 90% reduction of oxidation at 1000 µg/ml. The result further showed that sample 4 (DCM/methanol 95:5) had over 80 % reduction of oxidation at 150, 250, 500, and 1000 µg/ml which showed good activity. Also, the result showed that sample 5 (DCM/methanol 80:20) had over 90 % reduction of oxidation at 500 and 1000 µg/ml while at 150 they had less than 70 % reduction in oxidation. Sample 6 (methanol 100%) had the least reduction in oxidation with over 50 % reduction in oxidation at 500 and 1000 µg/ml while at 250 and 150 µg/ml the percentage reduction was less than 50%. Sample 7 (DCM/methanol 5:95) had over 80 % reduction in oxidation at 500 and 1000 µg/ml while at 150 and 250 was less than 75% reduction in oxidation. The result of the positive control showed over 90 % reduction in oxidation at 150, 250, 500, and 1000 µg/ml respectively. This result showed samples 3, 4, 7, and 5 had the best percentage reduction in oxidation and implied that compounds from this sample have great potency as sources of drugs useful for treating oxidative stress-related illness.



	Sampl.3	Sampl.4	Sampl.5	Sampl.6	Sampl.7	Quercetin
15.625	28.6	58.48	21.8	28.77	33.33	91.2
31.25	35.9	71.3	20.4	31.02	51.07	89.89
62.5	43.7	76.8	52.7	33.8	51.07	90.28
125	69.8	88.43	54.6	39.81	53.85	96.76
250	72.1	86.57	61.1	34.72	71.3	92.13
500	86.2	90.74	94.4	50.72	85.65	92.13
1000	95.7	87.96	97.2	51.39	87.04	91.67

Figure 2 The antioxidant assay result of a fraction of the crude extract of *O. gratissimum* endophytic fungus *Fusarium equiseti* (Note sample.3 means n-Hexane /ethyl-acetate fraction 50:50; sample.4 means DCM/ Methanol fraction 95:5; sampl.5 means DCM/ Methanol fraction 80:20; sample.6 means Methanol fraction 100; sample.4 means DCM/ Methanol fraction 5:95)

3.3. Inhibition concentration

The result of the inhibition concentration of fractions of the crude extract of *O. gratissimum* endophytic fungus *Fusarium equiseti* is presented in figure 3. This agrees with the result of the antioxidant activities (Figure 2) which showed that sample 4 had the best inhibition concentration of 20 µg/ml followed by sample 7 (30 µg/ml), sample 5 (60 µg/ml) and sample 3 (80 µg/ml) in that order while sample 6 had inhibition concentration of 500 µg/ml. The antioxidant evaluation identified fractions S7 and S4 as the most promising of all with IC_{50} values of 20 µg/ml and 30 µg/ml and therefore are potential candidates for the identification of the active agents.

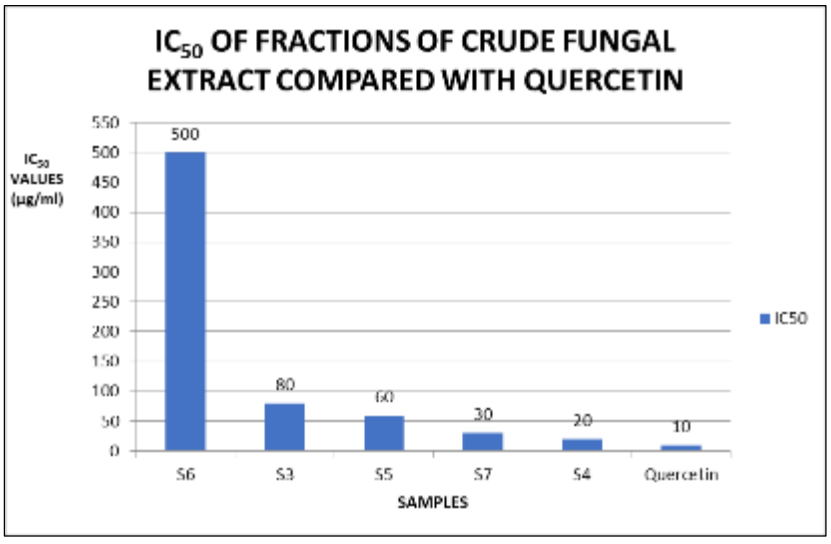


Figure 3 Comparative description of IC_{50} of Quercetin and fractions of the crude extract of *O. gratissimum* endophytic fungus *Fusarium equiseti*. (Note sample.3 means n-Hexane /ethyl-acetate fraction 50:50; sample.4 means DCM/ Methanol fraction 95:5; sampl.5 means DCM/ Methanol fraction 80:20; sample.6 means Methanol fraction 100; sample.4 means DCM/ Methanol fraction 5:95)

4. Discussion

The research revealed that *fusarium equiseti* crude extract has demonstrated cytotoxic action. The presence of several chemicals in the endophyte that have previously been identified to have cytotoxic activity may be the cause of this cytotoxic effect. Despite the fact that compounds isolated from the fungus have been previously reported for a variety of biological activities, the presence of compounds previously reported for cytotoxic activity, such as Enniatins (22–24) and Sarasinoside H1, an amino sugar containing saponins (25), may be the cause of the cytotoxic activity. The results also shown strong in vitro antioxidant activity against free radicals caused by DPPH. The IC_{50} of S4 subfraction was found to be twice that of quercetin and this indicates that the cytotoxicity activity could be due to the anti-inflammatory and antioxidant activity of the compounds previously isolated and detected from the endophytes such as Orientin, a flavone glycoside (26,27) previously shown to have antioxidant activity. Orientin has been previously isolated by [6] from *F. equiseti* and was previously isolated, detected and reported in *Ocimum sanctum* (28–30), *Passiflora* species (31,32), and *Jatropha gossypifolia* (33–36) to have antioxidant activity. Additionally, Orientin's anti-inflammatory capabilities have been mentioned (37,38).

5. Conclusion

According to the study's findings, endophytic fungi associated with *O. gratissimum* called *F. equiseti* produced substances that have strong antioxidant and cytotoxic properties and may serve as a source of new substances with potential for use in pharmaceutical applications for diseases associated with reactive oxygen species.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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