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In vitro antioxidant potential of endophytic fungi isolated from *Enicostemma axillare* (Lam.) *Raynal.* and *Ormocarpum cochinchinense* (Lour.) Merr.

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Abstract
To elucidate the antioxidant potential of ethyl acetate extracts of the endophytic fungi isolated from *Enicostemma axillare* and *Ormocarpum cochinchinense*. Four different endophytic fungal extracts were examined for the presence of various phytochemicals by qualitative and quantitative methods and its antioxidant activity was analyzed by DPPH free radical scavenging assay. Saponins, phenols, flavonoids, and cardiac glycosides were the main phytochemicals present in the endophytic extracts of *Enicostemma axillare* and phenols, cardiac glycosides and terpenoids in the case of *Ormocarpum cochinchinense*. There was a correlation found between the phenolic content and the antioxidant activity of the extracts. The endophytes from *Enicostemma axillare* was found to have the highest phenolic content and thereby had the highest antioxidant activity of 68% at 100µg concentration. The present results revealed that metabolites produced by the endophytic fungi isolated from *Enicostemma axillare* and *Ormocarpum cochinchinense* could be a potential source of novel antioxidant compounds.

Keywords: *Enicostemma axillare*, *Ormocarpum cochinchinense*, antioxidant, endophytic fungi, DPPH

Introduction
Microorganisms such as bacteria and fungi are known to colonize as endophytes in the interior of plants. They reside in the healthy tissues of the plant without causing any significant damage or injury to the host [1]. These diverse fungi are chemical synthesizers in the host plants [2]. Some endophytic fungi, while living inside the plant tissues, may produce several compounds which are of biological importance [3]. Recently, novel compounds from endophytes exhibited antibacterial activity against plant pathogens [4] and anti-cancer activity against tumor cell lines [5]. They also have the potential to be the source for antioxidants [6]. Hence, screening these compounds for the presence of active metabolites having medicinal applications is the focus of the current research.
Enicostemma axillare belongs to the family Gentianaceae. The plant is used to treat various diseases like Diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning [7]. It has been shown to possess in vitro anti-inflammatory activity [8] and hypoglycemic activity [9]. The whole plant is used in medicine as digestive, anti-inflammatory, liver tonic, antimalarial, antipyretic and as a laxative [10].
Ormocarpum cochinchinense is a medicinal shrub belonging to the family Fabaceae [11]. It is locally known as Elumbotti or Kattumoringai in Tamil. The root is utilized as a tonic, stimulant and used in treatment of lumbago. The leaves are included informations used for setting bone fractures and for nervous pain [12].
Reactive oxygen species (ROS) are associated with numerous human degenerative diseases like diabetes mellitus, cancer, and etc. [13]. Antioxidants are stable molecules which donate electrons to the free radicals and eliminate them to prevent cellular damage [14].
Endophytic microbes usually create a symbiotic relationship with the plant tissues and are potential medicinal sources. They are known to be a rich source of unique bioactive compounds that has gained importance in many fields such as pharmaceuticals [16]. Till date, a lot of work has been performed to determine the anticancer, antiviral, antibacterial, insecticidal and antidiabetic activity of endophytic fungi, but very little has been explored with respective to their antioxidant activity [17]. In the present study, the antioxidant potential of endophytic fungi isolated from *Enicostemma axillar* and *Ormocarpum cochinchinense* was investigated.

Materials and Methods
Endophytic fungi isolation
Fresh, disease free leaf samples were collected. They were washed and transferred in a sterile bag to the laboratory and stored at -20 ºC until use.

The leaves were rinsed with distilled water until the surface is cleaned thoroughly. The samples were kept over tissue paper for drying. After the samples were dried, they were sterilized with 70% ethanol for 60 s, followed by sodium hypochlorite for 4 minutes. It is then washed with 0.1% Mercuric chloride for 240s. Finally it is washed with distilled water to remove traces of Mercuric chloride for 5 minutes. They are cut over the alternative midrib pieces to avoid colonization of same organism. The samples were trimmed using sterile blade and inoculated in Potato Dextrose Agar plates. The plates were incubated at 28 °C for 7 days and observed for growth of fungus [18].

Endophytic fungi identification

The isolated endophytic fungi species were identified according to their microscopic and macroscopic structures. The fungal isolates that failed to sporulate were classified as mycelia sterilia. The taxa were assigned to different genera as specified by taxonomists [19, 20, 21, 22].

Endophytic fungal cultivation and extraction

The endophytic fungi were cultivated on PDA at 30 °C for 10 days. Five discs (6 mm diameter) were cut from the edges of growing cultures and inoculated into Potato Dextrose Broth (PDB) in 500 ml Erlenmeyer flasks containing 300 ml of Potato Dextrose Broth (PDB) containing 20% Potato infusion, 2% Dextrose and 2% agar and incubated at room temperature for 21 days under static conditions. The filtered broth was extracted exhaustively with ethyl acetate. The extract was dried over anhydrous sodium sulfate and then evaporated under vacuum in a rotary evaporator, to yield ethyl acetate extracts. The crude extracts were then dissolved in dimethyl sulfoxide (DMSO, Sigma) and stored at 4 °C as stock solution for phytochemical analysis and antioxidant bioassays [23].

Qualitative phytochemical analysis

The fungal extract was tested for the presence of phytochemicals such as carbohydrates, tannins, phenols, flavonoids, alkaloids, steroids, phytosteroids, phlobatannins, anthraquinones, saponins, glycosides, cardiac glycosides, terpenoids, coumarins and quinones [24-32].

Determination of total phenolic content

The amount of phenolic compounds in the extracts was determined by the Folin Ciocalteu colorimetric method and calculated from a calibration curve obtained with Gallic Acid as standard (10mg/10ml). Extract was added in a separate test tube at a concentration of 10 mg/ml and 5ml of folins-ciocalteu (1:10 dilution) was added and the contents were mixed thoroughly. 4ml of 0.7 M sodium carbonate was added and the mixture was incubated for 30 minutes. The absorbance was measured at 765nm in a UV-Visible Spectrophotometer. The results were expressed in Gallic acid equivalence of the samples (GE) µg/mg of the extract [33].

DPPH Radical scavenging activity

Various concentrations of ethyl acetate extracts of endophytic fungi (20-100 µg/mL) were mixed with methanolic solution containing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals (0.1 mM, 0.5 mL). The solution was allowed to react in dark for thirty minutes. The reduction in the DPPH radical concentration was determined by measuring the absorbance at 517 nm. DPPH solution without the extract was taken as control and methanol served as blank. The percentage of DPPH scavenged was calculated using the equation: % inhibition = [(Ac-As)/Ac] x 100 where, Ac is the absorbance of control, and as is the absorbance of solution containing sample extracts. 6-hydroxy - 2, 5, 7, 8 -tetramethylchroman-2-carboxylic acid (TROLOX) was used as standard. All experiments were carried out in triplicate [34].

Results

A total of 30 endophytes were isolated from the healthy leaves of *Enicostemma axillare* and *Ormocarpum cochinchinense.* The leaf along with grown mycelia was sub cultured into the plate containing potato dextrose agar. The isolated endophytic fungi were identified based on the culture characteristics, morphology and growth. Four isolates belonging to different taxa were identified as *Phialophora*, *Penicillium and Aspergillus*. The remaining isolates which failed to sporulate were categorized as mycelia sterilia.

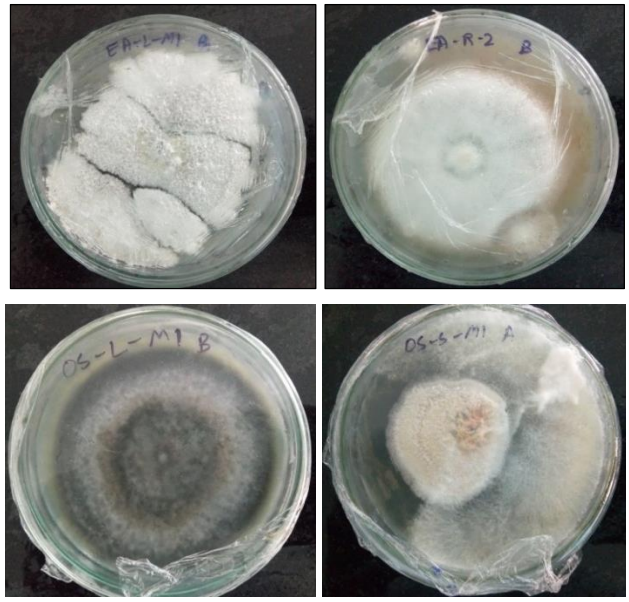
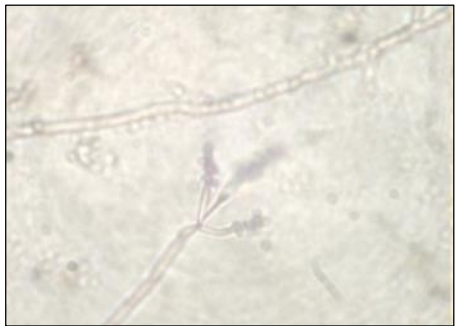
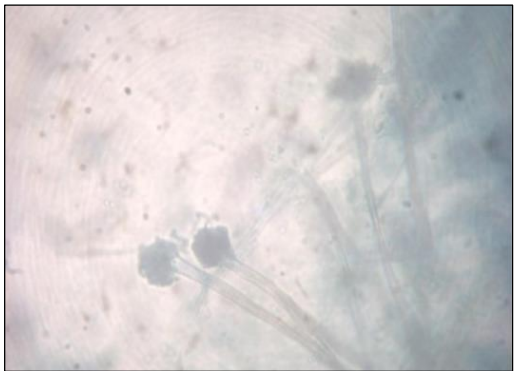


Fig 1: Some of the fungi isolated from the medicinal plants *Enicostemma axillare* and *Ormocarpum cochinchinense*.



a) *Phialophora*

b) *Penicillium*



c) *Aspergillus*

Fig 2: Identified endophytic fungal isolates from *Enicostemma axillare* and *Ormocarpum cochinchinense*.

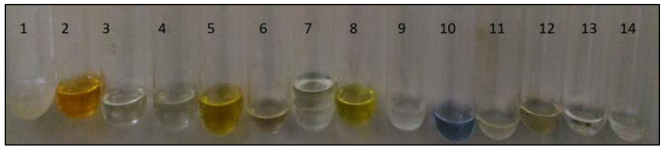
The crude extracts of the fungal isolates were examined for the presence of phytochemicals by qualitative and quantitative methods. The endophyte from *Enicostemma axillare* EA-R2 showed the presence of saponins, phenols, flavonoids, and cardiac glycosides, whereas EA-R1-B showed the presence of saponins, phenols, and cardiac glycosides. The endophyte from *Ormocarpum cochinchinense* OS-L-M1-A showed the presence of cardiac glycosides and phenols, whereas OS-L-M1-B showed the presence of phenols, and terpenoids. The phytochemicals present are shown in Table 1.

Table 1: Qualitative analysis of phytochemicals present in the endophytic isolates

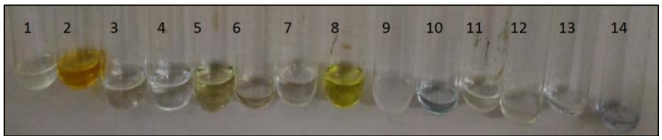
S. No	Phytochemical	Tests EA-R2	EA-R1-B	OS-L-M1-A	OS-L-M1-B
1.	Carbohydrates	-	-	-	-
2.	Tannins	-	-	-	-
3.	Saponins	+	+	-	-
4.	Flavonoids	+	-	-	-
5.	Alkaloids	-	-	-	-
6.	Quinones	-	-	-	-
7.	Glycosides	-	-	-	-
8.	Cardiac glycosides	+	+	+	-
9.	Terpenoids	-	-	-	+
10.	Phenols	+	+	+	+
11.	Coumarins	-	-	-	-
12.	Steroids and phytosteroids	-	-	-	-
13.	Phlobatannins	-	-	-	-
14.	Anthraquinones	-	-	-	-

(+) indicates presence and (-) indicates absence.

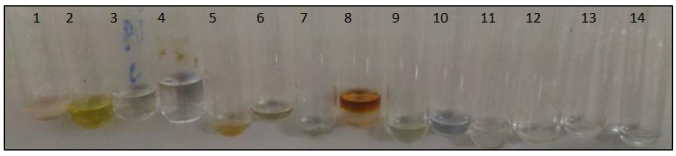
Ea-R2



EA-R1-B



OS-L-M1-A



OS-L-M1-B

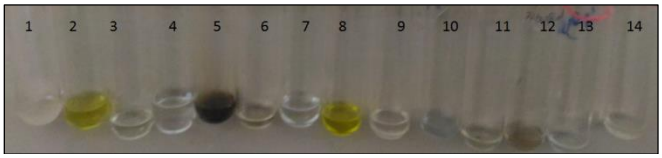


Fig 3: Qualitative analysis of phytochemicals present in the endophytic isolates

Table 2: Phenol estimation of Gallic acid and endophytic isolates at 510nm

Volume Concentration Absorbance at 510 nm (µL)(µg)				
Standard	**Gallic Acid**	**Solution 20**	**200**	**0.2155**
40	400	0.3184		
		60	600	0.6575
		80	800	0.9143
		100	1000	0.9843
	EA-R2	100	1000	0.1989
	EA-R1-B	100	1000	0.1396
	OS-L-M1-A	100	1000	0.0792
	OS-L-M1-B	100	1000	0.1347

The total phenolic contents were determined using the Folin Ciocalteu method terms of the Gallic acid equivalent (GAE) in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in Figure 4, and the standard curve equation was y = 0.0107x-0.0221, where R^2 = 0.9577. The total phenolic contents (Gallic acid equivalents, mg/g) in the ethyl acetate extracts of EA-R2, EAR1-B, OS-L-M1-A and OS-L-M1-B were calculated to be 2.06, 1.511, 0.946 and 1.465 mg/g, respectively.

The scavenging activity of four ethyl acetate extracts of the endophytic fungi increased with increasing concentration (Fig. 6-9). The extract EA-R2 shows the highest antioxidant activity among all four extracts with a maximum of 68.2% scavenging activity at 100µg concentration.

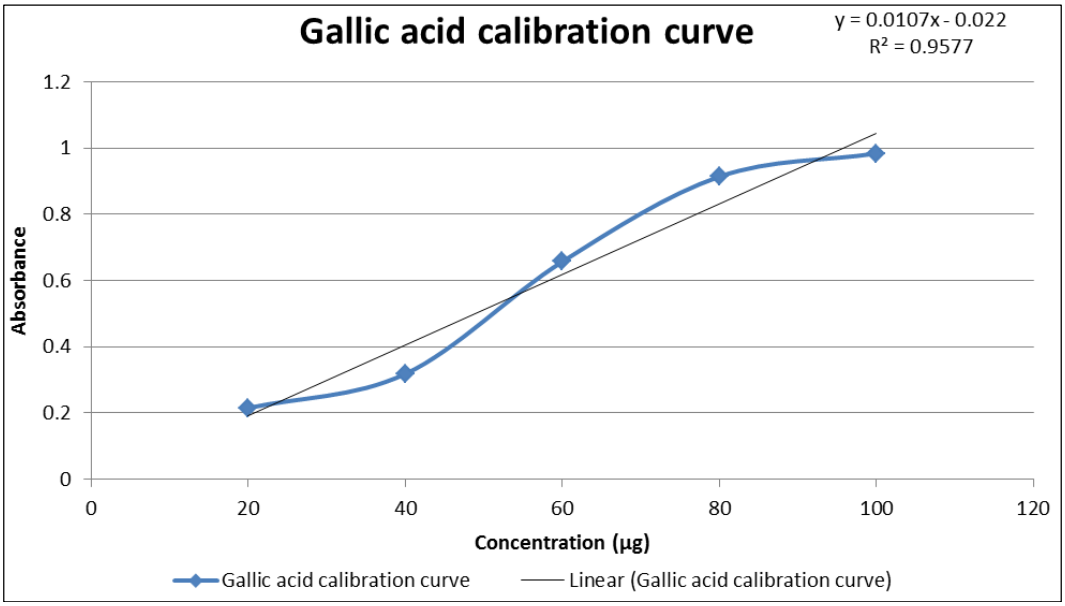


Fig 4: Standard curve of gallic acid

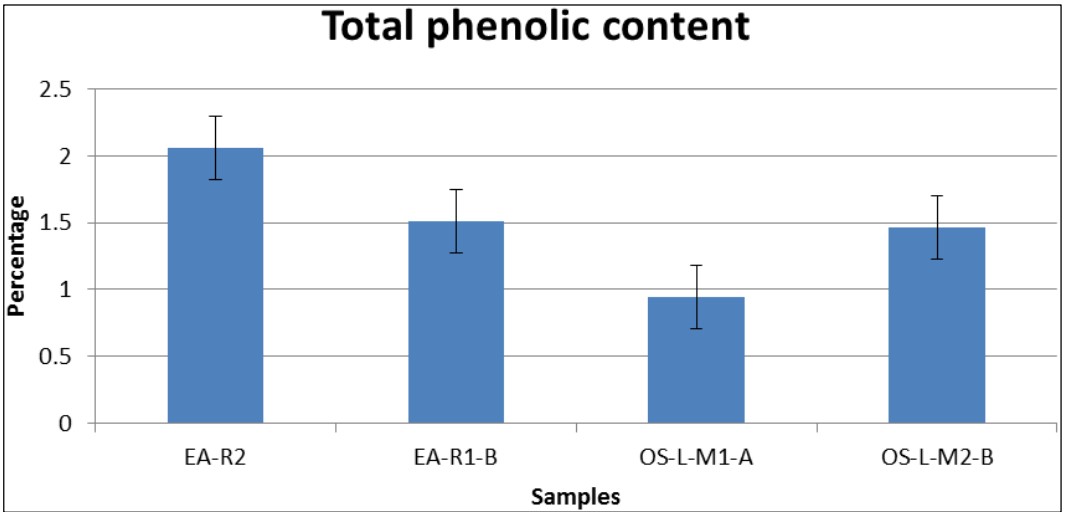


Fig 5: The phenolic content of endophytes isolated from *Enicostemma axillare* and *Ormocarpum cochinchinense*

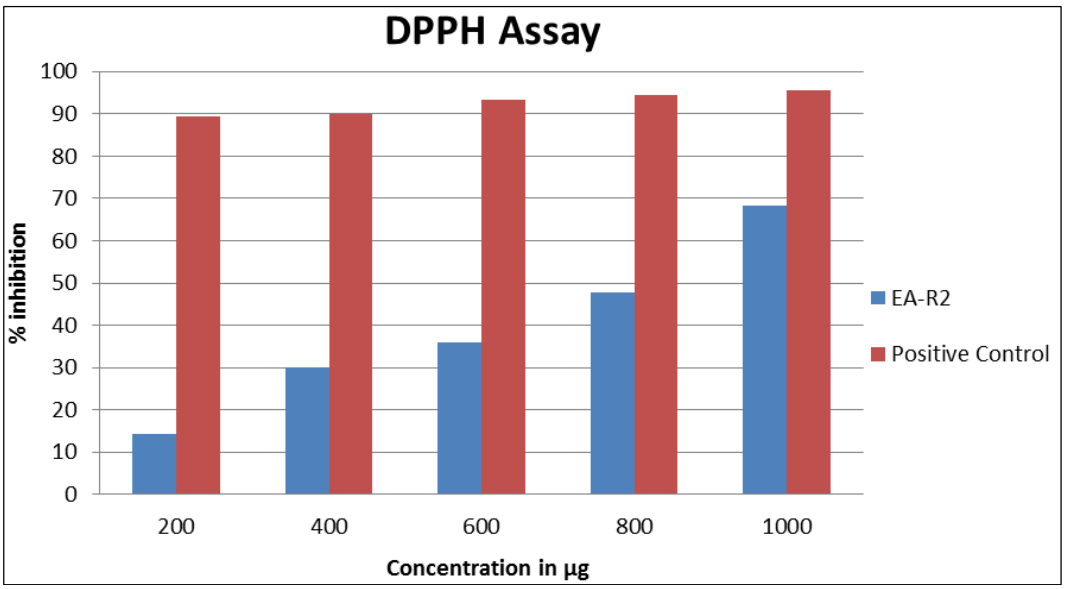


Fig 6: The antioxidant capacity of the endophytic isolate EAR2 was quantified in terms of % inhibition

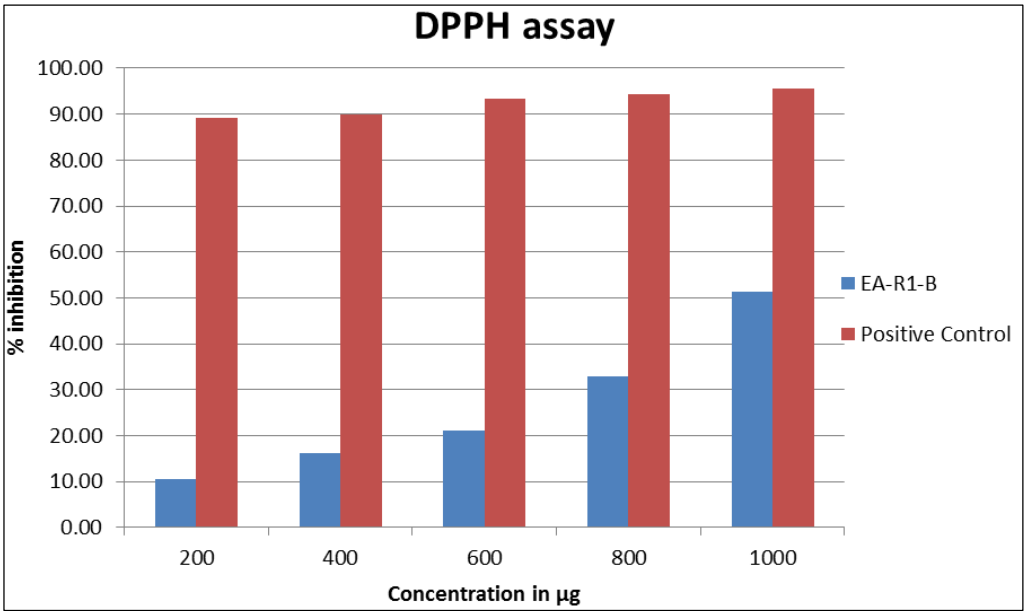


Fig 7: The antioxidant capacity of the endophytic isolate EA-R1-Bwas quantified in terms of % inhibition

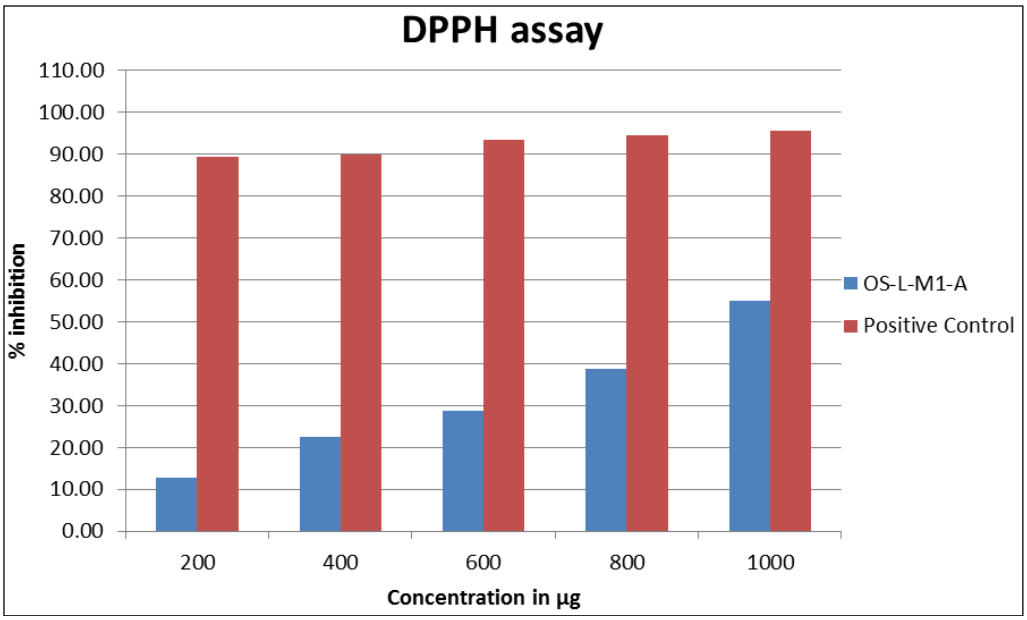


Fig 8: The antioxidant capacity of the endophytic isolate OS-L-M1-Awas quantified in terms of % inhibition

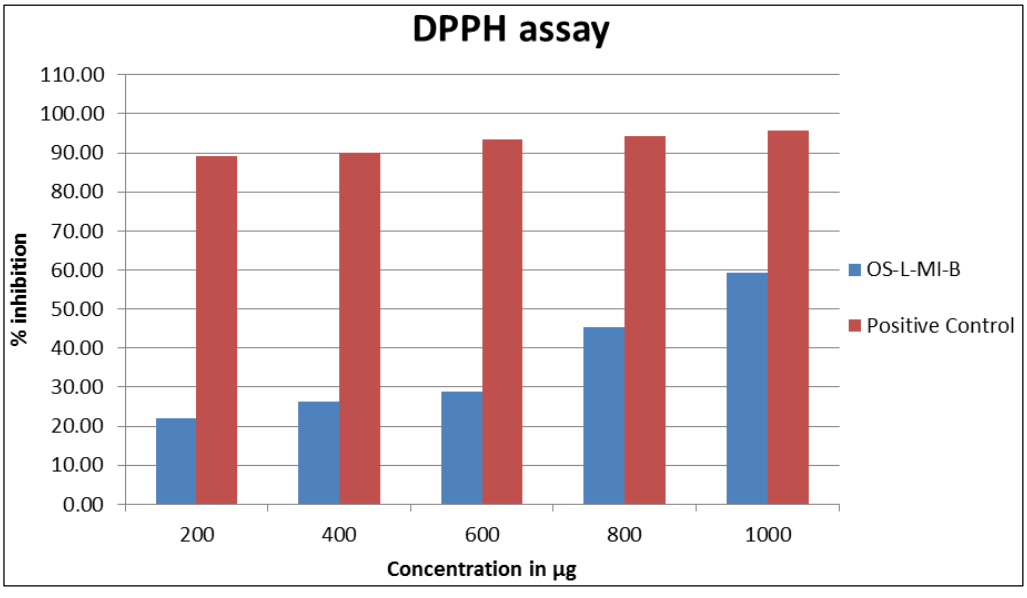


Fig 9: The antioxidant capacity of the endophytic isolate OS-L-M1-Bwas quantified in terms of % inhibition

Discussion

Most medicinal plants are hosts to endophytic fungi which may possess similar medicinal properties or secondary metabolites [35]. Endophytes have been reported to produce numerous bioactive metabolites which served as an outstanding source of drugs for treating various diseases. The bioactive compounds have potential applications in agriculture, medicine, food and cosmetics industries. One of the remarkable discoveries of taxol from *Taxomyces andreanae*, endophytic fungi isolated from *Taxus brevifolia* has been a breakthrough in endophytic bioactive compounds research studies [36]. *Enicostemma axillare* is a well-known medicinal plant used in the traditional system of medicine with various phytochemicals like glycosides, alkaloids, sterol, phenol and flavonoids [37-40].

In this study, preliminary phytochemical analysisof ethyl acetate extracts of endophytic fungi isolated from *Enicostemma axillare* and *Ormocarpum cochinchinense* confirms the presence of Phenols, flavonoids, saponins, cardiac glycosides and terpenoids. Phenols and terpenoids are of primary importance as they make up the main constituents responsible for lowering lipid peroxidation and hence are powerful antioxidant source [41, 42]. In the current study, extracts showing high phenolic content possess significant antioxidant activity. It has been proven through earlier studies that there has been a linear correlation between phenolic content and the antioxidant capacity of any given sample [43].

Extraction of the endophytic fungi from the medicinal plants was performed with ethyl acetate, which has been proven to be the most efficient method of isolating fungal secondary metabolites [44]. Ethyl acetate is a selective solvent that extracts low and high molecular weight polyphenols [45]. In the present study, among other endophytic fungi, Aspergillus was identified as one of the fungus from *Enicostemma axillare*. Previously, this fungus has been reported as common endophytic species in various plant species like *Withania somnifera, Calotropis procera, Tripterygium wilfordii, Calotropis gigantea, Azadirachta indica A. Juss*. and *Melia azedarach* L. [46].

DPPH assay is considered the most widely utilized and accurate technique for measuring the antioxidant capacity of a sample. In reducing power assay, reducing ability of a compound depends on the electron donor and free radical scavenging capacity [47]. Many endophytic fungi isolated from a number of medicinal plants have been shown to have high potential as an antioxidant. Endophytes isolated from *Salvadora oleoides* and, *Tabebuia argentea* showed antioxidant potential in different assays [48, 49]. Metabolites produced by endophytic fungi isolated from *Eugenia jambolana* have also been indicated as a potential source of novel natural antioxidant compounds [50]. Endophytic fungi isolated from *Nerium oleander L*. and liverwort *Scapania verrucosa* were shown to possess excellent antioxidant potential [51].

Fungal endophyte is a repository of novel secondary metabolites including antibiotic, antioxidant, anticancer, antiviral, antidiabetic, antimicrobial and immunosuppressant compounds [52]. A phenolic compound named Graphislactone A is produced by an endophytic fungus, Cephalosporium sp., residing in the root of *Trachelospermum jasminoides* (Apocynaceae). It has been shown to possess strong free radical scavenging and antioxidant activity [53]. *Pestalotiopsis microspore,* an endophytic fungi isolated from *Terminaliamorobensis*, produces two antioxidants, petasin and isopetasin. The results of this study represent that endophytic fungi may serve as a potential source of natural antioxidants. This is the first report on the antioxidant activity of endophytic fungi isolated from *Enicostemma axillare* and *Ormocarpum cochinchinense*.

References

1. Petrini O. Fungal Endophytes of Tree Leaves. In: Andrews JH, Hirano SS. (Eds.) Microbial Ecology of Leaves. Brock/Springer Series in Contemporary Bioscience. Springer, New York, 1991, 179-197.
2. Owen NL, Hundley N. Endophytes-the chemical synthesizers inside plants. Sci. Prog. 2004; 87:79-99.
3. Stierle A, Strobel G, Stierle D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science. 1993; 260:214-216.
4. Talontsi FM, Dittrich B, Schüffler A, Sun H, Laatsch H. Epicoccolides: Antimicrobial and antifungal polyketides from an endophytic fungus Epicoccum sp. associated with Theobroma cacao. Eur. J Org. Chem. 2013; 15:3174-3180.
5. Wu LS, Hu CL, Han T, Zheng CJ, Ma XQ, Rahman K *et al*. Cytotoxic metabolites from Perenniporia Tephropora, an endophytic fungus from Taxus chinensis var. Mairei. Appl. Microbiol. Biotechnol. 2013; 97(1):305-315.
6. Huang W, Cai Y, Xing J, Corke H, Sun M. A potential antioxidant resource: endophytic from medicinal plant. Econ Bot. 2007; 61(1):14-30.
7. Kirtikar KR, Basu BD. Indian Medicinal Plants, Bishen Sing, Edn 2. Mahendra Pal Sing publication, Dehradun, 1999, 1655-1656.
8. Leelaprakash G, Mohan Dass S. *In vitro* Anti-Inflammatory activity of Methanol extract of *Enicostemma Axillare*, Int. J Drug Dev. & Res. 2011; 3(3):189-196.
9. Jyoti M, Vasu VT, Ravikumar A, Sarita G. Glucose lowering effect of aqueous extract if *Enicostemma Littorale* Blume in diabetes a possible mechanism of action. Journal of Ethnopharmacol. 2000; 81:199-204.
10. Varier PS. Indian medicinal plants. Orient longman (Pvt.) ltd, Chennai. 1994; 2:374.
11. Shanthi P. *In vitro* propagation of Ormocarpum sennoides (Wild) DC. Prodr. From shoot tip explant. Indian Journal of Plant Physiology. 2008; 13:29-32.
12. Dinesh Kumar M, Maria John KM, Karthik S. The bone-healing potential of Ormocarpum Cochinchinense, methanolic extract on albino wistar rats. Journal of Herbs, Spices & Medicinal Plants. 2013; 19:1-10.
13. Gulcin I. Comparison of *in vitro* antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids. 2007; 32(3):431-8.
14. Halliwell B. How to characterize an antioxidant: anupdate. Biochem Soc Symp. 1995; 61:73-101.
15. Tan RX, Zou WX. Endophytes: A rich source of functional metabolites. Nat Prod Rep. 2001; 18:448-59.
16. Strobel G, Ford E, Worapong J, Harper JK, Arif AM, Grant DM *et al*. Isopestacin, a unique isobenzofuranone from Pestalotiopsis microspora possessing antifungal and antioxidant properties. Phytochemistry. 2002; 60(2):179-183.
17. Singh B, Thakur A, Chadha BS, Kaur S, Kaur A. Acetylcholinesterase inhibitory potential and insecticidal activity of an endophytic Alternaria sp. From Ricinus Communis. Appl. Biochem Biotechnol. 2012; 168:991-1002.

18. Wang Y, Xu L, Ren W, Zhao D, Zhu Y, Wu X. Bioactive metabolites from Chaetomium globosum L18, an endophytic fungus in the medicinal plant Curcuma wenyujin. Phytomedicine. 2012; 19:364-368.
19. Ellis MB. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, 1971, 608.
20. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi, Edn 4. APS Press. St. Paul, MN, 1998.
21. Ainsworth GC, Sparrow FK, Sussman AS. The fungi: an advanced treatise, A. Academic Press, New York, USA, 4, 1973.
22. Von Arx JA. The genera of fungi sporulating in pure culture. In Gantner AR, Verlag KG (eds.) FL-9490 Vaduz, Liechtenstein, 1978.
23. Sofowora A. Medicinal Plants and Traditional Medicinal in Africa. Edn 2. Sunshine House, Ibadan, Nigeria: Spectrum Books Ltd. 1993, 134-156.
24. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis, Edn 3, Chapman and Hall, New York, 1973, 279.
25. Smolenski SJ, Silinis H, Farnswoth NR. Alkaloids screening. V. *Lloydia*. 1974; 37:506-536.
26. Kapoor LD, Singh A, Kapoor SL, Shrivastava SN. Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. Lloydia. 1969, 32:297-302.
27. Jana S, Shekhawat GS. Phytochemical analysis and antibacterial screening of *in vivo* and *in vitro* extracts of Indian medicinal herbs: *Anethum graveolens*, Research Journal of medicinal plants. 2010; 4(4):206-212.
28. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, Atangbayila TO. Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria, Tropical Journal of Pharmaceutical Research. 2008; 7(3):1019-1024.
29. Sureshkumar CA, Varadharajan R, Muthumani P, Meera R, Devi P, Kameswari B. Pharmacognostic and Preliminary Phytochemical Investigations on the stem of Saccharum spontaneum, J Pharm. Sci. & Res. 2009; 1(3):129-136.
30. Boxi M, Rajesh Y, Rajakumar V, Praveen B, Mangamma K. Extraction, phytochemical screening and *in vitro* evaluation of anti-oxidant properties of commicarpus chinensis (aqueous leaf extract). International Journal of Pharma and Bio Sciences. 2010; 1(4):547.
31. Kolawole OM, Oguntoye SO, Agbede O, Olayemi AB. Studies on the efficacy of *Bridelia ferruginea* Benth. Bark extract in reducing the coliform load and BOD of domestic waste water. Ethnobotanical Leaflets. 2006; 10:228-238.
32. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-158.
33. Babu DR, Rao GN. Antioxidant properties and electrochemical behaviour of cultivated commercial Indian edible mushrooms. Journal of Food Science and Technology. 2011; 50:301-308.
34. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol. Biol. Rev 2003; 67(4):491-502.
35. Stierle A, Strobel G, Stierle D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science. 1993; 260(5105):214-6.
36. Daniel M, Sabnis SD. Chemical systematic of family Gentianaceae. Current. Science. 1978; 47:109-111.
37. Magora HB, Rahman MM, Gray AI, Cole MD. Swertiamarin from Enicostemma axillare subsp. axillare (Gentianaceae). Biochemical Systematics and Ecology. 2003; 31:553-555.
38. Vishwakarma SL, Rajani M, Bagul MS, Goyal RK. A rapid method for the isolation of swertiamarin from Enicostemma littorale. Pharmaceutical Biology. 2004; 42:400-403.
39. Ghosal S, Jaiswal DK. Chemical constituents of Gentianaceae XXVIII: flavonoids of Enicostemma Hyssopifolium (Wild). Verd. Journal of Pharmaceutical Sciences 1980: 61:53-56.
40. Gulcin I. Antioxidant and antiradical activities of L-carnitine. Life Sci. 2006; 78(8):803-811.
41. Hajdú Z, Hohmann J, Forgo P, Martinek T, Dervarics M, Zupkó I *et al*. Diterpenoids and flavonoids from the fruits of Vitexagnuscastus and antioxidant activity of the fruit extracts and their constituents. Phytother Res. 2007; 21(4):391-394.
42. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, and *Eugenia jambolana* Lam. trees. Food Chem. 2007; 104(3):1106-1114.
43. Garcia A, Rhoden SA, Bernardi-Wenzel J, Orlandelli RC, Azevedo JL, Pamphile JA. Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant *Sapindus saponaria* L. J Appl Pharm Sci. 2012; 2(10):35-40.
44. Scholz E, Rimpler H. Proanthocyanidins from Krameria Triandra Root. Planta Med 1989; 55(4):379-384.
45. Rezwana K, Saleem S, Choudhary MI, Shakeel AK, Aqeel A. Communities of endophytic fungi In Medicinal plant *Withania somnifera*. Pak. J Bot. 2010; 42(2):1281-1287.
46. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995; 28(1):25-30.
47. Dhankhar S, Kumar S, Dhankhar S, Yadav JP. Antioxidant activity of fungal endophytes isolated from Salvadora Oleoides Decne. Int J Pharm Pharm Sci. 2012; 4(2):380-385.
48. Govindappa M, Channabasava R, Sunil Kumar KR, Pushpalatha KC. Antioxidant activity and phytochemical screening of crude endophytes extracts of Tabebuia argentea Bur. & K. Sch. Am J Plant Sci. 2013; 4(8):1641-1652.
49. Yadav M, Yadav A, Yadav JP. *In vitro* antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia *jambolana* Lam. Asian Pac J Trop Med. 2014; 7(1):256-261.
50. Huang WY, Cai YZ, Hyde KD, Corke H, Sun M. Endophytic fungi from Nerium oleander L. (Apocynaceae): main constituents and antioxidant activity. World J Microbiol Biotechnol. 2007; 23(9):1253-1263.
51. Zeng PY, Wu JG, Liao LM, Chen TQ, Wu JZ, Wong KH. *In vitro* antioxidant activities of endophytic fungi isolated from the liverwort Scapania Verrucosa. Genet Mol Res. 2011; 10(4):3169-3179.
52. Song YC, Huang WY, Sun C, Wang FW, Tan RX. Characterization of graphislactone A as the antioxidant and free radical scavenging substance from the culture of Cephalosporium Sp. IFB-E001, an endophytic fungus in Trachelospermum Jasminoides. Biol Pharm Bull. 2005; 28(3):506-509.

53. Harper JK, Arif AM, Ford EJ, Strobel GA, Porco JA, Tomer DP *et al*. Pestacin: a 1, 3-dihydro isobenzofuran from Pestalotiopsis Microspora possessing antioxidant and antimycotic activities. Tetrahedron. 2003; 59(14):2471-2476.