



## Cytotoxicity and antimicrobial activity of alkaloids extracted from *Catharanthus roseus* associated endophytic fungi

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### ABSTRACT

**Aims:** The aim of this study was to isolate the endophytic fungi from *Catharanthus roseus* and screen the cytotoxicity and antimicrobial activity of alkaloids extracted from these fungi.

**Methodology and results:** A total of 56 endophytic fungal isolates were screened from *C. roseus* plant parts. *Aspergillus fumigatus* and *Fusarium oxysporum* were the most frequent species. Determinations of alkaloids extracted from the most dominant endophytic fungal species were done. The highest significant total alkaloids productions were by *A. fumigatus* and *Aspergillus niger*, while the least significant one was by *Botrytis cinera*. Antimicrobial assay of endophytic fungal extracts indicated that both *A. niger* and *F. oxysporum* exhibited significant antimicrobial activities, while *A. fumigatus* exerted the least activity. *In vitro* cytotoxicity assay of the endophytic fungal extracts was done against human breast cancer (MCF-7) and liver cancer (HEPG-2) cell lines using SRB assay method. *A. niger* extract showed potential cytotoxic effect on MCF-7 cell line with IC50 value of 42.1 µg/mL, while the least cytotoxic effect was exhibited by *F. oxysporum* on MCF-7 cell line with IC50 value of 66.9 µg/mL. Gas Chromatography Mass Spectroscopy (GC-MS) was used for analysis of alkaloids in mycelial and filtrate extracts of *A. niger*, where eleven compounds were detected.

**Conclusion, significance and impact of study:** Alkaloids extracted from *Catharanthus roseus* associated endophytic fungi had cytotoxic and antimicrobial activities.

**Keywords:** Endophytic fungi, *Catharanthus roseus*, alkaloids, antimicrobial activity, cytotoxicity

### INTRODUCTION

The original source for both ancient and modern medicine is mostly the medicinal plant (Karimi and Jaafar, 2011). More than 70% of the antimicrobial and 60% of the anticancer drugs in medicinal uses are natural products or their derivatives (McAlpine *et al.*, 2005).

Alkaloids are heterogeneous group of 2500 basic nitrogen containing compounds, found in about 15% of all vascular land plants constituting more than 150 plant families, widely distributed in higher plants particularly the dicotyledons (Mohan *et al.*, 2012).

*Catharanthus roseus* (Madagascar Periwinkle) is an important medicinal plant belong to family Apocyanaceae. It is a main source for alkaloids which have anticancer, antimicrobial and antidiabetic activities (Jaleel *et al.*, 2006; Gajalakshmi *et al.*, 2013). Vincristine and vinblastine are the most common terpenoid indole alkaloids produced by *C. roseus* and are used as anticancer (Balaabirami and Patharajan, 2012; Kumar *et al.*, 2015; Yamamoto *et al.*, 2016) and anti-microbial agents (Grellier *et al.*, 1999). Vincamine and vinpocetine are used for treatment of

cerebrovascular and cognitive disorders, and to reduce the effects of stroke types (Chen *et al.*, 1995; Chevallier, 1996). Ajmalicine and serpentine are monoterpene indole alkaloids which are used as anti-hypertensive and anti-neuro-inflammatory agents. However, yohimbine is mainly used in treatment for erectile dysfunction. Vindoline used for the development of antidiabetic therapeutics (Almagro *et al.*, 2015). Extracts of different parts of *C. roseus* (leaves, stem, root and flower) having potential against bacterial strains (Muhammad *et al.*, 2009).

Cropping of these plants is taking long time and high cost to extract alkaloids and their metabolites. The extraction of chemical plant products from intact plants has also several inherent problems, including seasonal variations, pests, diseases and inconsistent product quality and yield. Therefore, if an alternative method to plant extraction can be found for producing the active constituents, the need for this slowly growing plant would be reduced, and the pharmaceutical demand could be better satisfied. So many studies and researches were

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done to discover the other route for production of these compounds (Kargi and Potts, 1991; Yin and Sun, 2011; Ayob and Simarani, 2016).

The products from microbial source are frequently easier and inexpensive (Yin and Sun, 2011). Microorganisms are important sources of natural products with activity in drug discovery, agricultural applications and industrial uses (Demain, 1999; Keller *et al.*, 2005; Strobel, 2006; Porras-Alfaro and Bayman, 2011). Forty percent of new chemical entities (NCEs) collected from 1981 to 2005 have been produced from microorganisms (Khosla, 1997; Clardy and Walsh, 2004; Sieber and Marahiel, 2005).

Endophytes are microorganisms that live inside various parts of plant tissues without causing harm or disease to the hosts (Petrini *et al.*, 1992; Azevedo and Araujo, 2007). These species diversity actually remains unexplored (Srinivasan and Muthumary, 2009). Endophytic microorganisms can be divided into two groups; group that do not develop external structures outside the host, while the other group develops external structures as the nodules of nitrogen fixing bacteria and mycorrhizal fungi (Azevedo and Araujo, 2007).

Host plant relationship with endophytes depend on that host plant supply the endophytes with nutrient and habitation for their survival. While endophytes produce number of bioactive compounds for helping the host plants to resist biotic and abiotic external stresses and useful in the host growth (Firáková *et al.*, 2007; Rodriguez *et al.*, 2009).

Terpenoids, alkaloids, phenyl propanoids, aliphatic compounds, poly ketides and peptides were produced by endophytes that have been reported to have antimicrobial activities (Mousa and Raizada, 2013; Pinheiro *et al.*, 2013). Endophytic *Fusarium* species were reported to produce a variety of bioactive secondary metabolites such as fusarubin, javanicin, and marticin which have antimicrobial, insecticidal, phytotoxic, and cytotoxic properties (Kornsakulkarn *et al.*, 2011).

The endophytic *Xylaria* species produce several types of secondary metabolites such as sordaricin, antiplasmodial (+)-phomalactone which act as antifungals and (+)-mycoepoxydiene, and deacetylmicoepoxydiene which are cytotoxic. Endophytic fungi screened from *Garcinia hombroniana* are a rich source of new and bioactive secondary metabolites with different structures (Rukachaisirikul *et al.*, 2013).

Endophytic fungi are able to produce active constituent similar to which produced by their host plants (Gunatilaka, 2006; Zhou *et al.*, 2009). Stierle *et al.* (1993) found that taxol and taxane bioactive substance produced by endophytic fungi isolated from *Taxomyces andreanae* is the same as that produced by host plant. Similarly, vinblastine which is a common alkaloid produced by *C. roseus* was detected by using HPLC in the endophytic extract of *Fusarium* sp. isolated from *C. roseus* (Handa, 2015). An endophytic fungus isolated from *Gentiana macrophylla* produces the same bioactive compounds (gentiopicrin or other secoiridoids) so the host plant could be protected from harvest as it is a rare plant (Strobel and Daisy, 2003).

The ability of fungi to grow and produce their metabolites maximally from 2 to 4 weeks let them more economic than their host plants. *C. roseus* associated endophytic fungi could be a promising source of new compounds of industrial and pharmaceutical importance (Akpotu *et al.*, 2017). Thus, this study was carried out to survey the endophytic fungal species isolated from *C.*

*roseus* and screen the alkaloids producing species which have antimicrobial and antitumor activities.

## MATERIALS AND METHODS

### Plant collection

Fresh plant parts (stem, root and leaves) of *C. roseus* L. (G) Don. (Apocynaceae) were collected from the Garden in Cairo University, Egypt.

### Isolation of endophytic fungi

The plant parts were separated, washed thoroughly with tap water, surface sterilized with 70% ethanol for 1 min, 4% sodium hypochlorite for 3 min and again with 70% ethanol for 1 min, then rinsed twice with sterile distilled water. Samples were dried with sterile filter paper and cut into small pieces with sterile scalpel (Nath and Joshi, 2015). These pieces were then placed on Martin's and Sabouraud's media containing chloramphenicol to suppress bacterial growth. Plates were then incubated at 25-27 °C until the outgrowths of endophytic fungi from the explants were observed. The outgrowths were subcultured to produce pure culture on Czapek-dox's plates. All isolates were maintained in Czapek-dox's slants and keep at 4 °C.

### Identification of endophytic fungi

The endophytic fungal isolates were identified morphologically and microscopically according to Moubasher (1993).

### Alkaloids extraction

Endophytic fungi were inoculated into 250 mL Erlenmeyer flasks containing 100 mL Czapek-Dox's broth media and incubated at 25 °C for 10, 15 and 21 days. Both extracellular and intracellular alkaloids were extracted according to Yin and Chen (2008) with a minor modification. Extracellular alkaloids were extracted with absolute ethanol in water bath at 60 °C for 30 min, then concentrated to about 5-10 mL. The grinded dried mycelia were extracted with ethanol containing 3% HCl at 95 °C for 1.5 h. Filtrations were then done and the filtrates were concentrated to about 5-10 mL. Extracellular and intracellular alkaloids were extracted twice with ethyl acetate after alkalization with NH<sub>4</sub>OH to pH 8. Ethyl acetate layers were collected, concentrated and evaporated to dryness. Residues were stored for subsequent analysis.

### Qualitative examination of alkaloids

Dragendorff's and Mayer's reagents are used to detect both extracellular and intracellular alkaloids production by changing in the color of the reagents. An orange precipitate with Dragendorff's reagent and a white precipitate with Mayer's reagent indicated alkaloids-producing strains (Chen, 2004; Wu, 2004).

### Quantitative analysis of alkaloids

Extracellular and intracellular alkaloids concentrations were determined by absorbance measurement at 280 nm (Kargi and Potts, 1991).

## Antimicrobial activity

Antimicrobial activities of the endophytic fungal alkaloid extracts were tested against two Gram-positive bacterial species (*Staphylococcus aureus* and *Bacillus subtilis*), one Gram-negative bacterial species (*Escherichia coli*) and one fungal (yeast) species (*Candida albicans*). Nutrient agar (NA) media was used for test of bacteria, while Sabouraud Dextrose agar media (SDA) was used for fungi. The test organisms were inoculated by streaking over the surface of the sterilized media. The assay was performed using Kirby-Bauer disc diffusion method (NCCLS, 2002, 2003) at fungal extract concentration 5 mg/disc, using DMSO as a solvent. A control test for the solvent was also performed. The petri dishes were incubated at 37 °C for 48 h for bacterial species and at 25 °C for candida for 72 h. Sensitivity was then determined by measuring the mean diameter of the inhibition zones in mm.

## In vitro cytotoxicity assay

Analysis was done at National Cancer Institute. *In vitro* cytotoxicity of endophytic fungal extracts isolated from *C. roseus* was performed by using sulforhodamine B (SRB) assay against human breast cancer (MCF-7) and liver cancer (HEPG-2) cell lines using different concentrations of fungal extracts (0, 12.5, 50, 100 µg/mL). The surviving fraction and IC<sub>50</sub> values were determined (Skehan *et al.*, 1990).

IC<sub>50</sub> is defined as the concentration which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor (Sun *et al.*, 2011).

## GC-MS analysis of fungal alkaloids

Analysis was done at Agricultural Research Center, Giza, Egypt. The analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass selective detector (MSD Agilent 7000) equipped with an apolar Agilent HP-5ms (5% phenyl methylpolysiloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium with linear velocity of 1 mL/min. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with reported in the literature (Santanal *et al.*, 2013).

## Statistical analysis

The results were expressed as the mean ± standard deviations (mean±SD). Data were analyzed by one-way analysis of variance (ANOVAs) using SPSS statistical program. The differences were considered significant at  $p \leq 0.05$ . Letters were compared across the same column.

## RESULTS AND DISCUSSION

### Isolation and identification of endophytic fungi

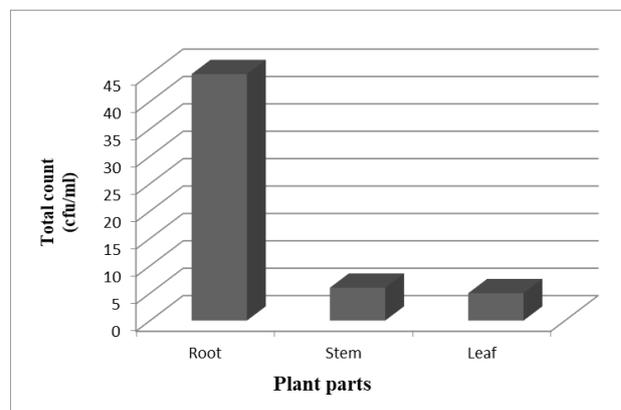
In the current study, endophytic fungi were isolated from different parts (roots, stems, and leaves) of *C. roseus* using two isolation media; Martin and SDA. Table 1 revealed that Martin media was more suitable for isolation

of endophytic fungi (83.92% frequency) than SDA media (16.07% frequency).

A total of 56 endophytic fungal isolates were surveyed from *C. roseus* parts represented by 12 species. *Aspergillus* was the most frequent genus represented by 23 isolates and 3 species, followed by *Fusarium* with 19 isolates and 4 species. The least dominant genus was *Alternaria* constituted one species with 2 isolates. In relation to our study, the fungal genera *Alternaria*, *Fusarium*, *Penicillium*, *Schizophyllum* and *Xylaria* were isolated from *C. roseus* as endophytes (Handa, 2015).

With regard to fungal species, *A. fumigatus* and *F. oxysporum* were the most dominant species represented by 11 isolates each with frequency 19.64% of the total isolates (Table 1). *A. niger* came in the second rank with 10 isolates and 17.85% frequency. *Botrytis cinerea* and *F. solani* came next with 5 isolates and frequency 8.92%. However, the least frequent species was *F. chlamydosporum* with 1 isolate and 1.78 frequency.

Figure 1 showed that endophytic fungi were more frequent in roots (45 isolates) than both stems and leaves (6 and 5 isolates, respectively). This is convenient with results indicating that endophytic colonization differs with different plant organs (Schulz and Boyle, 2005).



**Figure 1:** Total count of endophytic fungi isolated from different parts of *C. roseus*.

### Alkaloid determination

Both Dragendorff's and Mayer's reagents were used for qualitative screening of both intracellular and extracellular alkaloids produced by the most five dominant endophytic fungal species isolated from *C. roseus*. They were *A. fumigatus*, *A. niger*, *B. cinera*, *F. oxysporum*, and *F. solani*. The results in Table 2 showed that all the five fungal species produce intracellular and extracellular alkaloids. Results also indicated that Dragendorff's reagent was more precise for checking alkaloids production than Mayer's reagent. Yin and Sun (2011) isolated 10 endophytic fungal strains from stem, leaves and roots of *Vinca minor*. The extracts from strain Vm-J<sub>2</sub> only had positive results with both Dragendorff's and Mayer's reagents.

In this study, an experiment was done for quantitative screening of alkaloids in mycelial and filtrate extracts of the most dominant endophytic fungal species isolated from *C. roseus*. Table 3 showed that; by increasing the incubation period the intracellular alkaloid concentrations decreased, while the extracellular ones increased. The most significant total alkaloids productions were by *A. fumigatus* and *A. niger* after 21 days of incubation, while

**Table 1:** Isolation of endophytic fungi from different parts of *C. roseus*.

Source Organism	Media	Root		Stem		Leaf		TC and F (%)					
		M	S	M	S	M	S	TC M	F (%) M	TC S	F (%) S	TC	F (%)
<i>Alternaria alternata</i>		0	2	0	0	0	0	0	0	2	22.22	2	3.57
<b>Total <i>Aspergillus</i> sp.</b>		13		5		5				-		23	41.07
<i>A. flavus</i>		0	0	0	1	0	1	0	0	2	22.22	2	3.57
<i>A. fumigatus</i>		7	0	0	4	0	0	7	14.89	4	44.44	11	19.64
<i>A. niger</i>		6	0	0	0	4	0	10	21.27	0	0	10	17.85
<i>Botrytis cinerea</i>		4	0	0	1	0	0	4	8.51	1	11.11	5	8.92
<b>Total <i>Cladosporium</i> sp.</b>		4		0		0				-		4	7.14
<i>C. oxysporum</i>		2	0	0	0	0	0	2	4.25	0	0	2	3.57
<i>C. sphaerospermum</i>		2	0	0	0	0	0	2	4.25	0	0	2	3.57
<b>Total <i>Fusarium</i> sp.</b>		19		0		0				-		19	33.92
<i>F. chlamydosporum</i>		1	0	0	0	0	0	1	2.12	0	0	1	1.78
<i>F. moniliforme</i> Var. <i>anthophilum</i>		2	0	0	0	0	0	2	4.25	0	0	2	3.57
<i>F. oxysporum</i>		11	0	0	0	0	0	11	23.40	0	0	11	19.64
<i>F. solani</i>		5	0	0	0	0	0	5	10.63	0	0	5	8.92
<i>Mycelia sterilia</i>		3	0	0	0	0	0	3	6.38	0	0	3	5.35
<b>Total count</b>		43	2	0	6	4	1	47	83.92	9	16.07	56	100

M, matrin medium; S, Sabouraud Dextrose Agar medium (SDA); TC, total count (cfu/mL); F (%), frequency.

**Table 2:** Qualitative assay of intercellular and extracellular alkaloids produced by endophytic fungal species isolated from *C. roseus*.

Incubation Period & reagent	10 days				15 days				21 days			
	I		E		I		E		I		E	
	D	M	D	M	D	M	D	M	D	M	D	M
<i>A. fumigatus</i>	++	++	+	-	++	+	++	++	+	-	+	+
<i>A. niger</i>	++	++	+	-	++	++	+	+	+	+	++	++
<i>B. cinera</i>	+	+	+	-	++	++	+	+	+	+	+	+
<i>F. oxysporum</i>	++	++	+	+	++	++	++	++	++	++	++	++
<i>F. solani</i>	++	++	+	+	+	+	++	++	+	+	++	++

I, intracellular alkaloids; E, extracellular alkaloids; D, Dagrardorf's reagent; M, Mayer's reagent.  
 -, No colored precipitate was detected; +, low color intensity; ++, high color intensity.

**Table 3:** Quantitative assay of intercellular and extracellular alkaloids produced by endophytic fungal species isolated from *C. roseus*.

Organism	10 Days Incubation		15 Days Incubation		21 Days Incubation	
	I	E	I	E	I	E
<i>A. fumigatus</i>	22.4 <sup>d</sup> ± 0.0	12 <sup>c</sup> ± 0.76	21.8 <sup>d</sup> ± 1.2	21.2 <sup>d</sup> ± 1.69	21.2 <sup>d</sup> ± 1.69	30.6 <sup>d</sup> ± 0.68
<i>A. niger</i>	25.2 <sup>e</sup> ± 0.17	10.2 <sup>b</sup> ± 0.3	24.8 <sup>e</sup> ± 0.2	11 <sup>b</sup> ± 0.33	24.6 <sup>e</sup> ± 0.75	27.4 <sup>b</sup> ± 1.2
<i>B. cinera</i>	6.2 <sup>a</sup> ± 0.37	5.6 <sup>a</sup> ± 1.5	2.8 <sup>a</sup> ± 1.45	8.4 <sup>a</sup> ± 0.6	1.6 <sup>a</sup> ± 1.21	10.6 <sup>a</sup> ± 1.63
<i>F. oxysporum</i>	21.4 <sup>c</sup> ± 0.98	17.4 <sup>d</sup> ± 1.8	19.6 <sup>c</sup> ± 2.4	19 <sup>c</sup> ± 1.0	17.8 <sup>c</sup> ± 1.89	29.2 <sup>c</sup> ± 1.66
<i>F. solani</i>	17.6 <sup>b</sup> ± 0.7	31.8 <sup>e</sup> ± 0.9	13.2 <sup>b</sup> ± 1.28	32 <sup>e</sup> ± 0.3	9.2 <sup>b</sup> ± 0.84	32.4 <sup>e</sup> ± 1.15

I, Intracellular alkaloids (mg/mL); E, Extracellular alkaloids (mg/ mL). Across the same column, mean values with different letters were considered significant at  $p \leq 0.05$  according to Duncan's multiple range test. <sup>a</sup>, least significant value, <sup>c</sup>, most significant.

the least one was by *B. cinera* (Figure 2). Different plant parts of *C. roseus* produce intracellular and extracellular indole alkaloids (Abdel-Rahman *et al.*, 2010). The ability of endophytic fungi to produce active components is closely related to their microenvironment (Li *et al.*, 1999; Yin *et al.*, 2009).

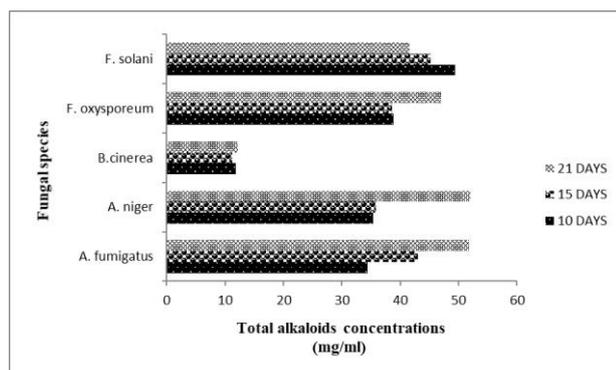
### Antimicrobial activity

Antimicrobial activities of mycelial and filtrate extracts of endophytic fungi isolated from *C. roseus* were assayed. The experiment was done against four pathogenic microorganisms; two Gram-positive bacterial species (*B. subtilis* and *S. aureus*), one Gram-negative bacterial species (*E. coli*) and one yeast species (*C. albicans*). As shown in Tables 4 and 5, all fungal extracts had antimicrobial activities against all pathogenic test

**Table 4:** Antimicrobial activities of filtrate extracts of endophytic fungi isolated from *C. roseus*.

Pathogenic test microorganism	Inhibition zone diameter (mm)					Control
	<i>A. fumigatus</i>	<i>A. niger</i>	<i>B. cinera</i>	<i>F. oxysporum</i>	<i>F. solani</i>	
Gram-positive bacteria						
<i>B. subtilis</i>	7 <sup>b</sup> ± 1.0	8 <sup>a</sup> ± 0.0	8 <sup>b</sup> ± 0.0	9 <sup>a</sup> ± 1.0	9 <sup>b</sup> ± 1.0	0 <sup>a</sup> ± 0.0
<i>S. aureus</i>	0 <sup>a</sup> ± 0.0	11 <sup>b</sup> ± 0.5	9 <sup>b</sup> ± 1.5	10 <sup>a</sup> ± 2.0	9 <sup>b</sup> ± 0.5	0 <sup>a</sup> ± 0.0
Gram-negative bacteria						
<i>E. coli</i>	6 <sup>b</sup> ± 0.5	8 <sup>a</sup> ± 1.0	0 <sup>a</sup> ± 0.0	10 <sup>a</sup> ± 0.0	7 <sup>a</sup> ± 1.0	0 <sup>a</sup> ± 0.0
Yeast						
<i>C. albicans</i>	9 <sup>c</sup> ± 0.5	9 <sup>a</sup> ± 0.0	11 <sup>c</sup> ± 1.0	11 <sup>b</sup> ± 1.0	10 <sup>b</sup> ± 0.0	0 <sup>a</sup> ± 0.0

Across the same column, mean values with different letters were considered significant at  $p \leq 0.05$  according to Duncan's multiple range test. <sup>a</sup>, least significant value, <sup>c</sup>, most significant.



**Figure 2:** Total alkaloids concentrations after 10, 15 and 21 days incubation periods.

microorganisms expect filtrate extracts of *A. fumigatus* and *B. cinera* against *S. aureus* and *E. coli*, respectively. Concerning filtrate extracts (Table 4), *B. subtilis* was highly sensitive to both *F. oxysporum* and *F. solani*, while *S. aureus* was inhibited strongly by *A. niger*. *E. coli* was very susceptible to *F. oxysporum*, while *C. albicans* showed high sensitivity to both *B. cinera* and *F. oxysporum*. Regarding to mycelial extracts (Table 5), *B. cinera* and *F. oxysporum* had the highest significant activities against *B. subtilis*, while *A. niger* was the most effective against *S. aureus*, *E. coli* and *C. albicans*. From the previous results, it was cleared that both *A. niger* and *F. oxysporum* had the most significant antimicrobial activities, while *A. fumigatus* had the least significant activity. In this field, five endophytic fungi isolated from *C. roseus* showed antibacterial activity when tested against *B. subtilis*, *E. coli*, *S. aureus* and *S. typhi* and antifungal activity when tested against *A. fumigatus* and *C. albicans* with inhibition zones ranging from 2 mm to 16 mm (Akpotu *et al.*, 2017).

Different parts of *C. roseus* (leaves, stem, flowers, roots and seeds) showed antibacterial activities against seven bacterial strains (*B. cereus*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. sonnei*, *S. paratyphi* and *S. typhi*) with different sensitivities (Jayakumar *et al.*, 2010). Shanmugaraju and Bhagyaraj (2016) tested the antibacterial activity of *C. roseus* leaf extract. They observed that *Staphylococcus* sp was more susceptible than *E. coli*, *Pseudomonas* sp. and *Streptococcus* sp. This was agreed with the hypotheses that endophytic fungi are having capacity to produce bioactive substances and secondary metabolites similar to that produced by their host plants (Gunatilaka, 2006; Zhou *et al.*, 2009). Endophytes should produce metabolites to compete first with other endophytes and then with pathogens to colonize the host (Schulz *et al.*, 2002). Most of these metabolites display antimicrobial and

cytotoxic activities (Sun *et al.*, 2011). So, endophytic fungi act as an important source for developing drug discovery process (Strobel, 2003; Firàková *et al.*, 2007; Debbab *et al.*, 2011).

### In vitro cytotoxicity assay

The *in vitro* cytotoxicity assay of extracts of endophytic fungal species (which had the highest antimicrobial activities) isolated from *C. roseus* was determined against breast cancer (MCF-7) and liver cancer (HEPG-2) human cell lines by using SRB assay method. As shown in Table 6, it was obvious that both fungal extracts showed cytotoxic effects on the two cell lines. *A. niger* extract displayed the highest significant cytotoxicity against MCF-7 cell line with IC<sub>50</sub> value of 42.1 µg/mL, while the least cytotoxicity was exhibited by *F. oxysporum* against MCF-7 cell line with IC<sub>50</sub> value of 66.9 µg/mL.

Similarly, mycelial extract of the fungus *Nigrospora sphaerica* isolated from *C. roseus* produced vinblastine with a concentration higher than produced by the plant leaf extract. Vinblastine produced by *N. sphaerica* had a higher cytotoxic activity when using MTT assay against breast cell line cancer MDA-MB 231 (Ayob *et al.*, 2017). Different extracts (methanol, ethanol, chloroform, ethyl acetate and acetone) of aerial parts of *C. roseus* showed *in vitro* cytotoxicity against MCF (breast cancer) cell lines by using MTT assay method (Ruskin and Aruna, 2014). This means that *C. roseus* had activities similar to their endophytic fungi, so further researches are needed to study the active constituent of both plants and their endophytes.

### GC-MS analysis of fungal alkaloids

Elucidation of the alkaloids in mycelial and filtrate extracts of *A. niger* was carried out using gas chromatography mass spectroscopy (GC-MS). As shown in Table 7, 11 alkaloid compounds were detected in both mycelial and filtrate extracts of *A. niger* with different retention times. They were Thebaine, (16s,19Z)-Isositsirikine, Pleiocarpamine, Aspidofractinine, Dihydrovallesiachotamine, Vincamine, Ajmaline, (-)-Vincadifformine, 20-Cycloaspidospermidine-3-carboxylic acid, 1-formyl-8-oxo-, methyl ester, (2α,3β,5α,12β,19α,20R), Reserpine and Epiibogamine. Percentages of Thebaine, Aspidofractinine, Vincamine and Epiibogamine alkaloid compounds in mycelial extracts were higher than in filtrate extracts. In contrast, percentages of (16s,19Z)-Isositsirikine, Pleiocarpamine, Dihydrovallesiachotamine, Ajmaline, (-)-Vincadifformine, 2,20-Cycloaspidospermidine-3-carboxylic acid, 1-formyl-8-oxo-, methyl ester,

**Table 5:** Antimicrobial activities of mycelial extracts of endophytic fungi isolated from *C. roseus*.

Pathogenic test microorganism	Inhibition zone diameter (mm)					Control
	<i>A. fumigatis</i>	<i>A. niger</i>	<i>B. cinera</i>	<i>F. oxysporum</i>	<i>F. solani</i>	
Gram-positive bacteria						
<i>B. subtilis</i>	7 <sup>a</sup> ± 1	10 <sup>a</sup> ± 0.0	11 <sup>c</sup> ± 1	11 <sup>c</sup> ± 1.5	9 <sup>a</sup> ± 1.5	0 <sup>a</sup> ± 0
<i>S. aureus</i>	9 <sup>b</sup> ± 0.0	10 <sup>a</sup> ± 0.5	9 <sup>b</sup> ± 0.5	9 <sup>b</sup> ± 0.0	8 <sup>a</sup> ± 1.5	0 <sup>a</sup> ± 0
Gram-negative bacteria						
<i>E. coli</i>	7 <sup>a</sup> ± 0.5	10 <sup>a</sup> ± 1	6 <sup>a</sup> ± 0.5	7 <sup>a</sup> ± 1	9 <sup>a</sup> ± 0.5	0 <sup>a</sup> ± 0
Yeast						
<i>C. albicans</i>	5 <sup>a</sup> ± 0.5	11 <sup>a</sup> ± 0.0	9 <sup>b</sup> ± 1	9 <sup>b</sup> ± 0.0	8 <sup>a</sup> ± 0.5	0 <sup>a</sup> ± 0

Across the same column, mean values with different letters were considered significant at  $p \leq 0.05$  according to Duncan's multiple range test. <sup>a</sup>, least significant value, <sup>c</sup>, most significant.

**Table 6:** Cytotoxicity assay of endophytic fungal extracts against MCF-7 and HEPG-2 human cell lines.

Concentration (µg/mL)	Surviving fraction			
	<i>A. niger</i>		<i>F. oxysporum</i>	
	MCF-7	HEPG-2	MCF-7	HEPG-2
0.0	1.000 <sup>a</sup> ± 0.000			
12.5	0.844 <sup>b</sup> ± 0.001	0.867 <sup>b</sup> ± 0.002	1.000 <sup>a</sup> ± 0.000	1.007 <sup>a</sup> ± 0.002
25.0	0.856 <sup>b</sup> ± 0.002	0.806 <sup>c</sup> ± 0.001	0.835 <sup>b</sup> ± 0.002	0.926 <sup>b</sup> ± 0.001
50.0	0.296 <sup>d</sup> ± 0.001	0.481 <sup>d</sup> ± 0.001	0.593 <sup>c</sup> ± 0.001	0.541 <sup>c</sup> ± 0.001
100.0	0.349 <sup>c</sup> ± 0.002	0.496 <sup>d</sup> ± 0.002	0.336 <sup>d</sup> ± 0.001	0.389 <sup>d</sup> ± 0.001
IC <sub>50</sub> (µg/mL)	42.1	48.2	66.9	61.9

Across the same column, mean values with different letters were considered significant at  $p \leq 0.05$  according to Duncan's multiple range test. <sup>a</sup>, least significant value, <sup>c</sup>, most significant.

**Table 7:** GC-MS analysis of alkaloids in mycelial and filtrate extracts of *A. niger*.

Alkaloid	Retention time (min)	Area sum% (Filtrate)	Area sum% (Mycelia)
Thebaine	4.4	0.42	1.67
(16s,19Z)-Isositsirikine	7.7	0.75	0.53
Pleiocarpamine	9.59	1.92	0.71
Aspidofractinine	13.6	0.4	0.58
Dihydrovallesiachotamine	14.3	1.48	0.65
Vincamine	16.92	0.39	4.37
Ajmaline	19.52	6.86	0.47
(-)-Vincadiformine	19.78	1.96	1.27
2,20-Cycloaspidospermidine-3-carboxylic acid, 1-formyl-8-oxo-, methyl ester, (2α,3β,5α,12β,19α,20R)	20.44	5.59	1.07
Reserpine	20.6	0.94	0.76
Epiibogamine	21.5	36.44	44.56

(2α,3β,5α,12β,19α,20R) and Reserpine were more in filtrate extracts than in mycelial extracts.

It was clearly observed that endophytic fungi and their host plants may produce similar bioactive compounds. This leads to hypothesis that the bioactivity of the host plant may be due to metabolites produced by the endophytic microorganisms.

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