



ORIGINAL ARTICLE

***In Vitro* Cytotoxicity and Antioxidant Activities of *Pestalotiopsis microspora* Culture Filtrate**

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**Abstract**

Endophytic fungi have been studied to provide protection and survival conditions to their host plant by producing a plethora of substances which, once isolated and characterized, may also have potential for use in industry, agriculture, and medicine. In this study, the culture filtrate of an endophytic fungus (*Pestalotiopsis microspora* (PM)) was evaluated for its cytotoxic and antioxidant activities in vitro. The cytotoxic activity of PM was determined using brine shrimp lethality assay (BSLA), while its antioxidant effect was investigated against DPPH, reducing power and hydroxyl radicals. Judging by the LC<sub>50</sub> value of 2.71 mg·mL<sup>-1</sup> for the BSLA, the culture filtrate could be considered highly potent. The PM also significantly scavenged free radicals and the effects elicited could be attributed to its phenolics and other phytoconstituents as revealed by the GC-MS results. It is thereby evident from the data presented that PM is endowed with chemotherapeutic constituents that could be potentially useful for the development of new lead anticancer agents.

**Keywords:** Bioactive metabolites; Cytotoxicity; Endophytic fungi; *Pestalotiopsis microspora*

**Introduction**

Endophytes are organisms, usually fungi and bacteria, that inhabit plant tissues without causing any manifestation of physiological abnormality in the plant (Bacon and White 2000; Harper *et al.*, 2003). They live in aerial parts (leaves, stems, barks, petiole, reproductive structures and roots) of the plant and as such makes them distinct from the mycorrhizal symbionts (Faeth and Fagan, 2002; Firakova *et al.*, 2007). Endophytes are promising source of natural bioactive compounds that are pharmaceutically important with ability to fight against pathogens and cancerous cells in humans (Agrawal *et al.*, 2014; Kusari *et al.*, 2014).

Fungi are achlorophyllous and hence heterotrophic. They establish different relationship with autotrophs, living in mutualistic as well as antagonistic co-existence (Dayle *et al.*, 2001). These class of organism have been identified to contain enormous bioactive compounds with potential antimicrobial properties and one of such was penicillin from *Penicillium notatum* as discovered by Alexander Fleming in 1928 (Agrawal *et al.*, 2014). Also,

the immunosuppressive drug known as cyclosporine was isolated from *Tricoderma polysporum* and *Cylindrocarpon lucidum* (Webber, 1981). An anticancer agent, taxol, was obtained from the fungus *Taxomyces andereanae* (Sterile *et al.*, 1993). Endophytic fungi, especially those that are associated with medicinal plants, are a cheap source of antioxidant and cytotoxic compounds (Agrawal *et al.*, 2014). *Fusarium pseudonygamai*, *Colletotrichum dematium*, *Acremonium strictum*, *Botryodiplodia theobromae*, *Myrothecium verrucaria*, *Nigrospora oryzae* and *Pestalotiopsis* spp. are common endophytic fungi (Nalini *et al.*, 2014). *Pestalotiopsis microspora* is one of the endophytic fungi that is most widely distributed and produce bioactive organic substances including taxol which is an anticancer drug (Strobel *et al.*, 1996). This fungus is known to produce certain natural metabolites such as isopestacin and pestacin (Zhang and Demain, 2007).

Despite the discovery of ability of endophytic fungi to produce bioactive secondary metabolites that are pharmaceutically and industrially useful, exploration of these organisms is still in its infancy. Hence, studies on the bioactivities of the active compounds from these endophytic fungi like the *P. microspora* have become imperative and have prompted the present research. This work evaluated the *in vitro* cytotoxicity and antioxidant potential of the culture filtrate of *P. microspora*. The GC-MS analysis of its culture isolate was also performed.

## Materials and Methods

### Preparation of Fungal Filtrate of *Pestalotiopsis microspora*

The pure cultures of *P. microspora* (Figure 1) was collected from Mycology Laboratory, Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria. The fungus was grown in 250 mL sterile conical flask containing 100 mL Potato Dextrose Broth and incubated at  $25\pm 2$  °C for 14 days (Garuba *et al.*, 2014). After incubation, the culture filtrates were filtered into pre-sterilized conical flasks using Whatman no. 1 filter paper. The filtrates were stored in a refrigerator at  $4\pm 2$  °C.

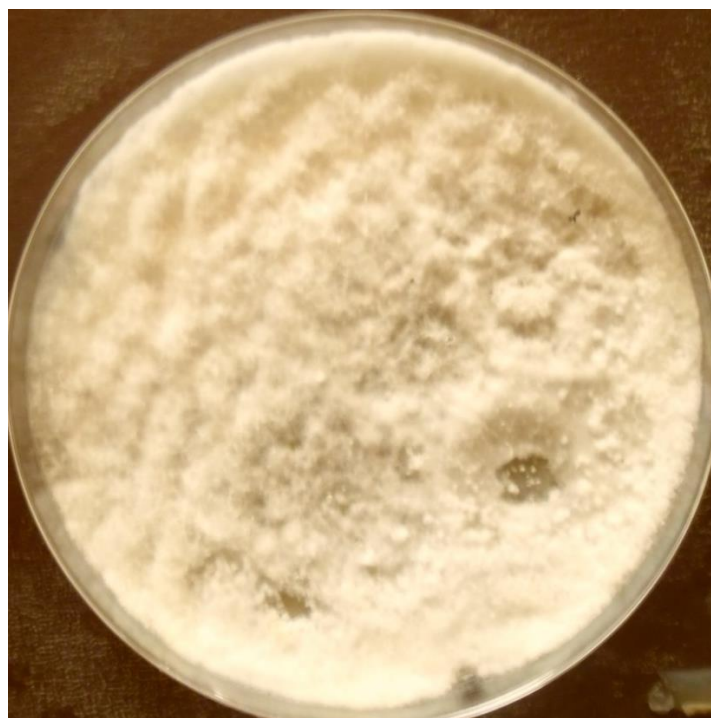


Figure 1. Pure culture of *Pestalotiopsis microspora*

### **Cytotoxic Activity of *Pestalotiopsis microspora* Culture Filtrate**

Adopting the method of Meyer *et al.* (1982), the Brine shrimp lethality test (BST) for the isolate was performed. Briefly, filtered seawater (500 mL) was put in a small hatching chamber with a partition for dark (covered) and light areas. Shrimp eggs (30 mg) were added into the dark side of the chamber while the lamp above the other side (light) was positioned to attract hatched shrimps. The set up was left for 48 h to allow shrimps to hatch and mature as nauplii (larvae). After incubation, when the larvae were ready, different concentrations (10 - 100 mg·mL<sup>-1</sup>) of the isolate were prepared from the stock solution and ten nauplii were transferred into each vial using Pasteur pipettes. The control vials were prepared using seawater only. Following 24 h of incubation under lamp at room temperature, the experiment was terminated and larvae were considered dead if they did not exhibit any observable movement during several seconds of observation. The number of survivors in each vial was counted and percentages of mortality calculated for each concentration. Using probit analysis, the lethality concentration (the concentration of the filtrate causing 50% mortality of nauplii (LC<sub>50</sub>) was evaluated at 95% confidence intervals. LC<sub>50</sub> values of less and greater than 100 mg·mL<sup>-1</sup> were respectively considered toxic and non-toxic (Moshi *et al.*, 2010).

### **Antioxidant Activities of *Pestalotiopsis microspora* Culture Filtrate**

#### **DPPH Radical Scavenging Assay**

Using the method described by Turkoglu *et al.* (2007), the antioxidant activity of the culture filtrate was determined by measuring its capacity of bleaching the purple- coloured ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Exactly 200 µL of varying concentrations (10 – 100%) of the culture filtrate in methanol was added to 500 µL of 0.2 mmol·mL<sup>-1</sup> sample of DPPH in methanol. After 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. Ascorbic acid was used for the control. Inhibition rate (I%) of the filtrate on the DPPH radical was calculated using the expression:

$$\text{Percentage Inhibition (I\%)} = \left( \frac{A_{\text{control}} - A_{\text{culture filtrate}}}{A_{\text{control}}} \right) \times 100 \quad \dots (1)$$

Where, A<sub>control</sub> is the absorbance of the control, A<sub>culture filtrate</sub> is the absorbance of the filtrate.

#### **Reducing Power Potential of *Pestalotiopsis microspora* Culture Filtrate**

The reducing power of the culture filtrate was evaluated by adding 200 µL of the culture filtrate (10 – 100% ) in 1 mL of distilled water and mixed with 500 µL of 0.2M phosphate buffer (pH 6.6) and 500 µL of 1% potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>). The mixture was incubated at 37°C for 30 min prior to addition of 500 µL of trichloroacetic acid (TCA). Following centrifugation at 3000 rpm for 10 min, 2.5 mL of the supernatant was mixed with an equal amount of distilled water and 0.5 mL of 0.1% FeCl<sub>3</sub>. Ascorbic acid was used for the control. The absorbance of the resulting solution was then read at 700 nm (Oyaizu, 1986).

#### **Hydroxyl Radical Scavenging Assay**

The reported procedure of Smirnoff and Cumbes (1996) was used for this assay. Briefly, 200 µL of varying concentrations (10 – 100%) of the culture filtrate was added to 600 µL of FeSO<sub>4</sub> (8 mM), 500 µL of H<sub>2</sub>O<sub>2</sub> (20 mM) and 2 mL of salicylic acid (3 mM) in a test tube. Following a 10 min incubation period at 37°C, distilled water (2.9 mL) was added and the resulting mixtures were shaken and allowed to settle for 15 min. The absorbance was subsequently read at 510

nm and percentage OH radical scavenging capacity of the filtrate was estimated as per the expression:

$$\text{Percent hydroxyl radical scavenged (\%)} = \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{culture filtrate}})}{A_{\text{control}}} \right) \times 100 \quad \dots (2)$$

Where,  $A_{\text{control}}$ ,  $A_{\text{sample}}$  and  $A_{\text{culture filtrate}}$  represent the absorbance of the mixture without filtrate, mixture with the filtrate and that of the filtrate alone, respectively.

#### Quantification of Phenol

Following the reported method of Wolfe *et al.* (2003), the total phenol contents in the culture filtrate was determined. Briefly, an aliquot of the filtrate was mixed with 1.58  $\mu\text{L}$  of distilled water and 100  $\mu\text{L}$  of Folin ciocalteu reagent. After 5 min at room temperature, 300  $\mu\text{L}$  of  $\text{Na}_2\text{CO}_3$  ( $75 \text{ g}\cdot\text{L}^{-1}$ ) was added. The tubes were vortexed for 15 sec and allowed to stand for 30 minutes at 40  $^\circ\text{C}$  for colour development. Absorbance was read at 765 nm using a spectrophotometer. The filtrate was evaluated at a final concentration of  $1 \text{ mg}\cdot\text{mL}^{-1}$ . Total phenolic content was expressed as  $\text{mg/g}$  gallic acid equivalent using the equation obtained from a calibration curve of gallic acid.

#### Detection of Bioactive Compounds by GC-MS Analysis

The isolate was subjected to GC-MS analysis using a Shimadzu GC-MS-QP 2010 Plus fitted with a RTX-5 ( $60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ) capillary column. The instrument was set to an initial temperature of 70  $^\circ\text{C}$ , and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280  $^\circ\text{C}$ , at the rate of an increase of 5  $^\circ\text{C}\cdot\text{min}^{-1}$ , and maintained for 9 min. Injection port temperature was ensured as 260  $^\circ\text{C}$  and Helium flow rate as  $1 \text{ mL}\cdot\text{min}^{-1}$ . The ionization voltage was 70 eV. The sample was injected in split mode as 10:1. Mass spectral scan range was set at 45–450 (m/z). Subsequently, the identification of bioactive compounds present in the isolate was performed by comparing its mass spectra with data from National Institute of Standards and Technology (NIST05, US), Wiley 8, and Flavour and Fragrance Natural and Synthetic Compounds (FFNSC1.3) libraries. The name, molecular weight and structure of the identified constituents of the sample were established.

## Results

The degree of lethality was directly proportional to the concentration of the culture filtrate. Maximum mortality (100%) was observed at the highest investigated concentration. Hence, the brine shrimp lethality of the *P. microspora* culture filtrate was concentration-dependent (Table 1).

**Table 1.** Cytotoxicity of *Pestalotipsis microspora* culture filtrate on brine shrimp nauplii

Conc. ( $\text{mg}\cdot\text{mL}^{-1}$ )	Log of Conc. ( $\text{mg}\cdot\text{mL}^{-1}$ )	Total no. of larvae	Number of dead nauplii / Test		Average mortality	% mortality
			T <sub>1</sub>	T <sub>2</sub>		
10	1.00	10	3	2	2.50 $\pm$ 0.71	25.00
20	1.30	10	8	7	7.50 $\pm$ 0.71	75.00
30	1.47	10	8	8	8.00 $\pm$ 0.00	80.00
40	1.60	10	9	7	8.00 $\pm$ 1.41	80.00
100	2.00	10	10	10	10.00 $\pm$ 0.00	100.00
LC <sub>50</sub>						2.71

T<sub>1/2</sub> values are means  $\pm$  standard deviation of two determinations.

The *in vitro* antioxidant effects of the culture filtrate of *P. microspora* are shown in Figures 2-3. The filtrate dose dependently inhibited or scavenged the generated radicals in all the assays investigated. The capability of the filtrate to scavenge DPPH and hydroxyl radicals revealed significant effects when compared with ascorbic acid (Figures 2a and 2b). Furthermore, at the highest tested concentration (100%), the reducing power effect of the filtrate on ferric ion competed well with that of ascorbic acid (Figure 3).

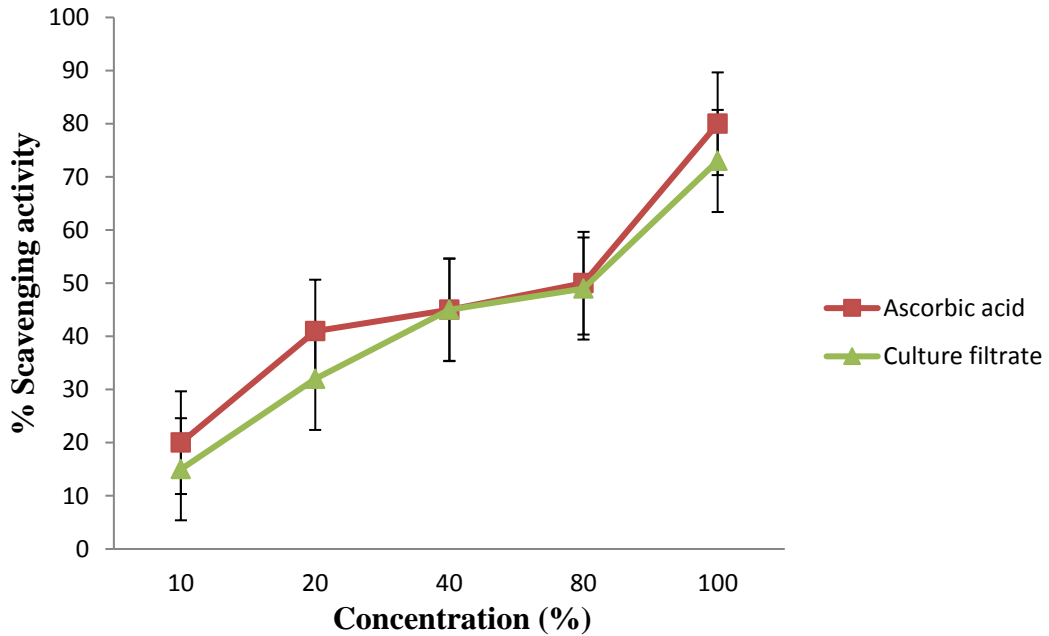


Figure 2a. DPPH radical scavenging effect of *Pestalotiopsis microspora* culture filtrate

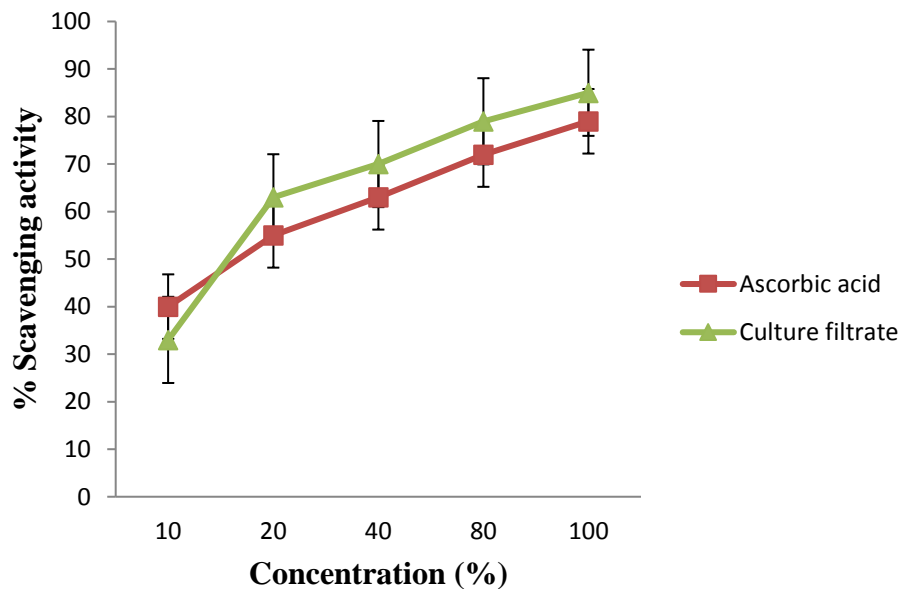
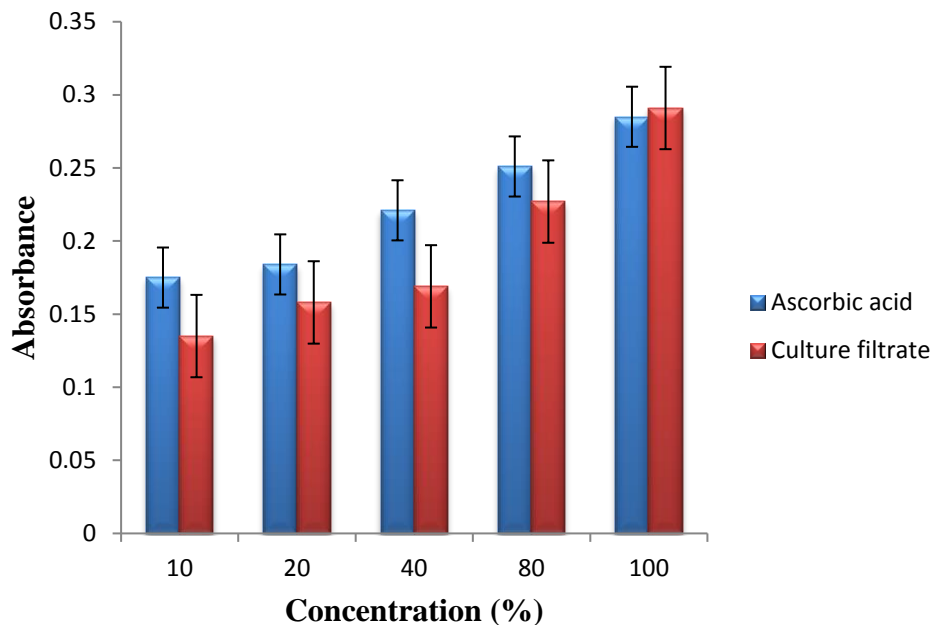


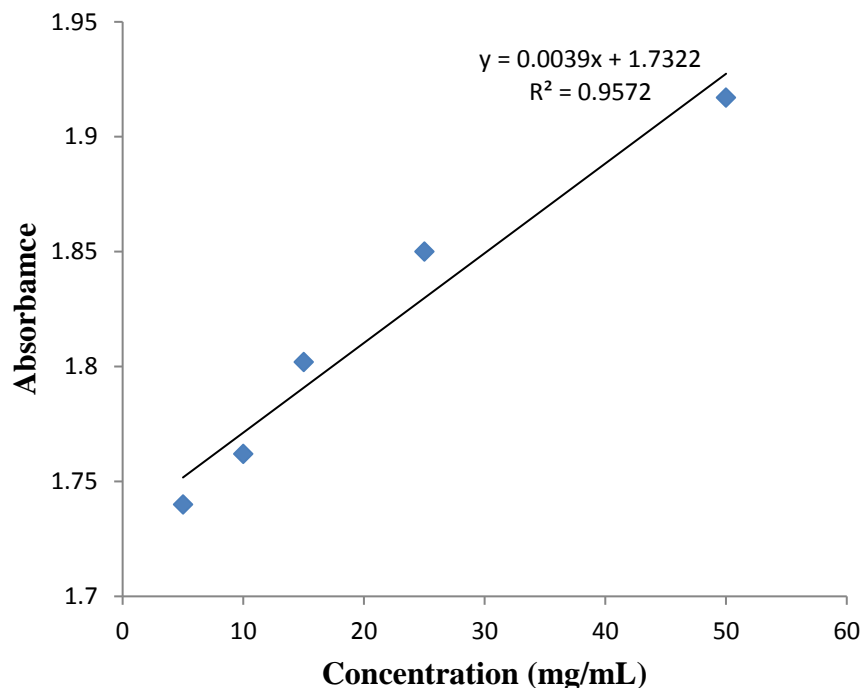
Figure 2b. Hydroxyl radical scavenging effect of *Pestalotiopsis microspora* culture filtrate



**Figure 3.** Reducing power potential of *Pestalotiopsis microspora* culture filtrate

The standard calibration curve for the estimation of the phenolic content of the *P. microspora* filtrate is presented in Figure 4. The estimated phenolic content of the filtrate was  $45.85 \pm 1.25$  mg gallic acid  $g^{-1}$ .

The GC-MS results of the fungal filtrate revealed the presence of 1-adamantyl fluoroformate (47.92%), 6-nitro-8-methoxy-2H-chromene (15.08%), 1-acetyl-3-methoxycarbonyl- $\beta$ -carbolin (8.21%), isoxazolidine (6.72%) and indolizine, 2-(4-methylphenyl)- as its major bioactive metabolites (Figure 5 and Table 2).



**Figure 4.** Gallic acid standard curve for the estimation of phenolic contents of culture filtrate.

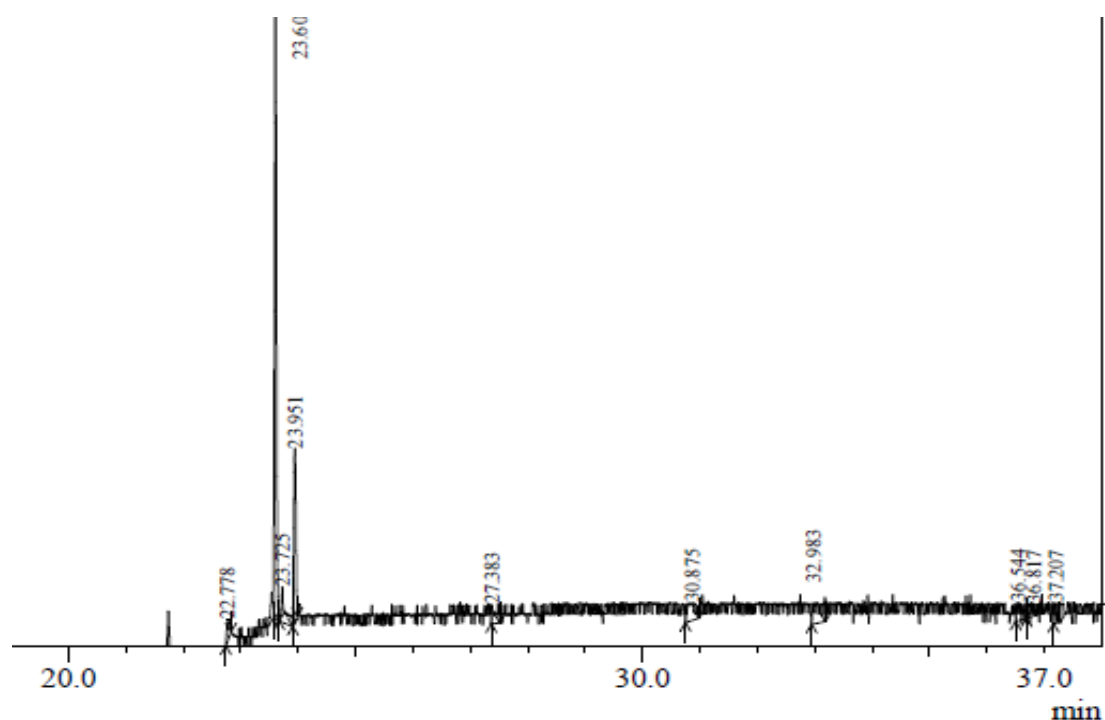


Figure 5. GC-MS chromatogram of *Pestalotiopsis microspora* culture filtrate

Table 2. Bioactive metabolites of *P. microspora* culture filtrate as revealed by GC-MS chromatogram.

Peak	RT	Area (%)	Compound	MF	Activity	Reference
1	22.78	2.54	Yadanzioside D	C <sub>22</sub> H <sub>28</sub> O <sub>11</sub>	Antimalarial, antidyentary	Weici and Gerhard (1992)
2	23.61	47.92	1-adamantyl fluoroformate	C <sub>11</sub> H <sub>15</sub> FO <sub>2</sub>	Antioxidant, antiviral	Maugh (1979)
3	23.73	5.90	Indolizine, 2-(4-methylpheny)-	C <sub>15</sub> H <sub>13</sub> N	Antitumor	Sathyaprabha <i>et al.</i> (2011)
4	23.95	15.08	6-nitro-8-methoxy-2H-chromene	C <sub>10</sub> H <sub>9</sub> NO <sub>4</sub>	Antioxidant, anti-inflammatory, immunomodulatory, anticancer	Zachariah and Thomas (2013)
5	27.38	3.50	1,2,2-triphenyl-3,3-bis(trimethylsilyl)phosphiran	C <sub>26</sub> H <sub>33</sub> PS <sub>12</sub>	-	-
6	30.88	6.72	Isoxazoline	C <sub>3</sub> H <sub>7</sub> NO	Anticancer, antiviral	Kokosza <i>et al.</i> (2015)
7	32.98	8.21	1-acetyl-3-methoxycarbonyl-β-carbolin	C <sub>15</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub>	Anticancer	Figueiredo <i>et al.</i> (2011)
8	36.54	3.59	Heptanol	C <sub>7</sub> H <sub>14</sub> O	Anti-Arrhythmic	Tse <i>et al.</i> (2016)
9	36.82	3.55	N-N-diethyl-2-formyl-3-methoxybenzamide	C <sub>13</sub> H <sub>17</sub> NO <sub>3</sub>	-	-
10	37.21	3.00	1-chloro-1,2,2,2-tetrafluoroethane sulfinylchloride	C <sub>2</sub> Cl <sub>2</sub> F <sub>4</sub> OS	Bronchodilator	Shulman and Sadove (1967)

RT= retention time (min.), MF= molecular formula



The lethality of a test sample against brine shrimps has been previously utilized and it is a very useful tool to screen a wide range of chemical compounds for various bioactivities (Meyer *et al.*, 1982). It has been demonstrated that BST correlates reasonably well with cytotoxic and other biological properties (Mclaughlin *et al.*, 1991) and has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound (Almeida *et al.*, 2002) as well as plant and microbial products of fungal origin (Meyer *et al.*, 1982; Sathyaprabha *et al.*, 2011; Sabiu and Ashafa, 2016). The significant correlation between the BST and *in vitro* growth inhibition of human solid tumor cell lines demonstrated by the National Cancer Institute, USA is significant because it shows the importance of this bioassay as a pre-screening tool for antitumor drug research (Anderson *et al.*, 1991). Many studies showing cytotoxic properties of either plant or fungal isolates in BST have also implicated their active metabolites in a diverse number of activities ranging from antifungal, pesticidal, teratogenic, antiviral, immunomodulatory, anti-inflammatory and antioxidant (Vanhaecke *et al.*, 1981; Sathyaprabha *et al.*, 2011; Zachariah and Thomas, 2013; Alayande *et al.*, 2017). In this study, the *P. microspora* culture filtrate exhibited good cytotoxic activity against the brine shrimps and this may be considered as significant. Such effect could be ascribed to the filtrate containing active or potent components as shown from its GC-MS chromatogram. A significant number of the identified constituents in the filtrate have been reported to potentiate either antitumor or anticancer properties (Table 2) and as such supporting the candidature of the *P. microspora* culture filtrate as a probable new lead in the pursuit of new anticancer drug. Previous studies on microbial cultures have also demonstrated similar cytotoxic activity and the effects were attributed to their bioactive metabolites (Sathyaprabha *et al.*, 2011; Sharma *et al.*, 2016).

Phenolic compounds have been studied to either inhibit cancer cells through induction of xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens or alter hormone production that subsequently inhibit aromatase to prevent the development of cancer cells (Prasad *et al.*, 2009). They could also reduce the amount of cellular protein, mitotic index and colony formation during cell proliferation (Gu and Sivam, 2006). Interestingly, the *P. microspora* culture filtrate investigated in this study was found to be rich in phenolics and this is another probable fact supporting its anticancer activity. This assertion also agrees with the report of Uddin *et al.* (2011), where antioxidant and anticancer effects were directly associated with a good number of secondary metabolites including phenolics. Furthermore, the relationship between generation of free radicals and the pathogenesis of cancer has been demonstrated (Jose and Janardhanan, 2000). In the present study, the culture isolate of *P. microspora* exhibited significant anti-radical activities when compared with the reference antioxidant (ascorbic acid) used. This observation is suggestive of the capability of the isolate to stall free radicals chain reactions associated with carcinogenesis. This effect may also be attributed to either the phenolic constituents of the isolate or its other phytonutrients as evidently identified in its chromatogram.

## Conclusion

This study indicated that *P. microspora* culture filtrate is rich in phenolics with significant antioxidant and cytotoxic properties. The observed effect of the culture filtrate on the brine shrimps also suggested the presence of potent cytotoxic and probably antitumor constituents. Overall, these results have supported the antioxidant potential of *P. microspora* culture filtrate and presenting it as viable candidate in the development of new anticancer agent.

## References

Agrawal, P. and Bhardwaj, A. (2014). A review fungal endophyte: as a store house of bioactive compound. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(9), 228-237



- Alayande, K. A., Sabiu, S. and Ashafa, A. O. T. (2017). Medicinal properties of *Abrus precatorius* L. leaf extract: Antimicrobial, cytotoxicity and carbohydrate metabolizing enzymes inhibitory potential. *Transactions of the Royal Society of South Africa*, 242-250.
- Almeida, P. A., Silva, T. M. S. and Echevarria, A. (2002). Mesoionic 5-alkyl-1,3- dithiolium -4 thiolates: Synthesis and brine shrimp toxicity. *He terocyclic Comm.*, 8, 593-600.
- Anderson J. E., Getz, C. M., Mclaughlin, J. L. and Suffness, M. (1991). A blind comparison of simple bench top bioassay and human tumor cell cytotoxicities as antitumor prescreen. *Phytochem Analysis.*, 2, 107-111.
- Bacon, C. and White, J. (2000). *Microbial endophytes*. Marcel Dekker, New York. 487p
- Dayle, E. S., Polans, N. O, Paul, D. S. and Melvin, R. D. (2001). Angiosperm DNA contamination by endophytic fungi: detection and methods of avoidance. *Plant Molecular Biology Report.* 19, 249-260.
- Faeth, S. H. and Fagan, W. F. (2002). Fungal Endophytes: Common Host Plant Symbionts but Uncommon Mutualists. *Integrative and Comparative Biology*, 42(2), 360–368,
- Figueiredo, P. O., Garcez, F. R., Maria de Fátima, C., Perdomo, R. T., Queiroz, L. M., Pott, A., Garcez, A. J. and Garcez, W. S. (2011). A New Cytotoxic  $\beta$ -Carboline Alkaloid from *Galianthe thalictroides*. *Planta Med.*, 77, 1852–1854.
- Firakova, S., Sturdikova, M. and Muckova, M. (2007). Bioactive secondary metabolites produced by microorganisms associated with plants. *Biologia*, 62, 251–257
- Garuba, T., Abdulrahman, A. A., Olahan, G. S., Abdulkareem, K. A. and Amadi, J. E. (2014). Effects of fungal filtrates on seed germination and leaf anatomy of maize seedlings (*Zea mays* L., Poaceae). *Journal of Applied Science and Environmental Management*, 18(4), 662-667
- Gu, Y. H. and Sivam, G. (2006). Cytotoxic effect of oyster mushroom *Pleurotus ostreatus* on human androgen-independent prostate cancer PC-3 cells, *Journal of Medicinal Food*, 9, 196–204.
- Harper, J. K., Arif, A. M., Eugene, J., Ford, E. J., Strobel, G. A., Porco, Jr., J. A., Tomer, D. P., Oneill, K. L., Heidere, E. M. and Granta, D. M. (2003). Pestacin: a 1,3-dihydro isobenzofuran from *Pestalotiopsis microspora* possessing antioxidant and antimycotic activities. *Tetrahedron*, 59, 2471–2476
- Jose, N. and Janardhanan, K. K. (2000). Antioxidant and antitumour activity of *Pleurotus florida*, *Current Science*. 79 (2000), 941–943.
- Kokosza, K., Andrei, G., Schols, D., Snoeck, R. and Piotrowska, D. G. (2015). Design, antiviral and cytostatic properties of isoxazolidine-containing amonafide analogue. *Bioorganic and Medicinal Chemistry*, 23(13), 3135-3146
- Kusari, S., Singh S. and Jayabaskaran, C. (2014). Biotechnological potential of plant-associated endophytic fungi: hope versus hype. *Trends in Biotechnology*. 32 297–303.
- Maugh, T. (1979). "Panel urges wide use of antiviral drug". *Science*, 206 (4422), 1058–1060
- Mclaughlin, J. L., Chang, C. J. and Smith, D. L. (1991). Bench top bioassays for the discovery of bioactive natural product. an update. In: Rahman, A. (ed.). *Studies in natural product chemistry*, 9, Elsevier, Amsterdam, Pp. 483 – 409.
- Meyer, B. N., Ferrigni, N. R., Jacobsen, I. B., Nichols, D. E. and McLaughlin, J. I. (1982). Brine shrimp: A convenient general bioassay for the active plant constituent. *Plant Medica*, 45:35-38.

- Moshi, M. J., Innocent, E., Magadula, J. J., Otieno, D. F., Weisheit, A., Mbabazi, P. K. and Nondo, R. S. O. (2010). Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, north western Tanzania. *Tanz J H Res.*, 12, 63-67.
- Nalini, M. S., Sunayana, N. and Prakash, H. S. (2014). Endophytic fungal diversity in medicinal plants of Western Ghats, India. *International Journal of Biodiversity*, 2014, 1-9
- Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activities of product of browning reaction prepared from glucosamine. *Japan. J. Nutr.* 44, 307-315
- Prasad, K. N., Hao, J., Yi, C. M., Zhang, D., Qui, S. and Jiang, Y. (2009). Antioxidant and anticancer activities of Wampee (*Clausera lansium* (Lour.) Skeels) peel. *Journal of Biomedicine and Biotechnology*, ID 612805: 1-6.
- Sabiu, S. and Ashafa, A. O. T. (2016). Cytotoxicity, antimicrobial and antidiarrheal activities of standardized aqueous root extract of *Elephantorrhiza elephantina* (Burch.). *Transactions of the Royal Society of South Africa*, 72(1),75-84.
- Sathyaprabha, G., Panneerselvam, A. and Kumaravel. S. (2011). Comparative study on Methanolic and Ethanol extracts of bioactive compounds in *Pleurotus platypus*. *International Journal of Chemical and Analytical Science*, 2(8), 111-114.
- Sharma, D., Pramanik, A. and Agrawal, P. K. (2016). Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D. Don. *3 Biotech.*, 6, 210
- Shulman, M. and Sadove, M. S. (1967). 1, 1, 1, 2-tetrafluoroethane: an inhalational agent of intermediate potency. *Anesthesia and Analgesia*. 46, 629-635
- Smirnov, N. and Cumbes, O. J. (1996). Hydroxyl radical scavenging activity of compatible solutes. *Phytochem.* 28, 1057-1060.
- Sterile, A., Strobel, G. A. and Sterile, D. (1993). Taxol And Taxane Production By *Taxomyces andereanae* an endophytic fungus of Pacific yew. *Science*, 260(5105), 214-216
- Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R. S. and Hess, W. M. (1996). Taxol from *Pestalotiopsis microspora*, an endophytic fungus of *Taxus wallachiana*, *Microbiology*, 142(2), 435-440
- Tse, G., Tse, V., Yeo, J. M. and Sun, B. (2016). Atrial Anti-Arrhythmic Effects of Heptanol in Langendorff-Perfused Mouse Hearts. *PLoS ONE*, 11(2): e0148858. <https://doi.org/10.1371/journal.pone.0148858>
- Turkoglu, A., Duru, M. E., Mercan, N., Kivrak, I. and Gezer, K. (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull). *Food. Chem.* 101, 267-273.
- Uddin, S. J., Grice, I. D. and Tiralongo, E. (2011). Cytotoxic effects of Bangladeshi medicinal plant extracts. *Evidence – Based Complementary and Alternative Medicine*. 7p.
- Vanhaecke, P., Persoone, G., Claus, C. and Sorgeloos, P. (1981). Proposal for a short-term toxicity test with *Artemia* nauplii. *Ecotox Env Saf.*, 5, 382-387.
- Webber, J. A. (1981). Natural control of dutch elm disease. *Nature*, 292, 449-451
- Weici, T. and Gerhard, E. (1992). *Bruea javanica* (L.) Merr. Chinese Drugs of Plant Origin. Springer, Berlin, Heidelberg. Pp 207-222
- Wolfe, K., Wu, X. and Liu R.H. (2003). Antioxidant activity of apple peels. *J. Agricul. Food. Chem.* 51, 609-614.
- Zachariah S. M. and Thomas N. (2015). Pharmacological activities of chromene derivatives: An overview. *Asian Journal of Pharmaceutical and Clinical Research*, 6(2): 11-15
- Zhang, L. and Demain, A. L. (2007). *Natural Products: Drug Discovery and Therapeutic Medicine*. Springer Science & Business Media. 345p.

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