NOVAL CYTOTOXIC COMPOUNDS FROM FUNGAL ENDOPHYTES

Karthika[1] M & Rasmi[2] A.R

(Post Graduate & Research Department of Botany, Government Victoria college (Affiliated to university of Calicut), Palakkad, India

[1]karthikanair.karthi@gmail.com & [2]rasmibotany@gmail.com

ABSTRACT

Endophytes are fungi or bacteria which invade the tissue of a living plant and cause unapparent and asymptomatic infections. Endophytic fungi synthesise metabolites that are structurally and functionally similar to those of their host plants. They are able to produce a variety of biomolecules of various classes with limitless possibilities. *Penicillium, Fusarium, Aspergillus, Sclerotium, Myxormia, Alternaria, Colletotrichum, Cladosporium, Diaporthe,* and other endophytic species are known to produce bioactive chemicals that have a vital role in the management of disease. Bioactive compounds derived from endophytic fungi were classified as alkaloids, steroids, flavonoids, phenolic acids, benzopyranones, quinines, tannins, xanthones, terpenoids,etc and they possess antimicrobial, antioxidant, cytotoxic, immunosuppressive, and anti-inflammatory activities.

The deadliest illness, cancer kills thousands of individuals annually. In this scenario, the scientific community is searching for new anticancer drugs to combat cancer. Studies on the discovery of anti-cancer drugs from endophytes began in the early 90s and gained prominence in the twenty-first century. More than 200 novel cytotoxic chemicals have been discovered in the last thirty years from endophytic fungi of both terrestrial and marine plants. These cytotoxic compounds are primarily classified as polyketides, terpenoids, sterols, macrolides, lactones, azaphilones, alkaloids, preussomerins, p-terphenyls, hybrid structures, and other substances. The most important anti-cancer drugs are from endophytes, including taxol, podophyllotaxin, camptothecin, vinca alkaloids, cytochalasin 1-3, malformin, graphislactone A, etc. Discovery of taxol and its analogue from endophytes marked a significant advance in the field of cancer drugs research. Studies have reported the production of taxol by *Pestalotiopsis versicolor*, and an apoptotic experiment using the isolated fungal taxol demonstrated high cytotoxic action against tested human cancer cells in in vitro culture, demonstrating that an increase in taxol concentration causes more cell death.

In this review we have referred a total of sixty-one research papers during the year 2010 to 2022 and tried to highlight the significance of fungal endophytes and the bioactive substances produced by them in cancer treatment.

Keywords - fungal endophytes, secondary metabolites, anti-cancer compounds.

1. INTRODUCTION

a) cancer

Uncontrolled and unchecked division of cells leads to cancer. Cancer can occur almost anywhere in the body, and these cells may form tumors. Based on the organ or tissue where the tumors form, more than 100 types of cancer have been reported (NIH). According to the statistics of the American Cancer Society, in 2022, there will be an estimated 1.9 million new cancer cases diagnosed and 609,360 cancer deaths in the United States. It is anticipated that by 2050, the annual death toll due to cancer will rise to 17.5 million [1]. The primary cause of the increase in cancer incidence can be attributed to the modern lifestyle, which includes drug use, smoking, exposure to toxins, environmental pollution, nutrition, drunkenness, viral infections, etc. Although the use of genomic, proteomic, and bioinformatics approaches will assist to grasp the intricacy of cancer, the scientific community has not yet found a complete cure for the disease [2].

For the treatment of cancer, numerous medicines have been proposed, many of which contain compounds derived from plants. Plants are a rich source of natural compounds that may have chemoprotective properties against cancer

and still hold great promise for the development of novel medications. Currently available plant-based anticancer medications are classified into four categories: vinca alkaloids (vinblastine, vincristine, and vindesine), epipodophyllotoxins (etoposide and teniposide), taxanes (paclitaxel and docetaxel), and camptothecin derivatives (camptotecin and irinotecan) [3,4].

b) Endophytic fungi and cancer

The term "endophyte" refers to bacteria and fungi that colonize internal plant tissue at some point in their life cycle without causing any pathogenic symptoms [5,6,7]. They can be found in root complexes and other parts of the host plant, and their host ranges from simple thallophytes to complex angiosperms [8]. Endophytes have been found in every plant studied to date. Endophytic fungi are considered an important component of microbial diversity. These endosymbionts contribute to the modification of ecosystem properties. They alter the growth and development of the host plant and increase its productivity and resistance to biotic and abiotic stress [9]. Fungal endophytes play a crucial role in biotechnology processes such as enzyme production, biocontrol plant growth promotion bioremediation, biodegradation, biotransformation, biosynthesis, and nutrient cycling. They are resources for novel biological compounds too [10].

The cytotoxic and anti- cancer activities of endophytic fungi were deemed the most important topics to the scientific community. Several fungal endophytic strains were reported for the production of taxol, vincristine, etoposide, irinotecan, topotecan, vinblastine, etc., and also new compounds that are in clinical use for the treatment of several human cancers [11]. Paclitaxel (commonly called "taxol"), derived from the Pacific yew tree *(Taxus brevifolia*), showed broad-spectrum activity against the treatment of several types of tumors, including breast, ovary, and Kaposi's sarcoma. *Taxomyces andreanae* is an endophytic fungus isolated from the barks of *Taxus brevifolia* that also produces taxol and related compounds [12]. Studies showed fungal endophytes from Taxus and non-Taxus plants were able to synthesize taxol and taxol-like derivatives. The major endophytic genera included in the production of taxol and its analogues belong to *Alternaria, Aspergillus, Botryodiplodia, Botrytis, Cladosporium, Ectostroma, Fusarium, Metarhizium, Monochaetia, Mucor, Ozonium, Papulaspora, Periconia, Pestalotia, Pestalotiopsis, Phyllosticta, Pithomyces,* and *Taxomyces* [11].

Similarly, podophyllotoxin is a plant-derived anticancer agent commonly used for the treatment of lung cancer and testicular cancer [13]. Fungal endophytes yielded podophyllotoxin. The common podophyllotoxin-producing endophytes include *Fusarium sp., Aspergillus sp., Mucor sp*., etc. Another fungal endophyte derived anti cancerous compound is camptothecin. The common camptothecin-producing endophytes includes *Fusarium sp,Phomopsis sp, Aspergillus sp, and Trichoderma sp.* Vinblastine, vincristine, vinleunosine, and vinrosidine are vinca alkoloids used to reduce the number of white blood cells in acute lymphoblastic leukaemia and nephroblastoma. endophytic fungi such as *Alternaria sp., Fusarium sp* are found to produce vinca compounds [11].

c) Cytotoxic activity of endophytic fungi

Studies by El-Kassem et al. (2019) [14]. demonstrated that endophytic fungal extracts of *Emericella. nidulans* (RSSSS-22, RSL24), *Fusarium oxysporum* SML-41, and *Penicillium sp.* RSL-43 exhibited strong cytotoxic activity against human breast cancer cell lines (MCF-7) with IC50 values of 10.8, 11.0, 12.5, and 13.7 g/ml, respectively; *E. nidulans* (RSSSSSS-22, RSL-43), and *Fusarium* exhibited a potent cytotoxic effect on human liver cancer cell lines (HEP-G2) with IC50 values of 14.8, 20.3, and 24.0 g/ml respectively. Furthermore, an ethyl acetate extract of *Colletotrichum gloeosporioides* showed potential cytotoxicity against the cancer cell lines HCT116, HeLa, and HepG2 with IC50 values of 76.59 g/mL, 176.20 g/mL, and 1750.70 g/mL, respectively [15]. Studies conducted by Sunkar et al. (2017) [16] on Hep2 cells showed that a fungal extract had a time- and dose-dependent effect on the cytotoxicity of cells.

Endophytic fungal extracts of *Ageratum myriadenia, Palicourea tetraphylla, Piptadenia adiantoides,* and *Trixis vauthieri* from Brazil were found to be cytotoxic to human cancer cell lines (ACC-62 (melanoma), MCF7 (breast), and TK-10 (renal), with IC 50 values ranging from >0.2 to 25 g/mL (Ros). Likewise, marine plant-associated fungal endophytes were potentially cytotoxic to M059J (brain), PC3 (prostate), DLD-1 (colon), MDAMD231 (breast), NCIH1299 (lung), B16F10 (melanoma), PC12 (pheochromocytoma), and Detroit 551 (fibroblast) cell lines [17]. Bacopa plant endophytes were cytotoxic to the HCT-116, MCF-7, PC-3, and A-549 cell lines, and extracts were more potent against the HCT-116 cells than the MCF-7, PC-3, and A-549 cell lines [18]. Human ductal breast epithelial tumour cell lines (T47D) and human colon carcinoma cell lines (WiDr) were significantly inhibited by endophytic

fungi derived from *Piper crocatum*, and MCF-7 and HCT116 cell lines were also inhibited by endophytic fungal extracts [19,20]. The growth of cells (human MCF7 breast cancer cell lines and A549 human lung cancer cell lines) was inhibited by AgNP nanoparticles made from endophytic fungus at an IC50 concentration of 100 g/mL[21].

d) Anti-cancer compounds from endophytic fungi

The secondary metabolites of endophytes have been used for the production of therapeutically important compounds. Endophytic fungi may play a leading role in the discovery of anticancer drugs at an affordable cost. Therefore, exploring and exploiting metabolites from endophytes provides an excellent source for the discovery of drugs against deadly human diseases. Research on anticancer properties and compound isolation from fungal endophytes has a significant impact (Table -1).

Table 1: Some anti-cancer compounds from fungal endophytes

Name of endophyte	Host plant with segment where endophyte isolated	Name of compound	Tested cell line and activity	Reference
Penicillium melinii and *Penicillium janthinellum*	Roots of *Panax ginseng*	Ginsenocin, methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate, 3,4,5-trimethyl-1,2-benzenediol, penicillic acid, mannitol, ergosterol, and ergosterol peroxide and brefeldin A	• human cancer cell lines-(MKN45, LOVO, A549, MDA-MB-435, HepG2, and HL-60) • IC_{50} values ranging from 0.49 to 7.46 μg/ml.	[22]
Aspergillus tubingensis	Radix of *Pongamia pinnata*	Rubasperone D, Rubasperone E, Rubasperone F, Rubasperone G, Naphtho-G-Pyrones - TMC 256 A1, Rubrofusarin B, Fonsecin, and Flavasperone	• Tumor cell lines of MCF-7, MDA-MB435, Hep3B, Huh7, SNB19, and U87 MG • C50 values between 19.92 and 47.98 mm	[23]
Talaromyces flavus	*Mangroves*	Talaperoxides A–D	• Human cancer cell lines MCF-7, MDA-MB-435, HepG2, HeLa, and PC-3. • IC50 values between 0.70 and 2.78 μg/mL.	[24]
Trichoderma harzianum	Leaves of *Cola nitida.*	4'-hydroxy-deacetyl-18-deoxycytochalasin H, deacetyl-18-deoxycytochalasin H, 18-deoxycytochalasin H	• Cytotoxic activity against the murine lymphoma (L5178Y) cell line and against human ovarian cancer (A2780 sens and A2780 CisR) cell lines • IC50 0.19-6.97 μM).	[25]
Penicillium brefeldianum.	Rhizome of *Pinellia ternate*	Indoloditerpene, 6,7-dehydropaxilline, spirotryprostatin F, N-demethylmelearoride A	• Cytotoxicities against HepG2 and MDA-MB-231 cells with IC50 values of 14.1 mmol/L and 35.5 mmol/L, respectively.	[26]
Lasiodiplodia theobromae	Leaf of the marine mangrove *A. ilicifolius*,	Chlorinated preussomerins, Chloropreussomerins A and B (1 and 2), Preussomerin analogues, 3−11	• Cytotoxicity against A549 and MCF-7 human cancer cell lines, with IC50 values ranging from 5.9 to 8.9 μM	[27]

Phomopsis sp.	*Mangroves*	Phomopchalasins D–O (1–3, 5–12, And 14)	• Cytotoxicity against human cancer cell line MDA-MB-435 with IC50 values ranging from 0.2 to 8.2 μM.	[28]
Epicoccum nigrum	Leaves Of *Entada Abyssinica*	Beauvericin, parahydroxybenzaldehyde, indole-3-carboxylic acid and quinizari	• LC_{50} values ranged from 40.42 to 86.56 μg/ml, 31.87 to 86.57 μg/ml and 21.59 to 67.27 μg/ml on Vero cells, THP-1 and RAW 264.7 respectively.	[29]
Coniochaeta sp	*Ageratina adenophora*	Phomoxanthone A and Penialidin A	• Cytotoxicity in mouse embryo fibroblasts cell line Balb/c3T3.	[30]
Penicillium chrysogenum	Marine Red Algal Species of the Genus *Laurencia*.	penicisteroids A and B	• Activity against the tumor cell lines HeLa, SW1990, and NCI-H460 with the IC50 of 15, 31, and 40 lg/mL	[31]
Fusarium chlamydosporium	Leaves of *Anvillea Garcinii*	Fusarithioamide A 2(2-aminopropanamido)-N-(1-hydroxy-3-mercaptopropyl) benzamide, 4), 1-O-acetylglycerol, 8-acetylneosolaniol, and ergosta-7,22-diene-3b,5a,6b-triol	• Potent and selective activity towards BT-549 and SKOV-3 cell lines with IC50 values of 0.4 and 0.8 mM,	[32]
Cladosporium sp	Leaves of *Rauwolfia Serpentina*	Anhydrofusarubin and methyl ether of fusarubin	• Human leukemia cells (K-562) showed potential cytotoxicity with IC_{50} values of 3.97 μg/mL and 3.58 μg/mL	[33]
Eutypella scoparia	Leaf of *Hevea Brasiliensis*	Cytochalasin derivative, scoparasin C (**1**), four cytochalasins (**2**–**5**), four pimarane diterpenes (**6**–**9**) and two chromene derivatives (**10** and **11**)	• Compounds **1**, **3**, **4** and **7** were strongly active against Vero cell lines with IC_{50} values of 1.19, 0.04, 1.01 and 2.50 μM, respectively. • compound **3** displayed potent cytotoxic activity towards KB-oral cavity cancer cell lines with the IC_{50} value of 2.46 μM.	[34]

Pleosporales sp.	Tuberous roots *of Siraitia grosvenorii*	Pleospyrones A-E, congener	• Colon cancer cells (HCT-116), liver hepatocellular carcinoma cells (HepG2), gastric cancer cells (BGC-823), nonsmall-cell lung carcinoma cells (NCI-H1650), and medulloblastoma cells (Daoy). • IC_{50} values of 1.26~47.5 μM.	[35]
Pleosporales sp	Pedicel of *Mahonia fortunei.*	Heptaketides, pleosporalins A–F an d pleosporalin G	• Against A549, SMMC-721, and MDA-MB-231 cancer cell lines. • Compound **7** showed moderate cytotoxicity against MDA-MB-231 with an IC_{50} of 22.4 ± 1.1 μM	[36]
Talaromyces flavus.	*Sonneratia apetala*	Talaperoxides A–D, teperoxide B	• Human cancer cell lines MCF-7, MDA-MB-435, HepG2, HeLa, and PC-3. Compounds 2 and 4 showed IC50 values between 0.70 and 2.78 μg/mL	[37]
Chaetomium globosum	*Ginkgo biloba*	Chaetoglobosins, C, E, F, Fex, 20-dihydrochaetoglobosin A	• Cytotoxic activities against HCT116 human colon cancer cells • IC50 values ranging from 3.15 to 8.44 μM, • Cytotoxic activity	[38]
Talaromyces sp	Stem bark of *Kandelia candel*	7-epiaustdiol (***1***) and 8-O-methylepiaustdiol (***2***), stemphyperylenol (***3***), skyrin (***4***), secalonic acid A (***5***), emodin (***6***), and norlichexanthone (***7***)	• Cytotoxicity against all tested cell lines.	[39]
estalotiopsis microspora	*Drepanocarpus lunatus*	Pestalotioprolides C (2), D−H(4−8), and 7-O-methylnigrosporolide,pestalotioprolide B (1), seiricuprolide (9), nigrosporolide (10), and 4,7dihydroxy-13-tetradeca-2,5,8-trienolide (11),	• Cytotoxicity against the murine lymphoma cell line L5178Y with IC50 values of 0.7, 5.6, 3.4, • and 3.9 μM, against the human ovarian cancer cell line A2780 with an IC50 value of 1.2 μM.	[40]
Trichoderma harzianum	*Physalis angulate*	Trichodestruxins A, destruxin E2 chlorohydrin (5) and destruxin A2	• Cytotoxicity against HT-29, A549, and/or P388 cell lines with IC50 values of 0.7–19.1 μM.	[41]

Penicillium brocae	*Avicennia marina.*	Brocazines A−F	• Cell lines, Du145, Hela, HepG2, MCF-7, NCI-H460, SGC-7901, SW1990, SW480, and U251 • IC50 values ranging from 0.89 to 9.0 μM	[42]
Phoma macrostoma	*Glycyrrhiza glabra*	Macrophin (1), rosellisin (2), 2-(2-hydroxy-5-6-methoxy-3-methylene-1,4-benzodioxin-2(3H)-one (3), and methoxyphenoxyacrylic acid	• Cytotoxic activity against the MDA-MB-231, T47D, MCF-7, and MIAPaCa-2 cancer • Cell lines with IC50 values of 14.8, 8.12, 13.0, and 0.9 μM, respectively.	[43]
Phialophora mustea	*Crocus sativus.*	Phialomustin A-D	• Cytotoxic potential against the human breast cancer cell line, T47D with an IC50 of 1 μM.	[44]
Mycoleptodiscus sp.	*Desmotes incomparabilis*	Mycoleptodiscin A (1) and mycoleptodiscin B	• Inhibiting the growth of cancer cell lines with IC50 values in the range 0.60−0.78 μM.	[45]
Curvularia verruculosa	Leaves of *Cathranthus roseus*.	Vinblastine analogous	• Cytotoxicity effect on HeLa cell line and it depicted a higher activity with IC50-8.5 μg/mL	[46]
Aspergillus niger	*Taxus baccata.*	Lovastatin	• Human cancer cells (HeLa and HepG2)	[47]
Alternaria alternata and Fusarium species	*Mappia foetida*	Camptothecin	• Cervical carcinoma (HeLa), breast carcinoma (MCF-7), non-small cell lung carcinoma (H1975), and hepatocellular carcinoma cell line (Hep G2)	[48]
Phoma sp.	-	α-pyrone derivatives	• IC50 values in the range 0.52–9.85 μM.	[49]

A. niger and A. fumigatus	*Cinnamomum mollissimum*	Hydroxyramulosin	• Cytotoxic against murine leukemia cells (IC_{50} 2.10 μg/mL).	[50]
Allantophomopsis lycopodina	-	Allantopyrone A	-	[51]
Aspergillus sp., Nigrospora sphaerica, Talaromyces purpureogenus, and Talaromyces stipitatus	*Argassum muticum*	Quinoline, indole, 2,4-bis(1,1-dimethylethyl) phenol, and hexadecenoic acid	• HeLa (cervical adenocarcinoma), MCF-7 (breast adenocarcinoma), Hep G2 (hepatocellular carcinoma), A-549 (lung carcinoma), A-431 (skin/epidermis carcinoma), and LN-229 (glioblastoma). • HeLa cells were most vulnerable to ASE treatment with an IC50 value of 24 ± 2 μg/ml.	[52]
Bipolaris sorokiniana	*Pogostemon cablin*	Isocochlioquinones D–E (1–2) and cochlioquinones G–H (3–4)	• Cytotoxic activities against MCF-7, NCI-H460, SF-268 and HepG-2 tumor cell lines	[53]
Phoma bellidis,	Leave tissue of *Tricyrtis maculate*	Bellidisins A-D, pinolidoxin (5), 5,6-epoxypinolidoxin (6), and 2-epi-herbarumin II (7)	• Human cancer cell lines HL-60, A549, SMMC-7721, MCF-7, and SW480. • IC50 value ranged from 3.40 to 15.25 μM, which is stronger than cisplatin (4.86–27.70 μM).	[54]
Pseudolagarobasidium acaciicola	*Bruguiera gymnorrhiza*	Merulin A and merulin D	• Cytotoxic activity (IC50 0.28 μM), and selectively exhibited activity against the HL-60 cell line.	[55]
Perenniporia tephropora	*Taxus chinensis var. mairei,*	Sesquiterpenoid, perenniporin A (1), ergosterol (2), rel-(+)-(2aR,5R,5aR,8S,8aS,8bR)-decahydro-2,2,5,8-tetramethyl-2H-naphtho[1,8-bc]genfuran-5-ol (3), and albicanol (4).	• Cytotoxic activity against three human cancer cell lines (HeLa, SMMC-7721, and PANC-1). EPT demonstrated significant cytotoxicity with IC50 values ranging from 2 to 15 μg/mL. • Compound 2 was the most cytotoxic constituent against the tested cell lines	[56]

			with IC50 values of 1.16, 11.63, and 11.80 μg/mL, respectively • While compounds 1, 3, and 4 exhibited moderate cytotoxicity with IC50 values ranging from 6 to 58 μg/mL	
Pestalotiopsis clavispora KJ677242, P. mangiferae	*Dendrobium officinale*	(4S,6S)-6-[(1S,2R)-1, 2-dihydroxybutyl]-4-hydroxy-4-methoxytetrahydro-2H-pyran-2-one (1), (6S,2E)-6-hydroxy-3-methoxy-5-oxodec-2-enoic acid (2), together with three known compounds, LL-P880γ (3), LL-P880α (4), and Ergosta-5,7,22-trien-3b-ol (5	• Compounds **1**–**4** possessed notable cytotoxcities against human cancer cell lines of HL-60 cells with the IC_{50} values of below 100 μM. • Compounds **1**, **2**, **4** and **5** showed strong cytotoxities on the LOVO cell line with the IC_{50} values were lower than 100 μM	[57]
Periconia sp.	-	Periconiasins A–C	• Cytotoxicity against human HCT-8 cancer cells.	[58]
Stachybotrys chartarum	*Pinellia ternate*	Stachybochartins A–G (**1–7**)	• MDA-MB-231 breast cancer cells and U-2OS osteosarcoma cells, with IC_{50} values ranging from 4.5 to 21.7 μM	[59]
Aspergillus oryzae	*P. polyphylla var. yunnanensis,*	Oryzaeins A–D(1-4),	• IC50 values in the range of 2.8–8.8 μM.	[60]
Marine-derived Mangrove Endophyte	Marine-derived mangrove	Marinamide (**1**) and its methyl ester (**2**), pyrrolyl 1-isoquinolone alkaloids	• Cytotoxic activity against HepG2, 95-D, MGC832 and HeLa tumour cell lines.	[61]

II. CONCLUSION

Endophytic fungi provide a boost to the synthesis of novel bioactive chemicals, but these efforts are hampered by low yields, an absence of exact knowledge of the biochemical interactions between fungi and plants, increased contamination. The cytotoxic activity of endophytic fungi was a well-explored area of research. There are many compounds extracted from these endophytes with significant anti-cancer properties. Production of anti-cancer compounds and novel drugs will help reduce the cost of medicine.

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