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Research article

Cytotoxic activities of endophytic fungi isolated from central region of Madhya Pradesh

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Abstract

A total of 496 endophytes were isolated from leaves of plants from the central region of Madhya Pradesh. Among them extract RKAAC11 of *Asclepias curassavica* and RKACQ13 of *Alternaria alternata* showed good cytotoxic activity. Methanolic extract against MCF-7 cell line showed significantly more cytotoxicity (IC_{50}: 31.53±1.04 µg/ml) than A549 cell line (IC_{50}: 55.26±1.41 µg/ml) ($P \leq 0.05$) as compared to paclitaxel as standard. Whereas, chloroform extract against MCF-7 cell line showed significantly potent cytotoxicity (IC_{50}: 15.63±0.65 µg/ml) than A549 cell line (IC_{50}: 19.56±1.25 µg/ml) ($P \leq 0.05$) as compared to reference compound paclitaxel. On the other hand, methanolic extract of *Alternaria alternata* (RKACQ13) showed dose dependent cytotoxicity against MCF-7 and A549 cell line. The MCF-7 cell line showed significantly more cytotoxicity (IC_{50}: 43.23±0.77 µg/ml) than A549 cell line (IC_{50}:46.33±0.9 µg/ml) ($P \leq 0.05$) as compared to reference compound paclitaxel with an IC_{50} value of 3.0 µg/ml and 4.9 µg/ml for MCF-7 and A549 cell line respectively. Results indicate the potential for production of bioactive agents from endophytes of the subtropical flora.

Key words: Cytotoxicity, Endophytic fungi, *Cissus quadrangularis, Asclepias curassavica*.

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1. Introduction

Endophytes are microbes inhabited in internal tissues of plants without causing any immediate, overt adverse effect [1]. Fungal endophytes are diverse group of microorganisms that colonize plants in the Arctic [2] and Antarctic [3] and in geothermal soils [4], rainforest [5], desert [6], ocean [7], mangrove swamps [8] and coastal forests [9]. A number of endophytes lived into each plant species [10]. Fungal endophytes harbored a broad variety of bioactive secondary metabolites with antibacterial, antiviral, anticancer, antioxidants, antidiabetic and immuno-suppressive activities [11]. Their study is expected to become an important component in the production of new natural bioactive products. Only a few studies of the endophytic fungi of these plants have been conducted. The current

study was conducted to isolate and screen endophytic fungi with cytotoxic activities from medicinal plants collected from the central region of Madhya Pradesh.

2. Materials and Methods

Source of endophytic fungi

Survey and collection of plant species leaves samples were obtained from the central region of Madhya Pradesh during the rainy season of 2011 and 2012 (July to September), coordinated between 22.53' N to 79°56' E. The plant's leaves were brought to the laboratory in sterile bags after surface cleaning with distilled water; kept on ice for transportation and stored at 4ºC for further acquisition. The plants were identified as per the description given in Ethno-medicinal diversity used by tribal's of central India [12]. The collected leaves samples were identified by faculty member and deposited at herbarium, Department of Botany, Govt. M.V.M., Bhopal and provided a specimen no: Bot/02/2012.

Isolation of endophytic fungi

Endophytic fungi were isolated from 10 plant leaf samples i.e. *Cissus quadrangularis* Linn (Harjor), *Asclepias curassavica* L. (Kakatundi), *Cassia fistula* Linn (Roxb.) (Amaltas), *Carissa carandas* Linn. (Karaunda), *Ocimum basilicum* Linn. (Common sweet basil), *Leucas aspara* (wiild) Link (Chhota halkusa), *Nerium oleander* Linn (Kaner), *Terminalia arjuna* Wight and Arn. (Kahu), *Vitex negundo* Linn. (Nirgundi) and *Plumeria rubra* Linn. (Golenci) as per method described by Strobel *et al*., [13] but with minor modifications. The collected leaf samples were rinsed in running water and air dried. Each sample was disinfected with 70% ethanol for 1 min followed by immersion in sodium hypochlorite (3.5%) for 3 minutes and again in 70% ethanol for 30 seconds along with surface sterilization with 0.01% mercuric chloride solution to remove contaminates. The samples were then rinsed thrice in sterile distilled water and blotted-dry on sterile blotting paper under laminar air flow [14, 15, 16]. Each plant leaves samples were cut aseptically into 5 x 5mm size and inner tissues were placed on the surface of Potato dextrose agar (PDA) media supplemented with penicillin G (100 U/ml) and streptomycin (100 µg/ml) (Bills *et al.,* 2002). Control plates were used for effective surface sterilization of leaf imprints on the agar surface [17]. The plates were incubated at 27 ± 2°C for 2-3 weeks. Pure cultures were then transferred to PDA plates free of antibiotics and maintained. The endophytes were cultivated for 14 days on PDA plates at 28°C for investigations of biological activity.

Extraction of fungal cultures

Harvesting of 21 days old Potato dextrose broth culture was done by passing through four layers of muslin cloth to separate the mycelial mat from the culture filtrate under aseptic conditions, centrifuged at 4000 rpm for 15-20 min (Kjer, 2006). Supernatant was collected and syringe filtered using 0.2 µm sterile Whatman microfilters (USA) [18] and vacuum concentrator (Eppendorf). The resultant extract was dissolved in 1 ml of dimethyl-sulfoxide (DMSO) (Sigma) and used for primary of cytotoxic screening.

Screening of solvents for crude endophytic fungal extracts

The crude extracts, which showed good cytotoxic activity, were further subjected to extraction using various solvents. For extraction of secondary metabolites from culture filtrates different solvents were screened i.e., hexane, toluene, chloroform, ethyl acetate, acetone, ethanol and

methanol (non-polar to polar). The crude extract was able to get dissolved in different solvents according to their polarities (1:1 v/v). Different solvent fractions were concentrated on vacuum concentrator (Eppendorf). The yields of different solvent fractions of crude extract were determined.

Cytotoxic activity

Human breast cancer cell line, MCF-7 (Passage No. 23) and Human lung cancer cell line, A549 (Passage No. 16) procured from the National centre for cell science (NCCS), Pune. These cell lines were maintained as per instructions provided by the supplier. The MCF-7 cell line was cultured in MEM (HImedia), whereas A549 in Ham's F12K media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Himedia).

Cultures were maintained in a humidified incubator at 37°C in an atmosphere of 5% CO2. Cytotoxicity of extracts at various concentrations (12.5-200 µg/ml) was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann, 1983 [19] but with minor modification, following 48 h of incubation. Assay plates were read using a spectrophotometer at 570 nm. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC50) was determined. Paclitaxel (Himedia) was used as standard and all data are expressed as means ± SD. All readings were taken in triplicate.

3. Results and discussion

A total of 496 colonies were isolated from 200 segments of 10 diverse plant species leaves samples from the central region of Madhya Pradesh (Table 1). They belonged to 20 species, where ascomycetes (43 colonies, 02 genera), coelomycetes (110 colonies, 4 genera), hyphomycetes (292 colonies, 9 genera) and sterile mycelia (51 colonies) (Figure 1). The isolate *Alternaria alternata* was most prominent followed by *Aspergillus terreus* and *Pestalotiopsis* sp. isolated from four to six different plant species and *Phoma* sp. was the second most isolated species. Among 10 plants, *Cissus quadrangularis* yielded the greatest fungal diversity and 11 different endophytic fungal taxa were obtained from its leaves.

Among the 85 fungal extract, RKAAC11 isolated from *Asclepias curassavica* and RKACQ13 *from Cissus quadrangularis* plant leaves exhibit good cytotoxic activity that was able to get dissolved in chloroform, methanol and methanol solvents respectively.

Study of morphological and cultural characteristics of fungal strain RKAAC11 was performed on fresh Potato sucrose agar at 25 ± 2 ºC attained a diameter 4 cm within 5 days which showed colonies producing white fluffy mycelium. The mycelia of the isolates were delicate, white and tinge red color. It showed sparse to abundant and margins were slightly lobed or smooth. Conidiogenous cells bearing micro- and macroconidia were monophialides type. Macroconidia were fusiform, thin walled, slightly curved and pointed at the tip. Microconidia were abundant, singly, oval, mostly non-septate, ellipsoidal or cylindrical, straight or curved (crescent shape). The size of the macroconidia ranged from 20.27-40.50 and 5.00-6.75 µm. Chlamydospores are terminal or intercalary, hyaline, smooth or rough-walled, 5-13 µm and identified as *Fusarium oxysporum*.

Morphology and cultural characteristics of fungal strain RKACQ13 was performed on Gause G-1 agar medium at 25 ± 2 ºC attained a diameter 3.5 cm within 5 days, which produced olivaceous dark blackish

green coloured colony. Hyphae were colourless and conidiophores arose singly or in small groups. They were smooth in appearances, often branched, straight and flexous. Conidia were obclavate and ovoid in shape. It has observed with several transverse and longitudinal or oblique septa and pale brown, smooth-walled cylindrical beak and identified as *Alternaria alternata*.

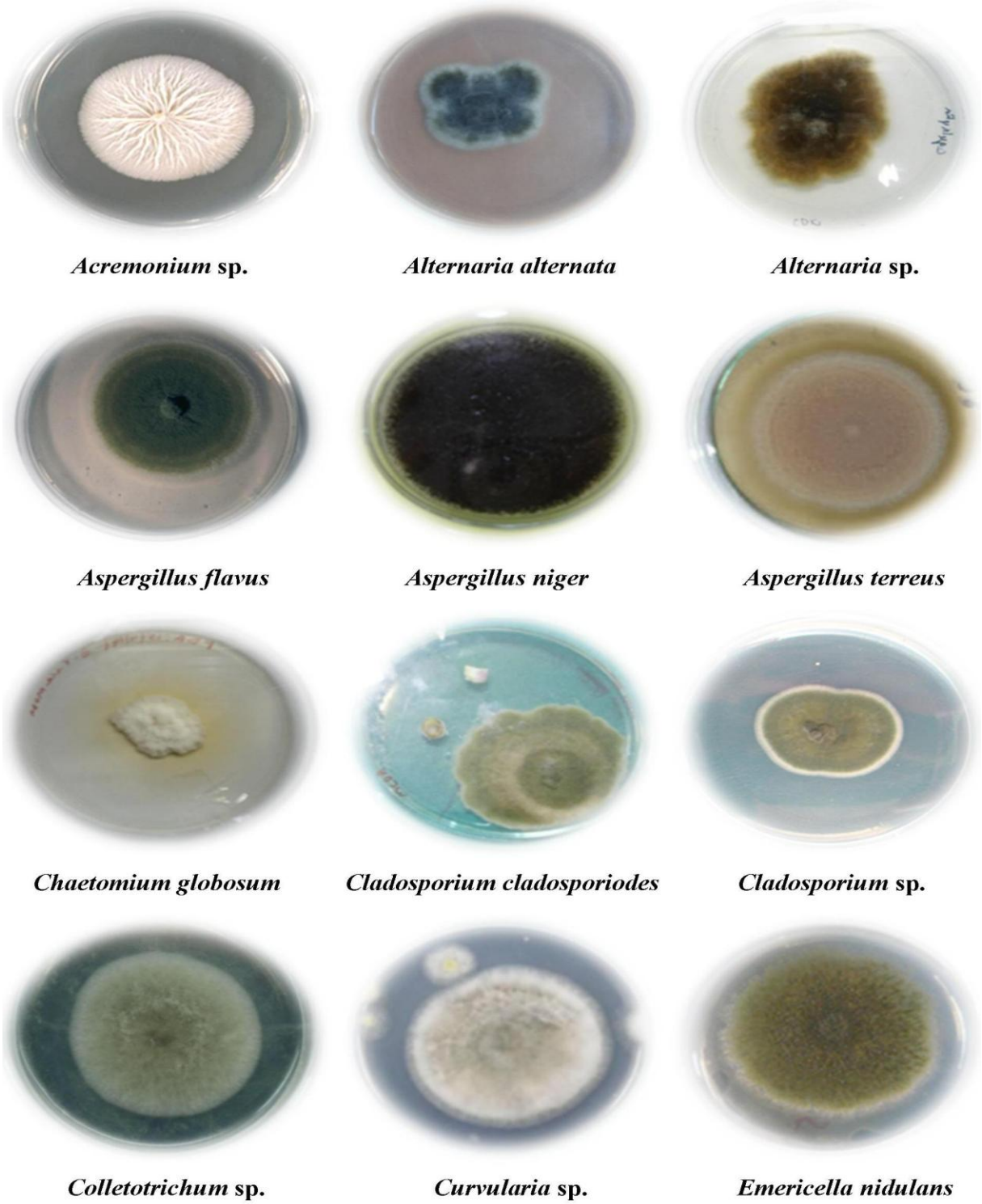
Table 1. Biodiversity of endophytic fungi in leaves of medical plants from central region of Madhya Pradesh.

Endophytic fungi	CQ	AC	CF	CC	OB	LA	NO	TA	VN	PR	Total
Ascomycetes											
Chaetomium globosum	3	-	2	3	-	4	-	7	4	-	23
Emericella nidulans	-	-	6	-	3	2	-	-	9	-	20
Coelomycetes											
***Colletotrichum* sp.**	-	14	-	4	-	-	10	-	-	-	28
***Pestalotiopsis* sp.**	2	-	-	9	5	-	-	20	-	-	36
***Phoma* sp.**	1	11	3	4	6	7	-	-	-	-	32
***Phomopsis* sp.**	-	-	10	-	4	-	-	-	-	-	14
Hypomycetes											
***Acremonium* sp.**	3	-	4	5	-	8	-	-	-	-	20
***Alternaria* sp.**	-	-	-	-	-	-	8	-	5	9	22
Alternaria alternata	9	17	-	5	8	-	-	-	-		39
Aspergillus niger	5	7	6	-	-	-	-	-	-	-	18
Aspergillus flavus	-	4	-	11	3	-	-	-	4	3	25
Aspergillus terreus	4	6	16	2	-	5	4	-	-	-	37
***Cladosporium* sp.**	-	-	-	-	-	-	-	-	6	-	6
Cladosporium cladosporioides	15	-	-	-	-	-	-	5	-	-	20
***Curvularia* sp.**	-	-	-	-	4	-	9	-	-	2	15
Epicoccum nigram	12	-	-	-	-	-	-	-	-	-	12
Fusarium solani	-	-	2	3	-	3	11	-	4	-	23
Fusarium oxysporum	4	4	-	6	-	-	-	-	-	-	14
***Penicillium* sp.**	6	3	3	-	-	-	-	-	4	-	16
***Trichoderma* sp.**	-	6	4	6	-	-	3	3	3	-	25
Sterile mycelia	7	5	3	10	5	4	5	2	3	7	51
Total no. of isolates	**71**	**77**	**59**	**68**	**38**	**33**	**50**	**37**	**42**	**21**	496

CQ-*Cissus quadrangularis* Linn, AC-*Asclepias curassavica* L., CF-*Cassia fistula* Linn, CC-*Carissa carandas* Linn, OB- *Ocimum basilicum* Linn., LA- *Leucas aspara* (wiild) Link, NO-*Nerium oleander* Linn, TA-*Terminalia arjuna*, VN-*Vitex nirgundo* Linn, PR-*Plumeria rubra* Linn.

Methanolic and chloroform extract of *Fusarium oxysporum* (RKAAC11) also exhibited dose dependent cytotoxicity against MCF-7 and A549 cell line. The methanolic extract showed 33.8, 42.64, 66.8, 83 and 85.77% cytotoxicity against MCF-7 cell line, whereas, A549 cell line exhibited 24.74, 35.8, 46.8, 79.7 and

81.8% cytotoxicity at 12.5, 25, 50, 100 and 200µg/ml concentration respectively. The MCF-7 cell line showed significantly more cytotoxicity (IC_{50}: 31.53±1.04 µg/ml) than A549 cell line (IC_{50}: 55.26±1.41 µg/ml) ($P \leq 0.05$) as compared to paclitaxel as standard (Table 2, Figure 2).



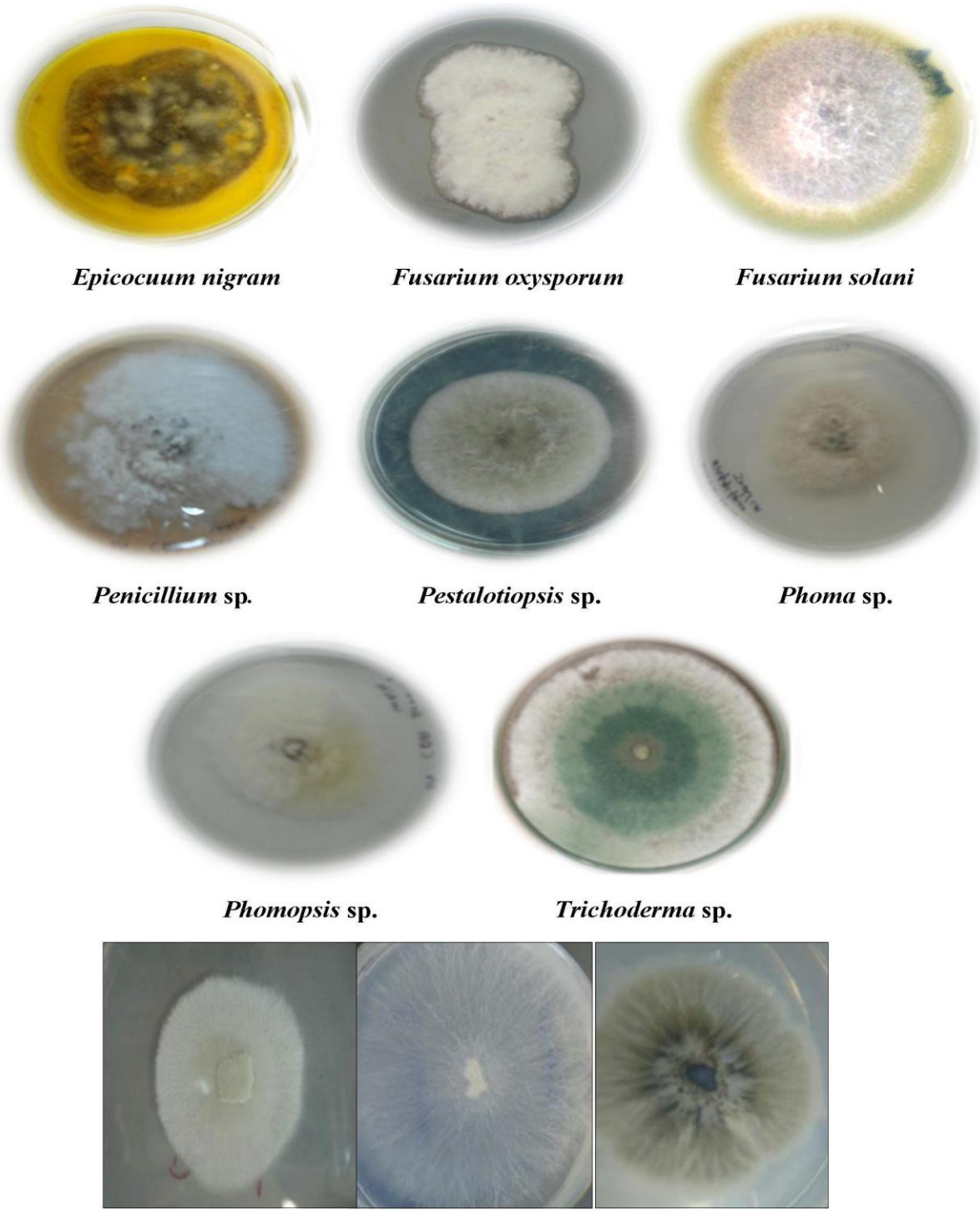


Figure 1. Endophytic fungi isolated from leaf sample of plants from central region of Madhya Pradesh

Similarly, chloroform extract showed 42.74, 67.8, 73.6, 81.9 and 87.7% cytotoxicity against MCF-7 cell line, whereas, A549 cell line exhibit 37.77, 59.94, 68.8 76.6 and 79.67% inhibition for 12.5, 25, 50, 100 and 200µg/ml concentration respectively. The MCF-7 cell line showed significantly potent

cytotoxicity (IC_{50}: 15.63±0.65 µg/ml) than A549 cell line (IC_{50}: 19.56±1.25 µg/ml) ($P \leq 0.05$) as compared to reference compound paclitaxel (Table 2, Figure 3). These values were within the cutoff point of the National Cancer Institute (NCI) criteria for cytotoxicity (IC50<30 µg/ml) in the screening of crude extracts [20].

Table 2. IC_{50} concentration of crude solvent fractions against cancer cell line

Endophytic fungi	Fraction	Cell line	Concent. (µg/ml)	Cell viability (%)	Cytotoxicity (%)	IC_{50} value (µg/ml)
***Fusarium oxysporum* (RKAAC11)**	**Methanol**	**MCF-7**	**12.5**	66.2±1.311	**33.8**	**31.53±1.04**
			25	57.36±1.28	**42.64**	
			50	33.2±0.721	**66.8**	
			100	17±0.7	**83**	
			200	14.23±0.68	**85.77**	
		A549	**12.5**	75.26±0.642	**24.74**	**55.26±1.41**
			25	64.2±1.113	**35.8**	
			50	53.2±0.8	**46.8**	
			100	20.3±1.276	**79.7**	
			200	18.2±1.311	**81.8**	
	Chloroform	**MCF-7**	**12.5**	57.26±1.301	**42.74**	**15.63±0.65**
			25	32.2±0.721	**67.8**	
			50	26.4±1.178	**73.6**	
			100	18.1±0.754	**81.9**	
			200	12.3±0.655	**87.7**	
		A549	**12.5**	62.23±0.776	**37.77**	**19.56±1.25**
			25	40.06±1.301	**59.94**	
			50	31.2±0.916	**68.8**	
			100	23.4±1.153	**76.6**	
			200	20.33±1.222	**79.67**	
***Alternaria alternata* (RKACQ13)**	**Methanol**	**MCF-7**	**12.5**	75.13±1.703	**24.87**	**43.23±0.77**
			25	63.3±0.7	**36.7**	
			50	48.2±1.41	**51.8**	
			100	27.43±1.209	**72.57**	
			200	24.23±1.078	**75.77**	
		A549	**12.5**	78.5±1.609	**21.5**	**46.33±0.9**
			25	67.33±1.222	**32.67**	
			50	47.06±1.01	**52.94**	
			100	32.43±0.986	**67.54**	
			200	29.16±0.96	**70.84**	
Paclitaxel (+) control		**MCF-7**	**IC_{50} value 3.0±0.464 µg/ml**			
		A549	**IC_{50} value 4.9±1.115 µg/ml**			

On the other hand, methanolic extract of *Alternaria alternata* (RKACQ13) showed dose dependent cytotoxicity against MCF-7 and A549 cell line. The cytotoxicity for the MCF-7 cell line was observed at 24.87, 36.7, 51.8, 72.57 and 75.77% for 12.5, 25, 50, 100 and 200µg/ml concentration respectively. Similarly, the cytotoxicity for A549 cell line was observed at 21.5, 32.67, 52.94, 67.54 and 70.84% for 12.5, 25, 50, 100 and 200µg/ml concentration respectively.

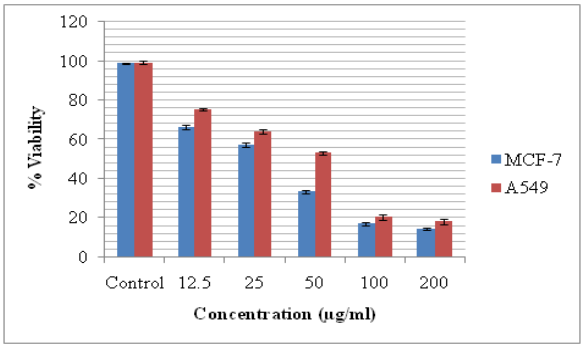


Figure 2. Effect of methanol fractions of *Fusarium oxysporum* (RKAAC11) on percentage viability of cancer cell line (MCF7 & A549).

All values are the mean ± S.D. of three replicates.

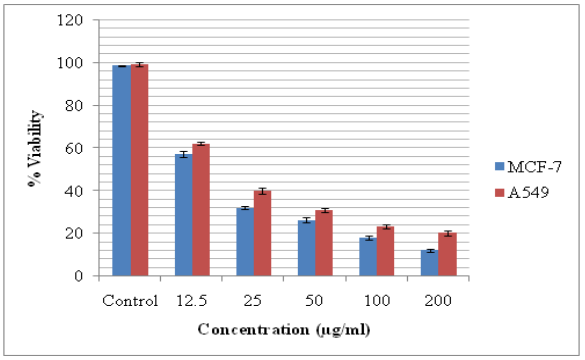


Figure 3. Effect of chloroform fractions of *Fusarium oxysporum* (RKAAC11) on percentage viability of cancer cell line (MCF7 & A549).

All values are the mean ± S.D. of three replicates.

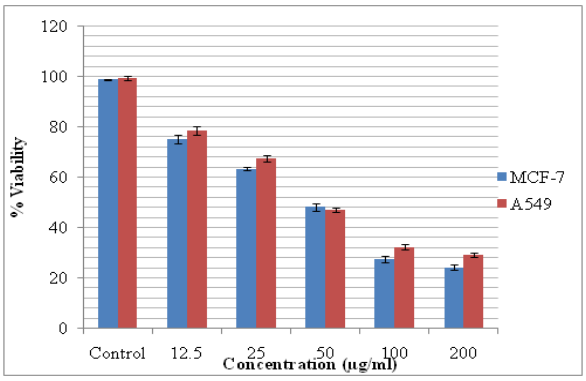


Figure 4. Effect of methanol fractions of *Alternaria alternata* (RKACQ13) on percentage viability of cancer cell (MCF-7 and A549).

All values are the mean ± S.D. of three replicates.

The MCF-7 cell line showed significantly more cytotoxicity (IC_{50}: 43.23±0.77 µg/ml) than A549 cell line (IC_{50}: 46.33±0.9 µg/ml) ($P \leq 0.05$) as compared to reference compound paclitaxel with an IC_{50} value of 3.0 µg/ml and 4.9 µg/ml for MCF-7 and A549 cell line respectively (Table 2, Figure 4)

A bioactive chloroform fraction of endophytic fungi, *Tubercularia* sp. of *Taxus mairei,* exhibit strong cytotoxic effect on KB and P388 cell lines [21]. Mohana Kumara *et al*., (2012) showed cytotoxic activity of methanolic extract of endophytic fungus *Fusarium proliferatum* from *Dysoxylum binectariferum* against HCT-116 and MCF-7 cancer cell lines [22]. The cytotoxic activity of endophytic fungal extract of *Bacopa monnieri* were found to be more effective against HCT-116 cells than the MCF-7, PC-3, and A-549 cell lines. Nearly one fifth (22%) of the extracts showed cytotoxic activity with IC_{50} of <20 µg/ml against HCT-116 cell lines, whereas only 5.5%, 11% and 11% of the extracts were found to be effective against MCF-7, PC-3, and A-549 cell lines respectively. These values were within the cutoff point of the National Cancer Institute's criteria for cytotoxicity in the screening of crude plant extracts [23]. When tested against HCT-116 cell line, B9_PinkO and B19O extracts were found to be more toxic than chaetominine isolated from the endophyte of *Adenophora axillifora* and less toxic than rubrofusarin B, isolated from the endophyte of *Cyndon dactylon* and cisplatin (Mayne Pharma) [24, 25].

Conclusion

In conclusion, this preliminary screening of fungal endophytes revealed their potential bioactive compounds for drug discovery programmes. Methanolic and chloroform extract of endophytic fungi RKAAC11 from *Asclepias curassavica* and

methanolic extract of endophytic fungi RKACQ13 from *Alternaria alternata* showed good cytotoxic effect indicating its possible potential for development as an anti-cancer drug. Further purification and targeted study of extract need to be carried out.

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References

1. Bacon CW, White JFJ: Physiological adaptations in the evolution of endophytism in the Clavicipitaceae. In: Bacon CW, White JFJ, eds. Microbial endophytes. New York, NY, USA: Marcel Dekker Inc. 2002; 237–263.
2. Fisher PJ, Graf F, Petrini LE, Sutton BC, Wookey PA: Fungal endophytes of Dryas octopetala from a high arctic polar semidesert and from the Swiss Alps. Mycologia 1995; 87:319–23.
3. Rosa LH, Vaz ABM, Caligiorne RB, Campolina S, Rosa CA: Endophytic fungi associated with the Antarctic grass Deschampsia antarctica Desv. (Poaceae). Polar Biol 2009; 32:161–67.
4. Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson, JM: Thermotolerance generated by plant/fungal symbiosis. Science 2002; 298: 1581.
5. Strobel GA: Rainforest endophytes and bioactive products. Crit Rev Biotechnol 2002; 22:315-33.
6. Bashyal BP, Wijeratne EM.K., Faeth SH, Gunatilaka, AAL: Globosumones A-C, cytotoxic orsellinic acid esters from the Sonoran desert endophytic fungus Chaetomium globosum. J Nat Prod 2005; 68:724–8.
7. Wang S, Li X, Teuscher MF, Li D, Diesel A, Ebel R, et al: Chaetopyranin, a benzaldehyde derivative, and other related metabolites from Chaetomium globosum, an endophytic fungus derived from the marine red alga Polysiphonia urceolata. J Nat Prod 2006; 69:1622–5.
8. Lin Z, Zhu T, Fang Y, Gu Q, Zhu W: Polyketides from Penicillium sp. JP-1, an endophytic fungus associated with the mangrove plant Aegiceras corniculatum. Phytochemistry 2008; 69:1273–8.
9. Suryanarayanan TS, Wittlinger SK, Stanley HF: Endophytic fungi associated with cacti in Arizona. Mycol Res 2005; 109:635–9.
10. Strobel GA: Endophytes as sources of bioactive products. Microbes Infect 2003; 5:535-44.
11. Tan R, Zou W: Endophytes: a rich source of functional metabolites. Nat Prod Rep 2001; 18:448-59.
12. Gangarde SK, Tripathi NK, Harinkhede DK and Mishra PK:. Ethno-medicinal diversity used by tribal's of central India, Sam Com Digital Graphics, Indore, 2003.
13. Strobel GA, Yang XS, Sears J, Kramer R, Sidhu RS, Hess WM: Taxol from Pestalotiopsis microspora, an endophytic fungus of Taxus wallachiana. Microbiology 1996; 142:435-40.
14. Ahmad, I.; Mehmood, Z.; Mohammad, P. and Ahmed, S: Antimicrobial potency and synergistic activity of five traditionally used Indian medicinal plants. J Med Arom Plant Sci 2000; 22:173-76.
15. Kjer, J.; Debbab, A.; Aly. A.H. and Proksch, P: Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nat Protoc 2010; 5(3): 479-90.
16. Tenguria, R.K.; Firodiya, A. and Khan, F: Biodiversity of endophytic fungi in leaves of Carissa carandas linn. from central region of Madhya Pradesh. Int. J. App. Bio. Pharm. Tech. 2012; 3(4):376-80.
17. Schulz B, Guske S, Dammann U, Boyle C: Endophyte-host interactions II. Defining symbiosis of the endophyte-host interaction. Symbiosis 1998; 25:213–27.
18. Walker, H.L. and Templeton, G.E.: In vitro production of phytotoxic metabolites by

Colletotrichum gloeosporioides F. sp. aeschynomene. Plant Sci. Lett. 1978; 13:91-99.
19. Mosmann, T: Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assay. J. Immunol. Methods 1983; 65(1-2):55-63.
20. Itharat, A.; Houghton, P.J.; Eno-Amooquaye, E.; Burke, P.J.; Sampson, J.H. and Raman, A: In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J. Ethnopharmacol. 2004; 90:33–8.
21. Wang, J.; Li, G.; Lu, H.; Zheng, Z.; Huang, Y. and Su, W.: Taxol from Tubercularia sp. strain TF5, an endophytic fungus of Taxus mairei. FEMS Microbiol. Lett. 2000; 193(2):249-53.
22. Mohana Kumara, P.; Zuehlke, S.; Priti, V.; Ramesha, B.T.; Shweta, S.; Ravikant, G.; Vasudeva, R.; Santoshkumar, T.R.; Spiteller, M. and Uma Shaanker, R.: Fusarium proliferatum, an endophytic fungus from Dysoxylum binectariferum Hook.f, produces rohitukine, a chromane alkaloid possessing anti-cancer activity. Antonie Van Leeuwenhoek 2013; 101(2):323-9.
23. Katoch, M.; Singh, G.; Sharma, S.; Gupta, N.; Sangwan, P.L. and Saxena, A.K: Cytotoxic and antimicrobial activities of endophytic fungi isolated from Bacopa monnieri (L.) Pennell (Scrophulariaceae). BMC Complement. Altern. Med. 2014; 14:52.
24. Song, Y.C.; Li, H.; Ye, Y.H.; Shan, C.Y.; Yang, Y.M. and Tan, R.X.: Endophytic napthopyrone metabolites are co-inhibitors of xanthine oxidase, SW1116 cell and some microbial growths. FEMS Microbial Lett. 2004; 241:67–72.
25. Jiao, R.H.; Xu, S.; Lin, J.Y.; Ge, H.M.; Ding, H.; Xu, C.; Zhu, H.L. and Tan, R.X: Chaetominine, a cytotoxic alkaloid produced by endophytic Chaetomonium sp. IFB-E015. Org Lett 2006; 8:5709–12.