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STUDIES ON ISOLATION AND CHARACTERIZATION OF *CATHARANTHUS ROSEUS* (WHITE) ENDOPHYTES FOR THEIR ANTIPROLIFERATIVE ACTIVITIES.

N. S. Mali and A. S. Gortyal
Lokmangal Science and Entrepreneurship College, Wadala.(MS)

ABSTRACT :

Eight different types of fungal endophytes were isolated from the different parts of white Catharanthus roseus. Three fungal endophytes were isolated from the leaves included Drechslera spp. Fusarium spp. and Colletotrichum spp. , one from the Stem i.e Helminthosporium spp. and three from the roots included -Bispora spp. Alternaria spp. Dematious hyphomycetes spp. All these were isolates were studied for their antiproliferative activities. The cytotoxicity of all isolates was tested on HeLa and MCF7 cell lines. Endophytic fungal extracts showed the cytotoxicity which varied from 10% to 80%. The isolates from leaf-2 and root –2, shown the highest activity against HeLa cell line. The cytotoxicity of endophytes varied from 15 to 41% against MCF-7 cell line. The isolates from leaf-1 and leaf-2, shown the highest activity against MCF-7 cell line.

KEYWORDS :*endophytes, isolates, cytotoxicity, antiproliferative HeLa, MCF7 Cell lines.*

INTRODUCTION:

The herb *Catharanthus roseus* (fa- Apocynaceae) is native and endemic to Madagascar. The plant is also known as *Vinca rosea, Ammocallis rosea* and *Lochnera rosea*. The plant is traditionally used for the treatment of different types of human diseases since ages.

Endophytic fungi grow in intercellular and intracellular spaces of the host without producing any disease symptoms. Within hosts, fungi inhabit all available tissue including leaves with petioles, stems, twigs, bark, root, fruit, flower and seeds. The endophytic fungi improve the resistance of host plants to adversity by secretion of bioactive metabolites. It is cultivated mainly for its alkaloids, A number of endophytic fungi have been isolated from many plants for, antidaibetic, anti tumor, anticancer activity etc. (Jaleel *et al.*, 2009). The extracts have demonstrated significant anticancer activity against numerous cell types (EL-Sayed and Cordell, 1981).

MATERIALS AND METHODS:

A) Collection of Plant Sample:

The mature White *Vinca rosea* plant was collected from Lokmangal Science and Entrepreneurship College, Wadala in sterile ploythene bag and brought to the laboratory for the isolation of endophytes. The plant was surface sterilized with two drops of Savlon, followed by frequently washing with tap water until the savlon removes. Then the plant was cut into different parts like root, stem and leaves. The explants -leaf, Stem and Root were treated with mercuric chloride 0.1%, 0.5% and 1% respectively for two minutes, and finally

rinsed with sterile distilled water for three times before sterilization separately.

B) Isolation of entophytes:

The midrib portion of leaf , stem and root segments about 0.5 cm each were placed on 20 ml PDA medium in a Petri dish and incubated for 15days at 27 ^{0}C ± 1 ^{0}C .The fungal growth was observed after incubation period on the culture medium.

Total 08 different endophytes were isolated from the plants. Table 1 showed the number of isolates, isolated from Leaf, Stem and Root explants. Kumar et al. (2013) also isolated 52 endophytic fungi from the leaves of *Catharanthus roseus* plant which were unusual and slow growing. The isolates from the different plant parts are shown in the plate-1

C) Identification and Characterization of isolates.

The slides of fungal species were prepared from fungal isolates by staining with Lacto phenol (Cotton Blue) and the identification was carried out using standard literature. All the isolates were identified by the expert mycologist, Plate-.1 shows the identified entophytic fungi from Leaf, Stem and Root and table -1 shows the list of isolates.

The identification of 08 endophytic fungi was carried out by staining with Lactophenol Cotton Blue and by observing under the microscope. For this, Kumar et al. (2013) and Mahajan et al. (2014) method was used.

Table 1: Isolated endophytic fungi from White *Catharanthus roseus.*

Isolate	No. of Isolates.	Endophytic Fungi
White Leaf – 1	4	Drechslera spp.
White Leaf – 2A		Drechslera spp.
White Leaf – 2B		Fusarium spp.
White Leaf – 3		Colletotrichum spp.
White Stem	1	Helminthosporium spp.
White Root – 1	3	Bispora spp.
White Root – 2		Alternaria spp.
White Root – 3		Dematious hyphomycetes.

D) Production of Alkaloids from Entophytic Fungi-

The fungal isolates were used for the production of alkaloids by two stage fermentation method.

Stage I:

i) The fungal isolates were grown in 500 mL Erlenmeyer flasks (100 ml MGYP composed of = 0.3 %, + glucose =1.0 %+yeast extract = 0.3 % +peptone = 0.5 %). The flasks were inoculated with the7 days old isolates grown on PDA and incubated at 25-27°C on a rotary shaker (240 rpm) for 7 days. (used as seed cultures).

Stage II:

i) 10 ml seed cultures were transferred to 500 mL flask (containing 100 mL vinca alkaloids (VM-1) medium.

ii) The flasks were incubated at 25-27°C on a rotary shaker (240 rpm) for 20 days

iii) After 20 days of incubation, the culture was harvested and passed through four layers of muslin cloth to separate the mycelia from the culture broth. The filtrates was lyophilized and extracted with equal volumes of ethyl acetate each time.

Alkaloids from Culture Filtrate:

All Eight endophytic fungi screened for *Vinca* alkaloids, the culture filtrates was extracted with equal volume of ethyl acetate yielded from the solvent, labeled and stored in vials. Plate- 1 shows different culture

filtrate of Vinca alkaloids (stored in vials).

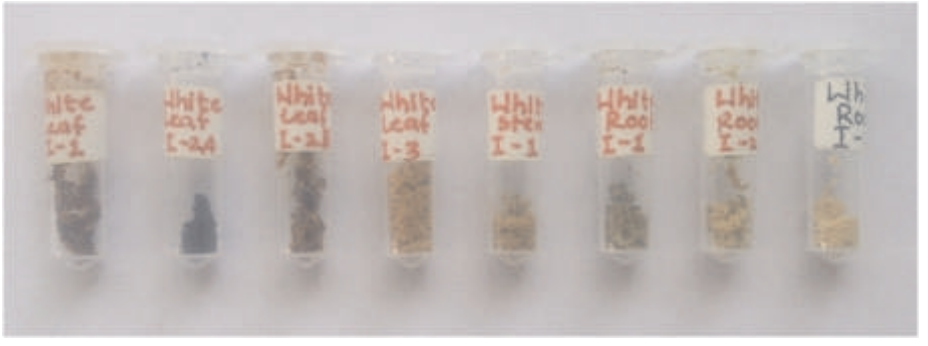

Plate- 1: Extraction of different isolates from Leaf, Stem and Root of Catharanthus roseus.

E) Cytotoxicity assay:

Materials:

The two cell lines HeLa and MCF – 7 obtained from NCL institute, Pune and were sub cultured in Eagles minimal essential medium with 10% FBS and maintained in a 5% CO_2 incubator at 37°C.

MTT assay:

HeLa cell line and MCF-7 cell lines were maintained in Eagle's minimum essential medium (2ml glutamine +Earle's salts). The cytotoxicity was evaluated by MTT [3-4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] according to Mossmann et al. HeLa and MCF-7 cell cultures ($5x10^5$ cells / mL) were cultured in 96-well flat bottomed microtitre plate and incubated for 48 h at 37°C in a humidified 5% CO_2 incubator. Different concentrations of the endophytic extracts were added to the wells. The plate was incubated for 48 h at 37°C in a humidified incubator with 5% CO_2.. MTT (5mg/ml) was prepared in phosphate buffer saline (PBS). MTT (10 µl) was added to each well and incubated in dark for 4 hours in CO_2 incubator. The supernatant was removed from the wells and the plate was washed three times with Dulbecco's formula PBS (pH 7.3). DMSO (100µl) was added to each well. The absorbance of the samples were measured at 570 nm after 30 min.

Cytotoxicity Assay:

The cytotoxicity of endophytic fungal extracts of *Catharanthus roseus* was tested on HeLa and MCF7 cell lines. Endophytic cytotoxicity varied from 11% to 80%. The isolates White leaf-2, root – 2, shown the highest activity against HeLa cell line. The isolated endophytes had shown the cytotoxicity from 15% to 41% against MCF-7 cell line. White leaf-1, White leaf-2, shown the highest activity against MCF-7 cell line. Similar types of results were obtained by Kuriakose et al. (2014) when the endophyte *Fusarium solani* isolated from *Datura metel* and tested on human cancer cells. The results of cytotoxicity activity of leaf stem and root of endophytic fungal extracts of White *Catharanthus roseus* is described in Table 2, 3 and fig.1, 2.

Table.2- Cytotoxicity Assay of white *Catharanthus roseus* against HeLa cell line:

Extract	Percent Inhibition (%)			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
White Leaf – 1	25.30	26.50	31.32	51.20
White Leaf – 2A	25.30	50.60	62.65	68.07
White Leaf – 2B	19.27	24.69	60.84	69.27
White Leaf – 3	08.43	35.54	47.59	65.66
White Stem – 1	15.66	29.51	60.24	63.25
White Root – 1	02.40	04.21	24.09	28.91
White Root – 2	22.89	27.10	34.93	36.14
White Root – 3	12.65	15.66	14.46	32.53

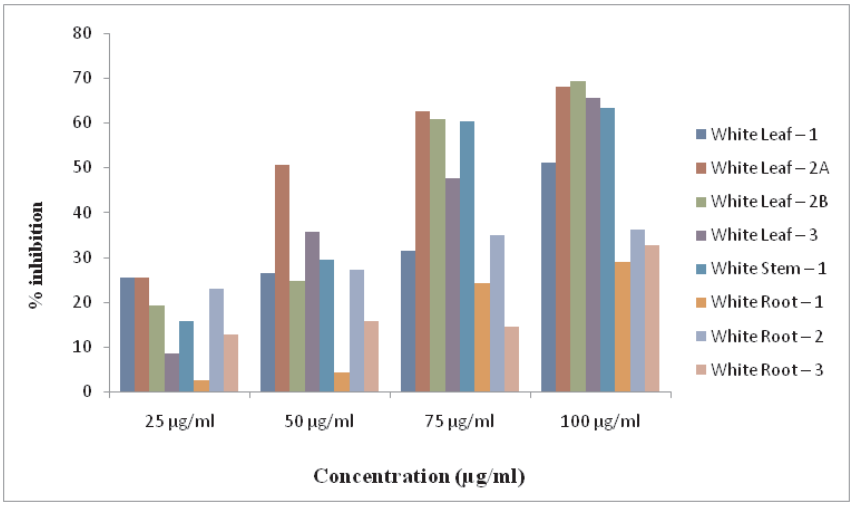

Fig 1: Cytotoxicity of endophytic fungal extract of leaf, stem and root of White *Catharanthus roseus* on HeLa cell line.

Table 3: Cytotoxicity of endophytic fungal extract of leaf, stem and root of White *Catharanthus roseus* on MCF 7 Cell line.

Extract	Percent Inhibition (%)			
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
White Leaf – 1	01.41	02.21	06.00	10.24
White Leaf – 2A	24.38	27.20	30.38	37.80
White Leaf – 2B	0.00	01.06	02.82	19.43
White Leaf – 3	12.72	24.02	30.38	34.27
White Stem – 1	24.38	25.44	27.20	28.97
White Root – 1	17.31	23.32	24.02	27.91
White Root – 2	21.20	22.96	28.26	32.86
White Root – 3	18.37	25.08	27.56	30.74

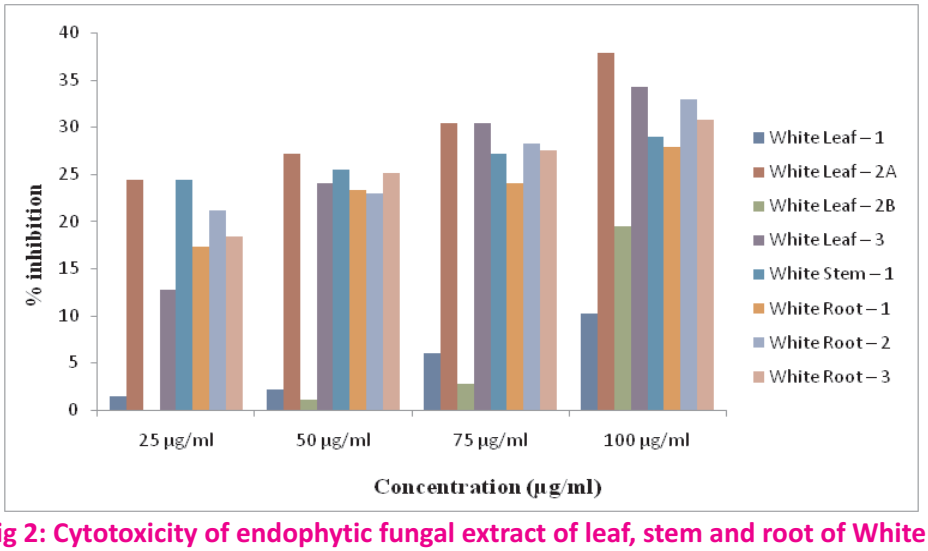

Fig 2: Cytotoxicity of endophytic fungal extract of leaf, stem and root of White *Catharanthus roseus* on MCF 7 Cell line.

Thus, all the endophytic extracts showed the potent cytotoxicity due to the presence of major vinca alkaloids like serpentine, catharanthine, ajmalicine, etc. along with flavonoids, glycosides and anthraquinones.

The Cytotoxicity of endophytic fungal extracts of White *Catharanthus roseus* was tested on HeLa and MCF7 (breast cancer cells) cell lines. Endophytic extracts showed the cytotoxicity from 11% to 80%.

RESULTS AND DISCUSSION:

The cytotoxicity of endophytic fungal extracts of White *Catharanthus roseus* was tested on HeLa and MCF7 (breast cancer cells) cell lines.

Cytotoxicity Assay of white *Catharanthus roseus* against HeLa cell line is shown in the table no-2 and fig-1.The minimum cytotoxicity (Percent Inhibition) was observed as 2.40 %from root-1 endohytes and maximum as 25.30 % from the leaf endophytes at 25 µg/ml, The minimum cytotoxicity was observed as 4.21 %from root-1 endohytes and maximum as 50.60 % from the leaf 2A-endophytes at 50µg/ml, The minimum cytotoxicity was observed as 14.46 %from root-3 endohytes and maximum as 62.65% from the leaf 2- A endophytes at 75 µg/ml and the minimum cytotoxicity was observed as 28.91 %from Leaf 2-B endohytes and maximum as 69.27 % from the leaf 2b-endophytes at 100µg/ml, (Table2) In the fig.2 same results are expressed. The cytotoxicity against HeLa cell line varied from 2.40 to 69.27 % .

Cytotoxicity Assay of white *Catharanthus roseus* against MCF 7 Cell line is shown in the table no-3 and fig-2.The minimum cytotoxicity (Percent Inhibition) was observed as 0.00 %from leaf 2-B endohytes and maximum as24.38 % from the leaf 2A and 2B endophytes at 25 µg/ml, The minimum cytotoxicity was observed as 01.06 %from leaf2 B endohytes and maximum a27.20 % from the leaf 2A-endophytes at 50µg/ml, the minimum cytotoxicity was observed as 02.82 %from Leaf 2 B endohytes and maximum as 30.38 % from the leaf 2- A endophytes at 75 µg/ml and the minimum cytotoxicity was observed as 10.24 %from Leaf -1 endohytes and maximum as 37.80 % from the leaf 2A-endophytes at 100µg/ml, (Table2) .In the fig.2 same results are expressed. The cytotoxicity against MCF 7 Cell line varied from 0.00 to 37.80 %. Similar results are also expressed graphically in fig.2.

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