Screening of fractions of endophytes of *Catharanthusroseus*against HT 29 induced Colon Cancer in Rats

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ABSTRACT

Malignanttumor cells expected to grow rapid, and chemo medications kill fast developing cells. Since these medications travel all through the body, they can influence normal, healthy cells that are fast developing as well. Harm to healthy cells causes adverse effects. Avariety of bioactive molecules, including anti-neoplastic agents, have been recognized from various fungi. The aim of this study is to explore theanticancer activity of fungal endophytesproduced by *Catharanthusroseus* in HT 29 colon cancer cells in rats.An anticancer activity of portions of endophytes of *Catharanthusroseus* was assessed in HT 29 (Human colorectal adenocarcinoma cell line)inducedcolon cancer in rats. Cell line was presented intraperitoneally and following 24 hours, treatment was given for 14 continuous days. After anticancer therapy, blood was procured and hematological variables like RBC, WBC and hemoglobin were assessed and colons were taken apart for histopathologicalinvestigations and aberrant crypt foci (ACF) assay was conducted.From ACF test result, % inhibition in ACF was determined for all rats and all the data obtained were investigated statistically.Treatment of chloroform extract of *Mucor* sp. remarkably displayed % inhibition in ACF, and normalized the hematological profiles contrasted with those of pathological control rodents. After finishing of treatment, rodents displayed huge increase in body weights.Chloroform extracts of endophytic fungi derived from *Catharanthusroseus* which was distinguished as *Mucor*sp. have significant free radical scavenging and anticancer action. This antioxidant action of endophytes might be answerable for their anticancer action and might be because of the secondary metabolites of fungus

catharanthusroseus. This data upheld the case that endophytes are considered as an elective hotspot for new secondary metabolites.

KEYWORDS: Endophytes,*Catharanthusroseus*, HT 29 cancer cells, Anticancer activity, *Mucor*sp antioxidant action, Secondary metabolites.

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I.INTRODUCTION

Developing new anticancer medications that tend to be effective on MDR cells is an achievable technique to beat MDR(Zhang *et al*.,2010).Colorectal disease (CRC) is the 3rd most standard neoplasm in men (10.0% of the full scale tumors, 663,000 cases) and the second in females (9.4% of the total cases, 570,000 cases) around the globe. For a significant longtime it has been reciprocated that the overwhelmingly vegetarian diet with enhanced fiber and reduced meat affirmation is subject for the reduced Colon cancer recurrence in our country(Kumar*et al*.,2018). Microorganisms, especially fungi, have for quite a while been seen as a huge wellspring of novel metabolites with promising foe of bacterial, against fungal and threatening to viral development. Moulds are one of the significant wellsprings of regular bioactive blends.

The achievement of finding parasitic taxol has made a perspective for of course other bioactive blends to be found in endophytic microorganisms. A few mixtures of endophytic growths were recognized as conveying biologically active mixtures with their utilization in metabolic measure (Kharwar*et al*.,2008).

The isolation of Taxol-delivering endophyte*Taxomycesandreanae* has given an elective method to manage gains a more affordable and more open thing through microorganism development (Ghosh*et al*.,1984). Starting now and into the foreseeable future, Taxol has furthermore been found in different genera of parasitic endophytes for instance, *Phyllostictaspinarum; Bartaliniarobillardoides; Botryodiplodiatheobromae; Taxodiumdistichum; Wollemianobilis; Pestalotiopsisterminaliae*(Aqubi*et al*.,2011).

As of late, it has been exhibited that manufactured mixtures in *Catharanthusroseus* may in like manner hinder the advancement of new veins that help tumor formation. It has been in like manner found that vinblastine is made by endophytic development, while another social event has kept vincristine from *Fusariumoxysporum*. Regardless, before these microorganisms can be utilized and made as accommodating thing, expansive business identified with boosting development for making the product, getting clinical examinations done and securing administrative underwriting are as yet essential. Be that as it may, these basic steps of thing revelation direct the way for future procedures toward be engaged by assessing helpful herbs for their endophytes and a short time later on a very basic level consideringproductseparation and structural characterization of bioactive compounds (Pimentel *et al*.,2011).

Some are listed as: Antineoplastic activity of *Catharanthusroseus* extracts against colorectal carcinoma cell line (Siddiqui*et al*.,2010). Influence of concomitant institution of methanolic extract of *Catharanthusroseus*on the antidiabeticefficacy of Metformin and Glyburide in rodents(Ohadoma*etal*.,2011). Sub-acute oral toxicity investigations of leaf extract of *Catharanthusroseus*(Kevin LY et al.,2012). *Ex-vivo*and *in vitro*pharmacological responses ofalkaloids from the roots of *Catharanthusroseus*in acetylcholinesterase blocking and parasympathetic transmission (Pereira *et al*.,2010).

Literature findings support the *in vitro* anticancer activity of *Catharanthusroseus*. There are reports of anticancer activity of *Mucor*species derived from *Catharanthusroseus* by chemically induced cancer in rats (Shukla*et al*.,2018). However there are no reports regarding the anticancer activity of *Mucor*species derived from *Catharanthusroseus* by human cell line induced cancer in rats. In addition to the time-

taking progress of chemical cancers, the main disadvantage of chemically induced cancer is the obstruction in noninvasive tumor burden evaluation in small living creatures(Wang *et al*.,2009). HT29 cells have emerged as anacceptable model for understanding the molecular mechanisms of intestinal cell proliferation. The utilization of these human cells also offers a valuable scope to perform studies when a variance in response between variant species is noticed (Martínez-Maquedaet *al*.,2015).

Accordingly the current research was intended to assess potential endophytic crude fractions of *catharanthusroseus*(*Mucor* species) for *in-vivo* anticancer action in HT 29 Cell line induced colon malignancy in rodents.

II.MATERIALS AND METHODS

1. Collection and substantiation of plant substance

*Catharanthusroseus*leaves required for investigation was gathered fromSurat's neighborhood district during December, 2012. *Catharanthusroseus* leaf sample was certified at Navsari Agricultural University and Herbarium model was given at SDPC, Kim, Gujarat.

2. Collectionof Materials

Neoplastic cell line HT 29 was purchased from NCCS, Pune.

Crude endophytic fraction was isolated at Shree Dhanvantary Pharmaceutical analytical and research facility.

5-fluorouracil and DPPH were received as an honorarium sample from Dhanvantary Pharmaceutical analytical and research center.

3.Animals

Wistar rats weighing between 80-120 gm obtained from SDPC were utilized for the study.The rodents were kept up at classical condition according to CPCSEA rules. The animal tests were performed by the guidelines of the IAEC. The investigation protocol was affirmed by IAEC(Ref No: SDPC-AFC/2013/66) committee.

4.Pharmacognosticalstudy

Various techniques for isolation of endophytes are depicted in literary works. A helpful and regular strategy acknowledged by numerous analysts includes plunging the tissues in alcohol (70%) for some seconds or in sodium hypochlorite (0.5-3.5%) for 1-2 minutes subsequently washes in clean twofold refined water prior to plating it on a supplement mode for isolation of endophytic microorganisms. Some isolates require months or additional time in culture former to their sporulation. For isolation of fungal endophytes surface sterilization of tissue requires 70% ethanol for 1-3 minutes, aqueous sodium hypochloride (4% available chlorine) for 3-5 minutes again rinse with 70% ethanol 2-10 seconds and final rinse with double distilled water and drying in Laminar air flow, also addition of 50mg/l chloramphenicol can be done to suppress bacterial growth. Sterile knife blade is required to remove outer tissues from sample and to excise inner tissues. Water agar, Potato dextrose agar, Yeast extract agar, Rose bengal chloramphenicol agar, Luria bertani agar, Humic acid vitamin agar are found to be suitable media for isolation of endophytic fungi, bacteria and actinomycetes respectively. Temperature of about 25- 30^{0}C is suitable for theirgrowth (Jalgaonwala*et al*.,2010).

The fungi were recognized in view of morphological characteristics as indicated by the techniques portrayed by Kong and Qi (Kong *et al*.,1985). Colony descriptions depended on observations on PDA under ambient daylight conditions. Development rates at 20, 25, 30, 35, and 40◦C were determined after 72 h following published protocols. Microscopic observations and estimations were made from preparations that were mounted in lactic acid.

5. Preliminary investigation of fungalcolony

Preliminary examination was performed in Shree Dhanvantary Pharmaceutical analytical and research center. The isolated fungi mounted on the sterile slides and furtherstained with lactophenol blue and afterward analyzed in 100X magnification utilizing light microscopy. Some endophytic fungi do not produce spores and it was grouped as a one species named "sterile form". The identified fungal isolates from the

respective plant tissue segments were isolated and then sub cultured in a petri dish which contains sterile PDA media. To preserve as a pure culture, the endophytic fungi was inoculated in PDA slant (Henzelyová*et al*.,2020;Vigneshwari*et al*.,2019).

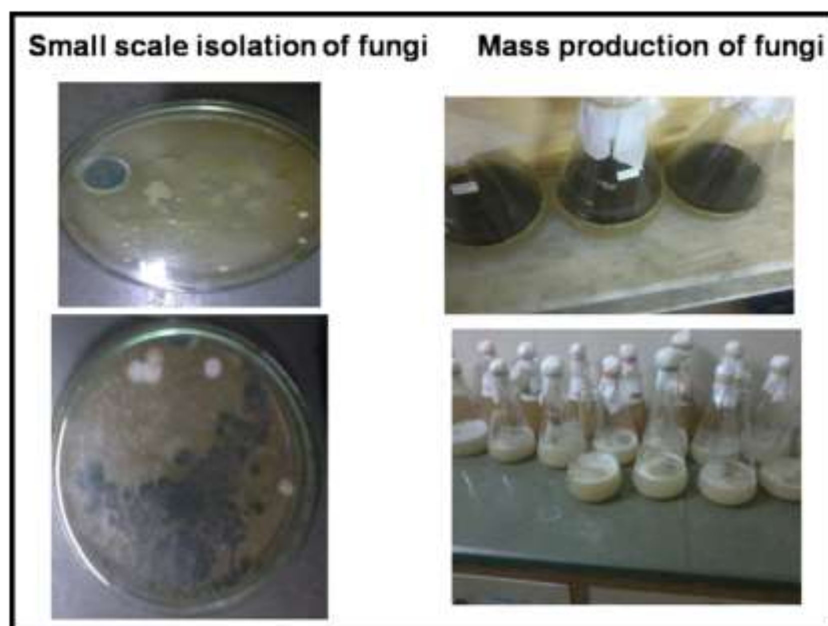

Fig-1: Isolation of fungi on small scale and large scale.

6. Preparation ofextract

The endophytic fungi was fermented in 3000 ml reagent bottle containing 2000 ml of PDA (potato infusion from 200g potatoes + 20g of dextrose, pH 5.1±0.2, 24g/L Hi- media) for 21 days at 23°C under static condition, in two replicates. Fungal mycelia were separated from the culture broth by passing through four layer of cheesecloth. The fermented broth was extracted with equal volumes of chloroform and dried by flash evaporation. The yield of the extract ranged from 0.1-0.2 mg/L fermented medium. The extract was tested for antioxidant and anticanceractivity.

7. In vitro antioxidant activity evaluation of fungalextract

***DPPH evaluation*(Tatiya*et al*.,2011)**

Percentage scavenging activity of free radicals of chloroform extract of endophytic fungi wasdetermined by technique given by Tatiya*et al*., 2007. DPPH (1, 1-diphenyl-2-picryl-hydrazyl)is a free radical (steady) and methanolic solution of it is utilized to assess the antioxidant activity of several natural compounds.100 µl of different concentrations (20-100 µg/ml in methanol) of hydroalcoholic extracts and 100 µl solution of DPPH (methanol 0.1 mM) were incubated at 37ºC for 30 minutes and change in absorbance of reaction mixture was read at 517 nm. An equal amount of methanol and DPPH was served as control.

TLC Profile or Fingerprinting Adsorbents forTLC

The two adsorbent materials frequently utilized for TLC are silica gel G and alumina G. G designation represents gypsum. Calcined gypsum, $CaSO_4$ ½ H_2O is also called Plaster of Paris. When exposed to water gypsum set in a rigid $CaSO_4.H_2O$, which binds the adsorbent, used for TLC. About 10- 13% by weight of gypsum is added as binder.

Stability of extract by TLC

This procedure also includes the utilization of adsorption system to separate a compound from a blend(Amalia*et al*.,2013;Jasprica*et al*.,2007). Separation depends on the interaction between the compounds in a blend and stationary phase. It is applicable in the separation of mixtures with low molecular weight.The stationary phase typically is 100g of silica gel dissolved in refined water to make a slurry. In the meantime, in certain occurrences Sephadex is relevant. The solution of silica gel is then poured in to a glass plate with dimensions 20cm × 20cm to

create a thickness of 1.5mm. It is then kept for 1h at 105°C to harden. Subsequently, 10mL of extract is infused into the lower part of the plate and permitted to spread. The plate is then carefully embedded into the separation chamber containing mobile phase and permitted to stand for 30 min. The mixtures contained in the combination will rise to different situations on the plate based on their solubility. Each compound separated is distinguished by working out its retardation factor which is the ratio of distance ventured out by the compound to the distance travelled by the solvent and compare it with that of a known compound. The compounds spotted are scrapped at various position utilizing spatula lastly re-extracted utilizing different solvents. Benefits incorporate less tedious, delivering clear spots, and stable to acid as solvent.

Preparing slurry of adsorbent

The slurry is most conveniently prepared in a wide mouth container. For smooth slurry without lumps, the silica gel is added to the solvent (water) and stirred. Finally stirred vigorously for thorough mixing.

Setting up the TLC plates

The glass plates were cleaned with soap and water and finally rinsed with 50% methanol and it was dried. Coating of plates has been done by pouring method.The plates were activated by heating in oven for 30 min at 105 ^{0}C.

Spotting of samples on plate

The small capillary is filled by dipping the pulled end into the solution to be examined and emptied the capillary by touching it tightly to the thin layer plate at a point about 1cm from thebottom.

Preparing a development chamber

The TLC glass chamber is saturated using mobile phase. The chamber was filled with mobile phase and covered the top. Permitted to saturate for around 30 min.

Developing TLC plates

After chamber development and spotting of samples on plate, it was placed in chamber. The level of the solvent in the chamber lower part should not be over the spot that was incorporated on the plate, as the material spotted will separate in the solvent pool instead of going through chromatography. Allowed the dissolvable (solvent) to run around 10 cm on the silica gel plate. Plates has been analyzed visually, using UV and after derivatization. $R_f$values of the spots were measured.

Table 1. *TLC of chloroform extract of Mucor sp..*

Adsorbent	Silica gel G (activated)
Plate size	10 x 20 cm
Thickness	0.4 mm
Activation temperature	110^{0}C for 10 minutes
Solvent system	Ethyl acetate: Ethanol: Formic acid 9 : 0.9 : 0.1
Detection	Modified dragendorff's reagent

Test solution:Dissolve 100mg of dried chloroform extract in chloroform.

8. Pharmacological investigations:

Acute ToxicityStudy

The acute toxicity investigation for endophyticfractions from *Catharanthusroseus* was carried out on male albino rats (20-30 gm) according to OECD principles. Rats were abstained overnight prior to investigation. Rats have not displayed mortality up to 5000mg/kg. So oral median lethal dose was thought to be above 5000mg/kg .The therapeutic doses selected were 200mg/kg and 150mg/kg (Stahl *et al*.,1962).

***Anticancer activity*(Sharmaet *al*.,1997; Zhuet *al*.,2007)**

The test rats were bifurcated into 5 groups with 6 rats in each group. All the rodents were held under standard research facility conditions. Rats were given standard pellet

diet and cleaned water *ad libitum*. The research protocol was approved by the IAEC. The initial body weights of all rodents in this test procedure were assessed.

Group I considered as a Normal control, for which tumor cell inoculation has not been done. The remaining sets of rats were inoculated with HT 29 tumor cells intraperitoneally. Group II served as a toxic control. Group I and II got saline (normal). Group III considered as reference medication, which was dealt orally with 5-flurouracil (5-FU, 30 mg/kg). Group IV and V were served with CEM fraction (150 and 200mg/kg) separately.

An examination time of 14 days was chosen to investigate and it is consider being sufficiently long to see ACF development. Animal weights were noted all through exploratory time and before sacrifice. All animals were put under Isoflurane anesthesia and relinquished toward the end of study. Blood has been accumulated by cardiac puncture to evaluate hematological parameters, such as hemoglobin (Hb), red blood corpuscles count (RBC), white blood cell number (WBC).

Statistical evaluation

Results are displayed in terms of mean ± SEM and examined subsequently by Dunnet's test. P values 0.001, 0.01, 0.05 were regarded as statistically significant. Data was explored using Graph pad prism (5.01).

III. RESULTS

1.Isolation of endophytic fungi from the leaves of Catharanthusroseus

The leaves of *Catharanthusroseus*weredivided into pieces of 1 cm and moved to Petri dishes containing water agar medium (2.5%) under aseptic environment. At regular intervals the plates were observed. Following one week the colonies (hyphal structures) of green color emerged from *Catharanthusroseus*leaves(Figure 1).

2. Preliminary identification ofcolony

Macroscopicidentification

Colony of fungi shows up precisely like Rhizopus, having free, cottony and cushy development. Colonies are white or brown colored in shading yet as it ages various pin headed structure grows on aerial mycelium.

Fig-2: Isolation and preliminary identification of endophytic fungi (*Mucor sp.*) from *Catharanthusroseus*.

Microscopicinvestigation

From the preliminary recognition of colony, the fungi was identified as *Mucor sp.***.**

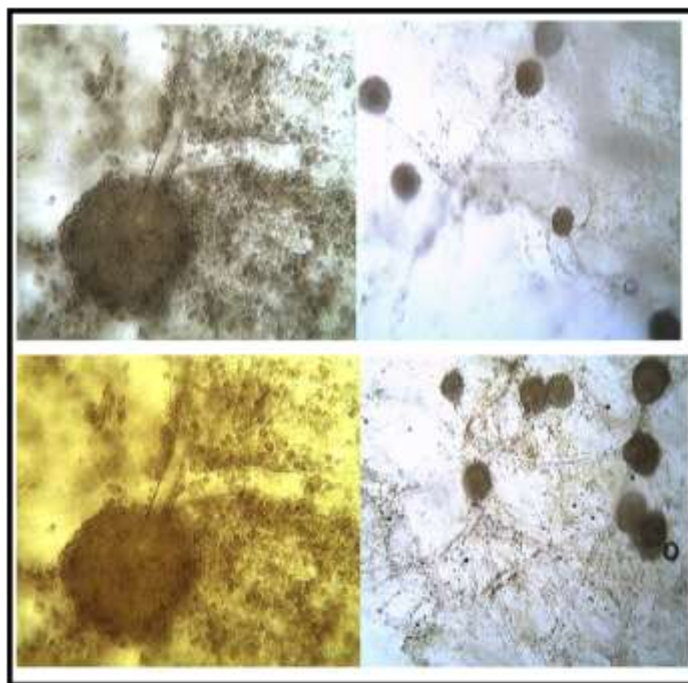

Fig-3: Microscopical observation of *Mucor*sp.

Mass production of Mucor sp..

PDA stock was utilized as large scale manufacturing medium. Fungal development mass was noticed in *Catharanthusroseus*(12-14 days) (Figure 3).

Fig-4: Mass production of *Mucor sp.*

Extraction

Fungal mycelia were isolated from the broth culture by permeating through four layer of cheesecloth. Extraction of fermented broth was done with chloroform by cool maceration and further evaporated for dryness.

0.1-0.2 g/L was the yield of the concentrate.

TLC Profile orfingerprinting

Fig-5: TLC profiling of Chloroform extract of *Mucor*sp.

Table 2. Colors and $R_f$values of spots by spraying with Modified dragendroff's reagent

Spot number	R_f Value	Color
1.	0.24	Dark brown
2.	0.37	Dark brown
3.	0.58	Dark brown
4.	0.73	Dark brown

In vitro antioxidant property assessment of fungalextract

DPPH scavenging activity

In this assay, Chloroform extract of *Mucor*sp.has shown a dose dependent rise in the DPPH radical scavenging property. Ascorbic acid (100µg) has exhibited 63.82 % scavenging property. Chloroform extract of *Mucor*sp.(100 µg/ml) has shown maximum scavenging activity i.e 82.85%. The results are summarized in Table 3.

Table 3. *In vitro* antioxidant property of Standard and Chloroform extract of *Mucor* sp.

Sample	Concentrations (µg/ml)	DPPH free radical supression (%) (Mean±SEM)	IC_{50} (µg/ml)
Control	-	-	-
Standard Ascorbic acid	20	37.72 ±0.45	40.72
	40	38.51 ±0.72	
	60	45.61 ± 0.14*	
	80	52.63 ± 0.21**	
	100	63.82 ± 0.86***	
Chloroform Extract of *Mucor* sp.	20	31.64 ± 0.71	38.14
	40	39.68 ± 0.61	
	60	56.81 ± 0.52**	
	80	68.16 ± 0.67**	
	100	82.85 ± 0.04***	

Data are represented as means ± SEM; comparison was made with the control group by Dunnett's test. The symbols represent statistical significance at *p<0.1, **p<0.01 and ***p<0.001.

Cytotoxic activity

Effect on body weight of animals

Table 4 addresses the body weight of the 4 groups of rats. Toward the finish of 2nd week, compare with initial body weights before treatment. Following 2 weeks,there is anincrease in body weight of all groups of animals.

Table 4. Body weights during the study

Treatment	Before treatment	Average increase in body weight (g)
Normal control	249.13 ± 2.61	253.23 ± 2.74
Pathological control	218.17± 11.81	251.4 ± 8.68[#]
Std. (5-Flurouracil 30 mg/kg)	206.3 ± 11.73	227.14 ± 10.31[*c]
CEM (150 mg/kg)	235.12 ± 13.43	248.17 ± 12.91[*c]
CEM (200 mg/kg)	217.2 ± 6.09	225.16 ± 6.74[*c]

CEM= Chloroform Extract of *Mucor* sp.

Values are mean ± SEM (n=6) data interpreted by dunnett's multiple comparison analysis as the post hoc test, #pathological control set vs normal control set; *all treated sets versus pathological control set; [c]p<0.05

Table 5. Effect of HT 29 and Chloroform Extract of *Mucor* sp. on ACF

	NO.OF FOCI CONTAINING				
SETS	**TOTAL NO. OF ACF**	**1 CRYPT**	**2 CRYPTS**	**>4 CRYPTS**	**% INHIBITION OF ACF**
Normal Control	--	--	--	--	--
Pathological Control	56.17 ± 1.83	22.11 ±1.27	18.3 ±0.71	10.74 ±0.24	--
Standard control 5-flurouracil	18.73 ±1.81[*]	12.17 ±1.15[*]	6.12 ±0.73[*]	1.78 ±0.21[*]	64.71
Low dose	32.86 ± 1.64[*a]	15.79 ±1.12[*a]	10.62 ±0.64[*a]	6.42 ±0.72[*a]	42.71
High dose	26.27 ±2.79[*c]	15.8 ±1.51[*c]	7.83 ±1.75[*c]	2.9 ±0.61[*c]	54.76

Values are mean ± SEM (n = 6), *all treated sets versus Pathological control set; [a]p<0.001; [c]p<0.05. All the data were evaluated by Dunnet's test**.**

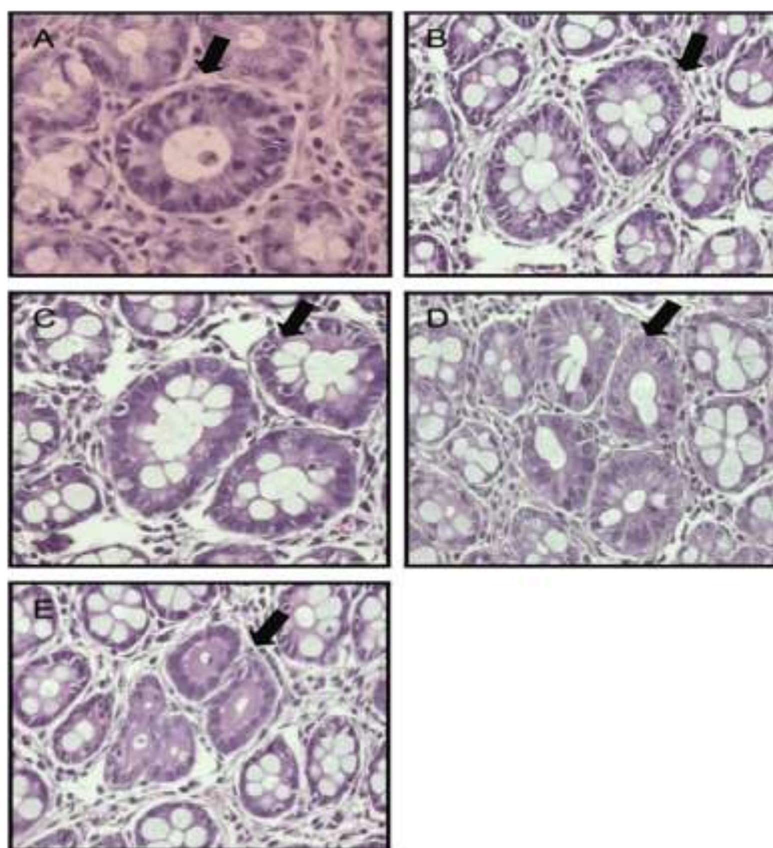

Fig-6: Microscopic observations of Aberrant crypt foci. A. NormalCrypt B. ACF displaying singleCrypts C. ACF displaying 2 crypts in wholemount D. ACF displaying 3 crypts in wholemount E. ACF displaying >4 crypts in wholemount.

HematologicalParameters

Hematological parameters of tumor bearing rodents found to be significantly alteredcompared to those of normal saline group. The total WBC count was found to be raised in pathological control rodents (p <0.001) when equated with the normal saline group. The WBC levels were brought down by both the extract groups in a dose dependent manner, however it was not effective as 5-flurouracil. RBC (Red Blood Corpuscles) counts were recovered back by both doses of endophytic crude fraction in a dose dependent manner, however it was less effective than 5-flurouracil. Treatment with Chloroform Extract of *Mucor sp*. 150 mg/kg and 200 mg/kg remarkably raised the hemoglobin percentage and RBC level towards the normal levels. The detailed data obtained from each hematological parameter is given inTable 6.

Table 6. Haematological Parameters

Groups	**Hb content (g%)**	**RBC (cells x 10^6/mm^3)**	**WBC (cells x 10^6/mm^3)**
Normal saline (5 ml/kg)	15.46 ± 0.17	7.26 ± 0.05	8.72 ± 0.05
Pathological control	9.93 ± 0.14$^{\#}$	4.02 ± 0.07$^{\#}$	19.64 ± 0.18$^{\#}$
Standard (5-FU 30 mg/kg)	14.82 ± 0.11^{*a}	6.21 ± 0.06^{*a}	8.62 ± 0.05^{*a}
CEM (150 mg/kg)	10.64 ± 0.13^{*c}	4.73 ± 0.01^{*a}	11.83 ± 0.24^{*a}
CEM (200 mg/kg)	11.76 ± 0.19^{*a}	5.59 ± 0.06^{*a}	9.01 ± 0.03^{*a}

Values are mean ± SEM (n = 6), *all treated groups vs Pathological control set; ^{a}p<0.001; ^{c}p<0.05. Entire data was evaluated by Dunnet's analysis**.**

IV. DISCUSSION

In present investigation preliminary evaluation was performed by directing qualitative chemical tests and the findings of screening exhibits that the endophytic crude fraction of *Vincarosea* possesses the significant class of constituents such as alkaloids. Moreover TLC was reformed with chloroform extract of *Mucor*sp. on trial and error basis. Finally the mobile phase was developed, in which maximum constituents were separated on form of bands having different colors. An effort has been made in the present investigation to evaluate antioxidant and anticancer property of chloroform endophytic fractions from *Catharanthusroseus*against HT-29 induced colon cancer in albino wistar rats. The chloroform endophytic fractions was subjected to *in vitro* antioxidant activity by DPPH scavenging activity and found to possess significant reducing power activity.

The endophytic fractions were subjected to acute toxicity investigations as per OECD guidelines. Since none of the animal died at 5000 mg/kg, two doses 150 mg/kg, 200 mg/kg were designated as effective doses for evaluating anticancer and antioxidantproperties. In this investigation the rats were treated with HT 29 cell lines for the development of aberrant crypt foci (Furtado *et al*.,2008).The results of current investigation indicated that endophytic crude fraction significantly inhibited the formation ofACF (Kumar*et al*.,2017). After completion of investigation, body weights of all animals recorded and compared with the initial body weights which indicates significant enhancement in body weights of all rodents due to genesis ofcancer. At the finish of investigation rats were sacrificed and development of pre-neoplastic lesions (ASC) was evaluated by histopathological examination. Blood was collected by heart puncture and all haematological parameters including WBC count, RBC count, Hb content were measured. Hematological parameters of tumor possessing rats were found to be significantlyalteredcompared to those of the normal saline group. The total WBC count was found to be increased in pathological control animals ($p < 0.001$) when compared with the normal saline group. The WBC levels were brought down by both the extract groups. RBC (Red Blood Corpuscles) counts were brought back by both the doses of endophytic crude fraction in a dose dependent manner. Treatment with CEM 150 & 200 mg/kg significantlyenhanced the hemoglobin content and RBC percentage towards the normal range.

Since the preliminary phytochemical examination of the fractions displayed the presence of alkaloidal compounds which have been recognized for their antioxidant and anticancer properties.It has been hypothesized that free radical generation is one of the principal cause of diseased condition. Endophytic fractions of Chloroform extract of *Mucor*sp.displayed remarkable antioxidant or inhibition of free radical generation and anticancer property.

V. CONCLUSION

Traditional methods of medications consistently assumed significant part in gathering the worldwide medical care needs. They are proceeding to do as such as of now and will assume significant part in subsequent years. The endophytic fungi *Mucor* sp. obtained from *Catharanthusroseus* which was recognized as *Mucor*sp. exhibited powerful antioxidant action in the current investigation. Antioxidant effect of fractions might be accountable for their anticancer action. The secondary metabolites present in endophytic fungus of the plant are accountable for free radical suppression and anti-neoplastic activity and is required to be investigated further.This information upheld the case that endophytes are an elective stock for novel auxiliary metabolites.

Abbreviations

ACF: Aberrant crypt foci; CEM: Chloroform extract of *Mucor*sp.; HT 29: Human colorectal adenocarcinoma cell line; ANOVA: Analysis of variance; DPPH:2,2-diphenyl-1-picrylhydrazyl; SEM: Standard error of Mean.

Conflict of Interest

Authors have declared that there are no conflicting interests with respect to this article.

Author's Contribution

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Author's Contribution

Kadiri Sunil Kumar andSamaresh Pal Roy carried out the research workand Rasapelly Ramesh drafted the manuscript, acquired and analyzed the data and revised the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

All data analyzed in the current research is available from corresponding author on reasonable request.

Declarations

Ethical issue

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed(approval No.SDPC-AFC/2013/66).All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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