Investigations of Myco-active Metabolites of two Endophytic Mushrooms; (*Daldinia concentrica* and *Irpex lacteus*) and their Mode of Actions Against Phytopathogenic Fungi

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Abstract

Endophytic fungi are refered to fungi that live in plant tissues throughout their entire or partial life cycle, establishing mutually beneficial symbiotic relationship with their host without causing any disease in their host but promoting their growth. Many fungal endophytes produce secondary metabolites which are anti-fungals and strongly inhibit the growth of plant pathogens through enhanced mechanisms of actions. This study aimed at assessing the mycoactive chemicals of two endophytic fungi for their antifungal properties against plant pathogens. Matured fruit bodies of Daldinia concentrica and Irpex lacteus were analyzed for the presence and quantity of alkaloids, tannins, flavonoids, phenols, saponins, terpenoids, oxalate and hydrogen cyanide, using standard methods as described by Obadoni and Ochuko. The results revealed that for D. concentrica, the contents of the mycochemicals were highest in tannins, followed by alkaloids and terpenoids; however, the contents of saponins and flavonoids were at appreciable ranges while oxalate which was not important in the study was the lowest. For I. lacteus, tannic acid and phenol had the highest contents of the mycochemicals. However, flavonoids and terpenoids had the lowest contents, while saponin was at an appreciable range. The findings of the study confirmed that with the presence of the mycoactive-chemicals inherent in the endophytes; they could protect plants against pests and pathogens through enhanced mechanisms as indicated in the study.

Keywords: *Endophytic fungi, Daldinia concentrica, Irpex lacteus, Mycoactive Chemicals, Phyto-pathogens, Mechanisms of actions, Antifungal.*

INTRODUCTION

Endophytic fungi are the group of fungi that live in plant tissues throughout their entire or partial life cycles. Some of them could establish mutually beneficial symbiotic relationship with their host plant without causing any adverse effect or disease on them (Chang, 1999; Sammee *et al.,* 2003). Fungal endophytes can be identified following comparison of their morphological characteristics with published literatures and online resources (Carbungco *et al.,* 2017).

They mostly belong to the Ascomycota and Basidiomycota class and are often found in the intercellular spaces of aerial plant parts; particularly in leaf sheath and barks as well as the root sytem, without inducing any visual symptoms of their presence (Sandro Rhoden *et al.,* 2014).

The endophytes are productive grounds for drugs discovery; hence fungal endophytes are considered as the hidden members of the microbial world that represent an underutilized resource for new therapeutic compounds (Chen *et al.*, 2020).

As fungal endophytes are associated to plants; so they are to mushrooms' growth promoting bacteria or microbes hence they help in mushroom growth.

Many fungal endophytes produce secondary metabolites which are reported to be effective anti-fungal agents and strongly inhibit the growth of phytopathogens. They produce some biocontrol substances reported as sensitive against phytopathogens, some of such compounds include terpenoids, tannins, alkaloids, saponins, aromatic compounds and polypeptides (Silva *et al.*, 2016; Gao *et al.*, 2010).

Hellwig *et al.* (2003) suggested that alkaloids from fungal endophytes could strongly suppress microbial activities; in which he cited an example on a new alkaloid isolated from endophytic *Alternaria* species that showed anti-bacterial activity against several pathogenic gram-positive bacteria. It has also been reported that endophytic fungi have the ability to protect host plant from diseases and limit the damages caused by pathogens. These are mainly reported effective in *in-vitro* controls (Arnold *et al.*, 2003); Gauley *et al.*, 2004; Kumar and Hye, 2004).

Some fungi serve as examples of common representatives of fungal endophytes for secondary metabolites. These include: *Aspergillus, Penicillium, Trichoderma, Fusarium, Phomopsis* species etc., hence recent studies have unraveled plant secondary metabolites' pathway genes in endophytic fungi (Gao *et al.*, 2010).

Additionally, some mushrooms are also considered as endophytes. Mushroom endophytes such as *Trametes versicolor, Daldinia concentrica* and *Irpex lacteus* are also reported to contain some mycoactive metabolites which are predominantly: Alkaloids, Flavonoids, Terpenoids, Polypide and Cytochalasin (Petrini and Muller, 1979).

The bioactive mycochemical compounds in fungal endophytes are reported as important in agricultural productivity; hence they are suggested to possibly serve as alternative and ecofriendly to manage phytopathogens which over the years have been managed by the use of synthetic chemicals. These field inventories may be effective in the control of phytopathogens; but they are not desirable due to their residual effects on crop consumers and the ecosystem, however, certain fungal endophytes have promising and proven anti-phytopathogenic organic

A **B**

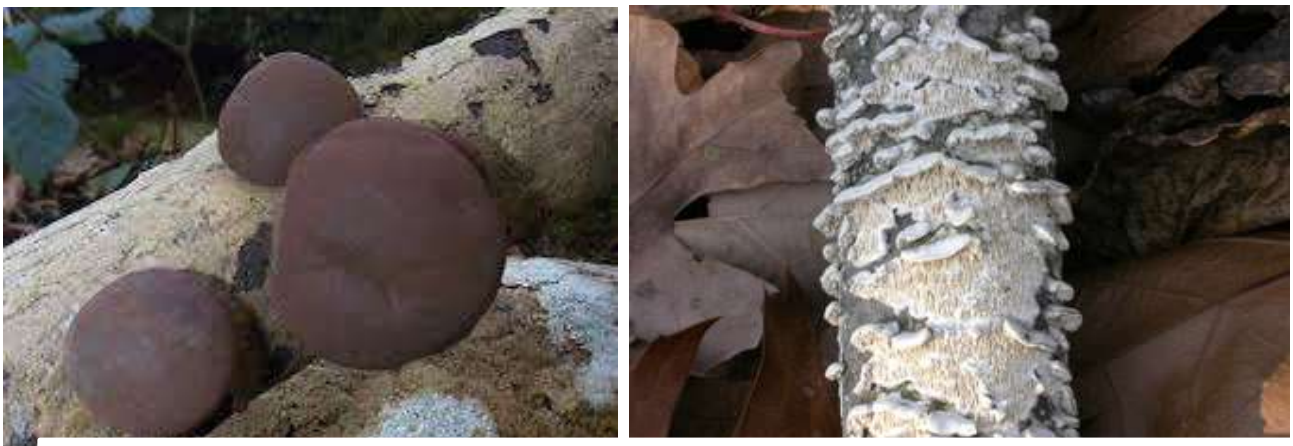

Fig. 1: Pictorial Image of *D. concentrica* (A) and *Irpex lacteus* on the branch of a hardwood (B)

Source: Gary Emberger

Daldinia concentrica, also known as cramp balls, King Alfred's cake and coal fungus is an ascomycete in the family; Xylariaceace. It has large globose stroma (Thangerej *et al.*, 2017). The genus *Daldinia* contains a variety of compounds that contribute to biological activities and has been found to exhibit notable antimicrobial effects against pathogens (Jonathan, 2002). Larzi *et al.* (2016), reported the impact of the mycochemical and volatile compounds of *D. concentrica* against post harvest crop infections; in which molds development were prevented on organic dried fruits and elimination of incidence of *Aspergillus niger* infection on peanuts.

Based on several reports of the impacts of the mycochemicals in the fungus which have been found effective to sustain crops at post harvest stage; it was suggested that the use of *D. concentrica* is potent as organic alternative approach to controlling phytopathogenic fungi in food industry and agriculture (Strobel and Daisy, 2003). The fungus is also reported to possess some mycochemical bioactive compounds as phenols, tannins, alkaloids, flavonoids, glycosides etc. for anti-pathogenetic fungi (Suwannarach *et al*, 2013).
Irpex lacteus is a white-rot fungus that majorly inhabits dead branches of angiosperm. It is a common crust and resupinate endophytic fungal polypore (Isaka *et al.*, 2016). The fungus which is versatile in lignin degradation is also implicated in biological activities such as anti-tumor, anti-bacterial, anti-inflammatory and anti-viral in humans. Additionally, the fungus causes soft rot and severe wounds on hard woods (Sanjay Parilhar *et al.*, 2015; Robert and Jeffery, 2015). It is possible that they possess these features due to the presence of some secondary metabolites in the endophyte. These secondary metabolites in the fungus includes alkaloids, glycosides, flavonoids, phytosterols, triterpenoids, saponins, tannins and phenolic compounds etc. (Sanjay Parilhar *et al.*, 2015).
It is severally reported that many mushrooms have mycotic compounds as secondary metabolites (as mentioned above), that exert effects on phytopathogenic fungi.

These compounds are reportedly noted for specific mechanisms to effect their efficacy in crop protection (Busby *et al.*, 2016).
Bostock *et al.* (1999) reported that the toxicity action pathway of tannins, attack phytopathogens directly on their cell membrane by metal depletion of the pathogen. Keukens *et al.* (1992) also suggested that the chemical efficacy of bioactive compounds contained in fungal endophytic material extract inhibit phytopathogens, from synthesizing laccases and cutinases with which they use to breakdown plant cutins. They further suggested that the steroidal glycol alkaloids in such extract, act against phytopathogens by interfering with the pathogen's membrane integrity. Additionally, they reported that saponin steroids complex interacts with sulfur residues for greater efficiency, because the sugar carbon attached to C_3 is often critical for both membranes.
Bar-Num and Meyer (1999) reported that the secondary metabolites of fungal endophytes are of immense importance; hence the cyclic triterpenoids (Saponins), steroidal alkaloids glycosalate, cucurbitans I and D in them efficiently hinder the induction of extracellular laccase formation of the pathogens.
Engelmeier and Hadacek (2006), suggested that the mycochemicals of the fungal endophytes interfer with the molecular targets of pathogens tissues and cells which major targets are the fungal biomembrane, protein and nucleic acid.
Nmom and Ajuru (2019), reported that saponins, tannins, resins, glycosides, flavonoids and anthracquinones contained in the extract of leaves of *Ficus exasperata* on *Vernonia amydalina*

were effective against powdery mildew on the plant by inhibiting the cutinases and laccasse production by fungal pathogen.
In the same vein, Luo *et al.* (2002) and Ito *et al.* (2005), reported that saponins as tomatin in fungal endophytes extracts, complexed with fungal membrane sterol with free hydroxyl group resulting in pore formation and subsequent loss of fungal membrane integrity in the complex. Dennis (1976 and 1977; Kindl, 2000) reported that terpenoid causes cytoplasmic granulate; disruption of plasma membrane and leakage of cellular contents. The summary of these mycochemicals and their mode of actions are summarized in Table 1.

There are various reports on the use of aromatic plants' leaf extracts and a few reports on the use of the extracts of endophytes for plant protection from phytopathogenic fungi; but there are dearths of information on such reports on their modes of actions. It is therefore on this premise that this study was intended to investigate the mycochemicals in the form of secondary metabolites of the two endophytic fungi and their possible modes of actions against phytopathogens for sustainable plant protection from diseases.

Table 1: Names of the Mycoactive Compounds and their Mode of Actions

S/N	Names of the Mycoactive Compounds	The Mode of Actions of Mycoactive Compounds
1.	Alkaloids	Interact into cell walls for disruptions and leakage of cellular contents.
2.	Tannins	Bind to protein enzymes of the pathogen, to inhibit the enzymes and cause substrate deprivation.
3.	Terpenoids	Disrupt the fungal pathogen membranes.
4.	Flavonoids	Bind to adhesions, complex with cell wall and inactivate fungal enzymes.
5.	Polypeptides	Also form disulphide bridges.
6.	Phenols	Disruption of membrane and deprive the pathogen of substrates.
7.	Phenolic acid	Bind to adhesion, complex with cell wall and inactivate fungal enzymes.

Source: Adapted from Cowan (1999)

Materials and Methods

Source of Sample:

The sampling area for this study was Ikwerre Local Government Area (Kelga) of Rivers State, in the Niger Delta Sedimentary Basin in Nigeria, covered on the surface by Basin Formation. *Daldinia concentrica* and *Irpex lacteus* were obtained from the wild, in a home garden; precisely at Omuanwa in Kelga.

Sample Collection

The samples, *Daldinia concentrica* and *Irpex lacteus* were obtained as fairly dried from the wild and identified by a plant mycologist, Dr. F. W. Nmom. The samples were further sun-dried at 36^0C to suit the nature of the study.

Sample Description

D. concentrica was identified based on its hard rounded dark-brown balls which the older ones were black as charcoal, being known as cramp ball or king Alfred's cake. *Irpex lacteus* was also identified based on its crust nature on wood, whitish in colour and fibrous in texture.

Sample Preparation

The samples were sun-dried at 36^0C, 5 hours daily for three days and later grounded to powder form for mycochemical analysis study.

Mycochemical Determination

Mature fruit bodies of the fungi, *D. concentrica* and *Irpex lacteus* were analyzed for determination of the presence and quantity of the contents of Alkaloids, tannins, flavonoids, phenols, saponins, terpenoids, oxalate and hydrogen cyanide in the samples using standard method as described by Obadoni and Ochuko (2001).

Sample Preparation and Mycochemical Analysis

The mature fruit bodies of the samples were obtained from the wild, sun-dried at 36^{0C} for five hours daily for three days; after which were grounded to powder form. The powder form of the samples was further sun-dried, for five hours daily at 36^0C for another three days to avoid clumping of the sample; after which, they were mycochemically analyzed.

Test for Alkaloids

The method described by Harbourne (1983) was used to determine the presence and content levels of alkaloids in the study samples.

Procedure for Analysis

One gram (1g) of each sample (Wo) was weighed into a volumetric flask and 40ml of 10% acetic acid in ethanol was added and shaken. This was allowed to stand for 4 hours and then filtered. The filtrate was evaporated to a quarter of its original volume. To it was added 3 drops of concentrated NH_4OH solution so as to precipitate the alkaloids. The formed precipitate was filtered through a weighed filter paper, labelled as (W_1).

The filter paper was placed in the oven and allowed to dry at 60^0C for 30-60minutes until it was constant. The filter paper was weighed and recorded as (W_2). Percentage alkaloids was determined using the formula:

% alkaloids = $W_2 – W_1/W$

Where

W_2 = Weight of filter paper + residue

W_1 = Weight of empty filter paper

W_0 = Sample weight

Test for Tannins

This was determined using the method of Jaff (2013).

Procedure

One gram (1g) each of the ground sample was weighed into a flask and 10ml distilled water added and agitated. This was left for 30 minutes at room temperature; after which the stand was centrifugated at 2500rpm for 15 minutes. 1ml of the supernatant was measured into a 10ml volumetric flask and 1ml folin – ceocalteu reagent was added, followed by 1ml saturated Na_2CO_3 solution and diluted to 10ml with distilled water. The experiment was incubated for 30 mins at room temperature.

To obtain standard tannic acid, the above procedure is repeated for tannic acid standards 20, 40, 60, 80, 100 and 120mg/l from a stock of 500ppm (i.e. 50mg) of tannic acid standard dissolved in 100ml of distilled water); but centrifugation is excluded in this procedure.

The absorbance of the above tannic acid concentration was read to a wavelength of 725nm and calibration curve for the tannic acid standards was drawn which standards as absorbance against concentration. To obtain the tannin acid concentration of the sample; the absorbance of the sample down the concentration axis was traced and extrapolated. To calculate the tannic acid content;

(mg/kg) = Conc obtained in mg/1x vol. of sample x DF x 100/1/sample weight (100g).

Where

DF = Dilution factor, but if not diluted

DF = 1

Test for Flavonoids

This test adopted the methods reported by Sofowora (1993) and Harbourne (1973).

Procedure

0.30g of the samples each was weighed into a beaker and extracted with 30cm^3 of distilled water for 2 hours and filtered, thereafter with filter paper number 42 (i.e. 125mm).

To 10cm^3 of the aqueous filtrate of the sample was added 5cm^3 of 1.0m of diluted ammonia solution, followed by the addition of 5cm^3 of conc. H_2SO_4. Appearance of yellow colouration which disappeared on standing, confirmed the presence of flavonoids in the samples. To calculate the contents of flavonoid in the samples, the fomular below was used.

(% w/w = RE x V x D x 10^{-6} x 100/weight of sample

Where

RE = Rutin equivalent (ug/ml)

V = Total voume of sample

D = Dilution factor

W = Sample weight

(Ezeonu, 2016)

Test for Total Phenol with Tannic-acid Standard

The method of Furkmen *et al.* (2016) was used to determine total phenol from the sample.

Procedure

1.00g of the ground samples, each, was weighed into a conical flask and 10ml ethanol was added and plugged with aluminium foil. This was vigourously shaken and left to stand for 30 minutes for proper extraction after which, the set up was filtered to obtain clear supernatant which was used for total phenolics; then 1ml solution of the supernatant was pipetted into a test tube and 0.5ml 2u folin-ceocalten reagent and 1.5ml of 7% $NaCO_3$ solution were added. This was made up to 10ml with distilled water and vigorously shaken and allowed to stand for 90 minutes. The absorbance was read at 765nm.

The following concentrations of tannic acid standard 20, 40, 60, 80, 100 and 120mg/l was prepared. The absorbance of the above tannic acid concentrations was read off and calibrated curve for the tannic acid standard was drawn for absorbance against concentration. And to obtain the concentrations of the samples, the absorbance of the samples down the concentration axis were traced and extrapolated and calculated as:
Phenol content mg/kg (TAE) = obtained in mg/l x vol. of sample x DF/sample weight.
Where:
DF = Dilution factor and if not
DF = 1

Test for Saponins
The method of Obadoni and Ochuko (2001) was used to determine the presence and quantity of saponins in the samples each.

Precdure
20g (W_O) of the ground samples each was weighed into a conical flask and 2.5ml of 20% aqueous ethanol was added. The content was heated in a hot water both for 4 hours stirring at 50^0C and filtered. The set up was re-extracted using 40ml of 20% ethanol to combine the extract.
The volume of the extract was reduced to 20ml by evaporating in a water bath at 90^{0C}. The concentrates were transferred into a 250ml separating funnel. The combined butanol layer was washed twice with 10.25ml of the 5% aqueous Nacl. The remaining solution was collected and weighed into a Petri dish and recorded as (W1). The Petri dish was dried in an oven at 90^0C and reweighed and recorded as (W2). Total saponin was calculated as:
% saponin content = W2 – W1/W0 x $^{100}/_1$
Where:
W_2 = Reweighed Wt of Petri dish
W_1 = Initial weight (wt) of petridish
W_0 = Sample weight

Test for Terpenoids: The methodology used for this test was that of Ejikeme *et al.* (2014).
Procedure: 0.03g of ground samples each was weighed into a conical flask and extracted with $30cm^3$ and component extracted for 2 hours. A mixture of $2cm^3$ chloroform and $3cm^3$ H_2SO_4 concentration were carefully added to $5cm^3$ of the extract to form a layer. The presence of reddish brown colouration at the interphase was an indication and posiive result for the presence of terpenoids. Percentrage terpenoid was calculated as:

% terpenoids = weight of terpenoids/weight of sample x $^{100}/_1$

Test for Oxalate: The methods of Munro (2000) was adopted for this test.
Procedure: 1g of the ground samples each was weighed ino a conical flask and 75ml of $3NH_2SO_4$ was added. This was allowed to stand for 30 minutes, after which, it was filtered, using whatman no 1 filter paper. 25ml of the filtrate was pipetted into a beaker and 2 drops of methyl red indicator was added. The set up was allowed to boil. A hot titration against 0.05m of $KMNO_4$ was made until a faint pink colour persisted for 30 seconds.
The concentration of oxalte in the sample was made by taking 1ml of 0.5ml $KMNO_4$ as equivalent to 2.2mg oxalate represented as follows:

Oxalate content (µg/100g) = Titre value x 2.2XDE/u x $^{100}/_{1}$
Where
2.2µg = Mass equivalent oxalate value of 1ml of 0.5ml of $KMNO_4$ solution
DF = Dilution factor
W = Sample weight in gramme

Test for Hyrogen Cyanide: The method of Railes (1992) was used.
Procedure: 5g of the ground samples each, was weighed into a conical flask and 50ml distilled water was added. This was allowed to stand over night and filtered. 2ml of the filtrate was pipetted into a test tube and 4ml of alkaline picrate was added and the tube corked. It was incubated in a water bath for 5 minutes at 80^0C. A change in colour from yellow to reddish brown after incubation indicated the presence of hydrogen cyanide.

After detection was made, the absorbance was read at 510nm and a blank containing 2ml distilled water which contained alkaline picrate solution was made; then the cyanide standard curve with 1, 2, 4, 6, 8, and 10mg/L cyanide standard as prepared. The set up was extrapolated and the graph was traced down the absorbance of the sample and concentration axisting graph was traced down the absorbance of the sample and concentration axis, so as to obtain concentration of the sample in mg/L. Calculation was made as follows:
Mg/kgHCN = Conc. Obtained in mg/l x vol of sample x DF, if present/sample wt x 1000.

Test for Phytate: This was determined using the methods of RUSS (1980).
Procedures: 2g ground samples each was weighed into a 250ml conical flask and 100ml of 20% conc. HCl was added and allowed to stand for 3 hours and filtered. 50ml of the filtrate was pipetted into a 250ml beaker. A 107ml of distilled water was added to improve acidity; while 10ml of 0.3% ammonium thiocyanate (NH_4SCN) was added as indicator. Standard iron III chloride ($FEcl_3$) solution which contained 0.00195fe/ml was titrated until brownish yellow colour appeared and persisited for 5 minutes to indicate the presence of phylate in the samples. Calculation was made as:
Phytic acid g/kg = 0.00195 x volume of $Fecl_3$ consumed x DF/sample weight x $^{100}/_{1}$

Results and Discussion

Table 1: percentage Concentration of Mycochemicals in *Daldinia concentrica*

Samples	**Mycochemicals (%)**							
	Oxalate mg/100g	**Tannins mg/100g**	**Flavonoids mg/100g**	**Alkaloids mg/100g**	**Saponnin mg/100g**	**Terpenoids mg/100g**	**Phylate mg/100g**	**Hydrogen Cyanide**
D. concentrica 1	2.69	94.73	8.91	35.0	6.54	14.85	10.338	12.87
D. concentrica 2	2.66	95.16	9.80	33.33	6.31	14.00	10.55	11.19

Legend: *D. concentrica* 1 and 2 indicate that the sample was assessed in Duplicate analysis.

Table 2: Mean Percentage Mycochemical Contents in *D. concentrica*

Samples	Mean Percentage Contents							
	Tannins	Alkaloids	Terpenoids	Flavonoids	Phytate	Hydrogen Cyanide	Saponnin	Oxalate
D. concentrica	94.945	34.165	14.425	9.355	10.444	12.03	6.425	2.675

Legend: DC = *Daldinia concentrica*

Table 3: Percentage Concentrations of Mycochemicals in *Irpex lacteus*

Sample	Mean Percentage Concentration					
	Flavonoids (%)	Alkaloids (%)	Tannin acid (%)	Terpenoids (%)	Total Phenols (%)	Saponins (%)
Irpex lacteus 1	4.43	9.90	68.54	5.88	66.63	14.77
Irpex lacteus 2	4.93	9.85	68.96	5.47	66.39	14.83

Legend: *Irpex lacteus* (1) and (2) showed that result was in duplicate of the sample

Table 4: Mean Percentage Mycochemical Contents of *I. lacteus*

Sample	Mean Percentage Mycochemicals					
	Flavonoids (%)	Alkaloids (%)	Tannin acid (%)	Terpenoids (%)	Total Phenols (%)	Saponins (%)
Irpex lacteus	4.68	9.875	68.75	5.675	66.51	14.8

Table 5: Comparison of level of Mychochemical Contents in *D. concentrica* and *Irpex lacteus* for antifungal potentials.

Samples	Oxalate mg/100g	Tannins mg/100g	Flavonoids (%)	Alkaloids (%)	Phytate g/kg	Hydrogen Cyanide mg/100g	Saponins (%)	Terpenoids	Tannic acid	Total Phenol mg/100g
D. C	2.675	94.945	9.355	34.165	10.444	12.03	64.25	14.425	-	-
I. lacteus	-	-	4.68	9.875	-	-	14.8	5.675	68.75	66.39

Legend: ***D.C = Daldinia concentrica***
I. lacteus = Irpex lacteus

The results of this study are presented on Tables 1 – 5. The results on Table 1 revealed that the contents of the mycochemicals (%) are highest in tannins, followed by alkaloids and terpenoids; however, the contents of oxalate were the lowest which is a bioactive substrate compound. Saponins and flavonoids contents are at appreciable ranges.

The results presented on Table 2 revealed the mean percentage of mycochemical concentrations. It showed that tannins had the highest values, this was followed by alkaloids and terpenoids. Oxalate showed the lowest value, though it is of less importance to the essence of this study which bothers on antifungal properties.

The resuls in Tables 3 and 4 indicated the percentage concentration and mean percentage of same respectively of mycochemical contents in *Irpex lacteus.* The results showed that tannic acid and total phenol had the highest contents of the mycochemicals in the sample. However, the presence of flavonoids and terpenoids was indicated, but had the lowest concentrations. Saponnins was at appreciable range.

While investigating the mycochemicals in *D. concentrica* and *I. lacteus*, the study was narrowed down to compare the presence and concentrations of the mycochemicals in the samples. The results presented on Table 5, indicated that oxalate, tannins, phytate and hydrogen cyanide were absent in *Irpex lacteus;* whereas tannic acid and total phenols were absent in *D. concentrica.* The results also indicated that in *D. concentrica* tannins had the highest concentration, followed by alkaloids, but tannin was absent in *I. lacteus.* Tannic acid and total phenols occurred highest in *Irpex lacteus* but absent in *D. concentrica.*

The results obtained from this study indicated the presence of tannins, alkaloids, terpenoids, saponins and flavonoids in the two endophytic fungi (*D. concentrica* and *Irpex lacteus*) studied. The results therefore obviously agreed with the submission of Strobel and Daisy (2003) who suggested the use of *D. concentrica* as a potent organic alternative approach to controlling phytopathogenic fungi in food industry and agriculture. They also confirmed the presence of the mycochemical compounds which occur as secondary metabolites, confirming those compounds as mentioned above. The results of the secondary metabolites in the fungi studied also agree with the report of Jonathan (2002) that the genus *Daldinia* contains a variety of mycochemical compounds such as mentioned above that contribute to biological activities and have been found to exhibit notable antimicrobial effects against pathogens. The result is also in line with the suggestions of Larzi *et al.* (2016), who also reported the impact of mycochemicals and volatile compounds in *D. concentrica* against post harvest crop infections in which molds development were prevented on organic dried fruits and the elimination of incidence of *Aspergillus niger* infection on peanuts.
The results also showed that mushroom components such as *Daldinia concentrica* and *Irpex lacteus* are endophytic fungi with active secondary metabolites such as mentioned above; in line with the report of Hardoim *et al.* (2015) who suggested that endophytic fungi form unique symbiotic relationships with their host plant where their mycelia grow in between cell walls and help them with nutrient absorption, fight pathogen infections in plants and predatory fauna. It is also in line with the report of Brossi and Arnold (2012) who reported that endopytic fungi promote plant growth, providing protection against pest and pathogens.

It is possible that the impact of these fungal endophytes is through the secretion of secondary mycochemicals mentioned above which may exert their effect using several mechanisms. Infact, this report aligns with the submission of Bushy *et al.* (2016) who reported that mushrooms have some active secondary mycochemicals which are noted for specific mechanism to effect their efficacy in crop protection.

It is also likely that these secondary metabolites act directly on the pathogens cells to effect their efficacy. This report relates to the reports of Bostock *et al.* (1999) who suggested that the toxicity action of tannins, attack phytopathogens directly on their cell membrane by metal depletion of the pathogen. Also in line with the submissions of Keukens *et al*. (1992 who reported that the chemical efficacy of the secondary metabolic compounds contained in the fungal endophytic material extracts inhibit phytopathogens from synthesizing laccasses and cutinases with which they use to breakdown plant cutins. They further suggested thtat the steroidal glycol alkaloids in such extracts act against the pathogens by interfering with their membrane integrity. Additionally, they reported that saponin-steroids complex interacts with sugar residues for greater efficiency because the sugar chain attached to C_3 is often critical for both the host and the pathogen's permeability and to the antifungal organic chemicals in the

sample which must be extracted with carefulness so that the sugar residues remain retained to avoid biological inactivity of the orgnic chemical if the sugar residues get lost according to their report.

The secondary metabolites of the endophytic fungi such as considered in this study are of immense agricultural importance in line with the report of Bar-Num and Meyer (1999) who suggested that cyclic triterpenoids, also known as saponins in steroidal form of alkaloids glucosalate and cucurbitacins I and D are effective to hinder the extracellular laccase formation of some fungi such as *Botrytis cinerea.*

The findings of the study also agree with the suggestions of Engelmeier and Hadacek (2006) who reported that organic chemicals such as extracted from the samples of this study as secondary metabolites act on phytopathogens by interfering with the molecular targets in the pathogens' tissues and cells. Additionally, they added that the major targets of the organic chemicals are the fungal pathogen's biomembrane, protein and nucleic acids.

Findings of the study as touching the secondary metabolites align with the reports of several authors of plant protection such as the reports of Nmom and Ajuru (2019), who submitted that saponins, tannins, resins, glycosides, flavonoids and anthracquinones contained in the leaf extract of *Ficus exasperata on Vernonia amydalina* with powdery mildew disease was effective in inhibiting cutinases and laccases production by the phytopathogenic fungi. Also in agreement with these study findings are the report of Luo *et al.* (2002) and Ito *et al.* (2015) who proposed that mushroom material which contains saponins in the form of tomatin formed complex with fungal membrane sterol with free hydroxyl group and the complex resulted in pore-formation and subsequent loss of fungal membrane integrity.

Conclusion

The findings of this study have shown that fungal endophytes are indeed the hidden members of the microbial world that represent an underutilized resource for plant protection from several phytopathogens hence, they are laden with active secondary metabolites with potentials against plant pathogens. The mycochemical compounds in the endophytes are very important in agricultural productivity as they may serve as alternative and ecofriendly means to check or manage plant diseases which over time have been managed by the use of synthetic chemicals which though may be effective; but undesirable due to their residual effects on consumers and the ecosystem.

Another interesting finding of this study is that it is a pointer to the pathologist and farmers the mechanism in which the organic/mycochemical or secondary metabolites tackle phytopathogens for sustainable agricultural productivity devoid of synthetic chemicals or field inventory in disease management.

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