INTERNATIONAL JOURNAL OF UNANI AND INTEGRATIVE MEDICINE

E-ISSN: 2616-4558
P-ISSN: 2616-454X
IJUIM 2018; 2(4): 26-34
Received: 15-08-2018
Accepted: 20-09-2018

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Antioxidant and anticancer activities of endophytic-crude fraction of Sadabahar [*Catharanthus roseus* (L.) G.Don] in rats

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Abstract
An attempt has been made to isolate, identify and screen the endophytic crude fractions of Periwinkle (*Catharanthus roseus* (L.) G.Don) leaves for antioxidant activity and also for 1, 2-Dimethylhydrazine (DMH) induced colon cancer. Endophytic fungi from *Catharanthus roseus* (L.) G.Don leaves were isolated and cultured aseptically in PDA media. The developed fungus cultures were fermented in potato dextrose broth and fractionated using chloroform. Isolated fungus was identified from *Mucur Sp.* in preliminary identification method. Chroloform extract-1 of *Mucor sp.* (CEM-1) was subjected to free radical scavenging activities against 2, 2-diphenyl-1- picrylhydrazyl, hydroxyl free radicals and reducing power assay. The fraction was further assessed against DMH induced colon cancer in rats. DMH was induced subcutaneous route twice a week for 2 weeks with 40mg /kg body weight. The crude fractions were administered to the rats five times a week for four weeks by gavages at doses of 100mg/kg and 150mg/kg body weight/day each, during and after DMH treatment. All animals were sacrificed in week 5 for the evaluation of ACF. The colons were dissected out for histopathological study to find out aberrant crypt foci (ACF) % inhibition. Fraction was found to be potential free radical scavenger with IC_{50} values of 104.07µg/ml & 341.13µg/ml for DPPH and hydroxyl radical respectively. It also shows increasing order reducing power activity with significantly % inhibition in ACF. Therefore these data support the claim that endophytes are an alternative source for novel secondary metabolites.

Keywords: *Catharanthus roseus* (L.) G.Don, endophytes, aberrant crypt foci, colon cancer

Introduction
Endophytes, which survive in the tissues of living plants, are mainly unstudied as potential sources of novel natural products for exploitation in medicine [1]. While the symptomless nature of endophytes occupation in plant tissue has prompted focus on symbiotic or mutualistic relationships between endophytes and their hosts, the observed biodiversity of endophytes suggests they can also be aggressive saprophytes or opportunistic pathogens [2]. The most frequently isolated endophytes are fungi, but bacteria also form endophytic relationships. The vast majority of plants have not been studied for the endophytes that may inhabit them [2]. Endophytes are likely to be a rich and reliable source of genetic diversity and biological novelty, and experience has shown that newly found endophytic microbes usually produce novel natural products [1, 3]. Recent interest has increased considerably in finding naturally occurring products for medicinal materials to replace synthetic drugs, which are being restricted due to their carcinogenicity [4]. Many medicinal plants contain large amounts of antioxidants such as flavonoids, polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides, thus endophytes can be a new field to evolve novel drug discovery and alternative source for natural products of various medicinal plants to conserve them.
Fungi are one of the major sources of natural bioactive compounds. Over 4000 bioactive metabolites fungal origin have been described. In some cases, plant associated fungi are able to make the same bioactive compound as the host plant itself. One of the best examples of this is the discovery of gibberelines in *Fusarium fujikuroi* in the early 1930s. Eventually it was learnt that the gibberelines are one of only five classes of phytohormones that are to be found in virtually all plants [5]. This observation led to the prospect that endophytic fungi, associated with *Taxus brevifolia,* may also produce Taxol. Taxol itself is the world's first billion dollar anticancer drug and its main source is *Taxus spp.* potentially,

a fungal source of taxol would reduce its price and save the plant from extinction in some areas. The success of finding fungal taxol has produced a paradigm for still other biodactive compounds to be found in endophytic microbes. Some compounds from endophytic fungi has been identified as producing bioactive compounds and their application in biotransformation process [6]. The diterpenoid Taxol interferes with the multiplication of cancer cells, reducing or interrupting their growth and spreading [7]. After that, Taxol has also been found in a number of different genera of fungal endophytes either associated or not to yews, such as *Taxodium distichum*; *Wollemia nobilis; Phyllosticta spinarum*; *Bartalinia robillardoides*; *Pestalotiopsis terminaliae*; *Botryodiplodia theobromae* [5]. Camptothecin a another important anticancer compound, a potent antineoplastic agent which was firstly isolated from the wood of *Camptotheca acuminata* Decaisne (Nyssaceae) in China. Vincristine the terpenoid indole alkaloid derived from the coupling of vindoline and catharanthine monomers, are two of the well-known anticancer agents. Yang *et al*. also found an unidentified vincristine producing endophytic fungus from the leaves of *C. roseus* in 2004 [8]. Zhang *et al*. successfully discovered an endophytic *Fusarium oxysporum* from the pholem of *C. roseus* that could produce vincristine [9]. Vinblastine the terpenoid indole alkaloids derived from the coupling of vindoline and catharanthine monomers, are two of the well-known anticancer agents. Guo *et al*. First reported an endophytic fungus *Alternaria* sp. isolated from the phloem of *Catharanthus roseus* (L.) G.Don that had the ability to produce vinblastine in 1998 [10].

Traditionally, *Catharanthus roseus* (L.) G.Don, has been used as a folk remedy to cure diabetes and high blood pressure. It was believed to promote insulin production or to increase the body's utilization of sugars from food. A few studies on injectable and oral *Catharanthus roseus* (L.) G.Don extracts have shown some antidiabetic effects in pet animals, but no human studies have revealed significant reduction in blood sugar. During the 1950s *Catharanthus roseus* (L.) G.Don was shown to contain a number of chemicals in the alkaloid class. Alkaloids are bitter-tasting plant compounds that contain nitrogen. Many of them have pain relieving or anticancer properties. At least two of the alkaloids in *Catharanthus roseus* (L.) G.Don (vinblastine and vincristine) have been developed into prescriptions for anticancer drugs. These injectable drugs and their derivatives (such as vinflunine) work in several ways that interfere with the division of cancer cells. Recently, it has been shown that chemicals in *Catharanthus roseus* (L.) G.Don might also prevent the growth of new blood vessels that support tumor growth. It has been also discovered that vincristine is produced by *Fusarium oxysporum*, an endophyte of this host, while another group has isolated vinblastine from an endophytic fungus [10]. However, before these microbes can be utilized and developed as useful product, extensive work related to maximizing fermentation for the production of the compound, getting clinical tests performed and obtaining governmental approval are still required. However, these initial steps of product discovery point the way for future approaches to be taken by examining medicinal plants for their endophytes and then systemically studying product isolation and characterization of bioactive products. As *Catharanthus roseus* (L.) G.Don is well documenented for its various medicinal attributes, such as its anticancer properties, as well as its wound healing and its antiseptic effects in the tribal areas of the Indian sub-contiment, it was selected for as a source plant to examine the population of endophytic fungi [5]. Endophytes are the chemical synthesizers inside plants. Many of them are capable of synthesizing bioactive compounds that can be used as potential sources of pharmaceutical leads. Endophytic fungi have been proven useful for novel drug discovery as suggested by the chemical diversity of their secondary metabolites. Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds belonging to the alkaloids, steroid, flavanoid and terpenoids [10]. Hence based on the previous literature that endophytes of medicinal plants are the potential source for secondary metabolites and possess the same biological activity, it was thought worthwhile to isolate fungal endophytes from *Catharanthus rosea* and assess the crude fractions scientifically for *in vitro* antioxidant and colon cancer activities in animal models.

Materials and Method

Plant Material

The plant required for the study i.e. leaves of *Catharanthus roseus* (L.) G.Don was collected from the local region of Surat in the month of December of year 2012. The plant, *Catharanthus roseus* (L.) G.Don has been authenticated at Navsari Agricultural University, Navsari, Gujarat and Herbarium specimen was submitted at Shree Dhanvantary Pharmacy College (SDPC), Kim, Gujarat, Surat.

Collection of Materials

1, 2-Dimethyle hydrazine and 5-fluorouracil were obtained as gift samples from Shree Dhanvantary pharmaceutical analytical and research centre (SDPARC), Kim, Surat. DPPH, Deoxy D-ribose, Potassium ferricyanide, Trichloroacetic acid, Ferric chloride and potato-dextrose-agar (PDA) were obtained from Himedia Laboratories, Mumbai, India.

Isolation of fungal endophytes

Leaves from Periwinkle plant were cleaned under running water to remove soil and adhering dust particles and then air dried. Leaves were surface sterilized under aseptic condition by 4% sodium hypochlorite for 5 min, 70% ethanol for 1 min and sterile distilled water for 1 min 2–3 times 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 leaves and to excise inner tissues. The surface sterilized leaves were transferred to an alcohol sterilized mortar and macerated separately in to suspension using distilled water and serial dilutions were made. The diluted aliquots were transferred on sterile potato-dextrose-agar (PDA) plate. After incubation at 25°C for 7–14 days, predominant isolates of fungi were picked up and purified. Culture purity was determined from colony morphology [11].

Preliminary identification of fungal colony.

Preliminary identification was done at microbiology laboratory of SDPARC. The morphology of surface texture, pigmentation and spores at the hyphal tips were used as tool to identify the endophytic fungi at species level. The fungal isolates mounted on the sterile slides then it was stained

with lacto phenol cotton blue and then examined in 100X 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 Petridis which contains sterile PDA media. To preserve as a pure culture, the endophytic fungi was inoculated in PDA slant [12, 13].

Fermentation and Extraction

Endophytic fungi isolates was grown on PDA plates at 25°C for 7–14 days depending on growth rate. Purified isolates fungus was inoculated and fermented separately into a 3000 ml Erlenmeyer flask containing 600 ml of potato-dextrose broth (potato infusion from 200 g potatoes+20 g of dextrose, pH 5.1± 0.2, 24 g/L). After incubation at 25°C for 21 days under stationary condition, the fungi culture was filtered through four layer of cheese cloth and homogenized at 4000 rpm to separate the mycelia from broth. The filtrate was extracted with 300 ml chloroform three times. The organic phase was separated to dryness under reduced pressure using rotary evaporator (Superfit Rotavap, PBU-6) and weighed to constitute crude extract then the crude fraction was tested for free radical scavenging activity. Fractions shown potential *in vitro* free radical scavenging activity thus considered for *in vivo* study [14, 15].

***In vitro* free radical scavenging activity**

Reaction with DPPH radical

The scavenging effect of CEM-1 (20–220 µg/ml) against DPPH stable radical was determined using ascorbic acid (ASC, 1–5 µg/ml) as standard. Plotting the percentage for DPPH scavenging against ASC concentration gave the standard curve [16].

Reaction with hydroxyl radical

Steady state hydroxyl radical scavenging activity of CEM-1 (50–500 µg/ml) was measured by degradation of deoxy-D-ribose method. Mannitol was used as standard for comparison. Different concentrations (0.5–5.0 µg/ml) of mannitol were mixed as explained above. Plotting the percentage inhibition of *OH scavenging against mannitol concentration gave the standard curve [16].

Determination of reducing power

The reducing power of CEM-1 (50–250 µg/ml) was determined according to the method previously reported. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, the absorbance of which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. Ascorbic acid was used as standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was taken and is expressed as mean± S.E.M [17].

Animals

Albino Wister rats weighing 80–120 g were used in the study. The inbred colonies of rats were collected from Mahaveera Enterprises Hydrabad, Andhra Pradesh. They were maintained in the Animal Facilitation Centre (AFC) of SDPARC, Kim, Surat for experimental purpose. The animals were maintained under controlled conditions of temperature (23±2°C), humidity (50±5%) and 12-h light-dark cycles. All the animals were acclimatized for 7 days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. Animals were habituated to laboratory conditions for 48 h prior to experimental protocol in order to minimize any, non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of SDPC, Kim, Surat (Ref No: SDPC-AFC/2013/66), according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India [11].

Pharmacological study

Acute Toxicity Study

The acute toxicity tests for endophytic fraction from *Catharanthus roseus* (L.) G.Don was performed on albino rats of female rats weighing between 180-200 gm as per OECD Guidelines 425. The animals were fasted overnight prior to the experiment. The animals did not show any mortality up to 5000mg/kg, thus oral LD50 was assumed to be more than 5000mg/kg. The doses were selected as 1/10th of 2000mg/kg (200mg/kg and 150mg/kg) [18].

Anticancer Activity

Experimental design

Group I: Normal control (Saline)
Group II: Pathological control (DMH)
Group III: Standard preparation (5-flurouracil 30mg/kg)
Group IV: CEM-1(150 mg/kg)
Group V: CEM-1 (200 mg/kg)

The experimental animals were divided into 5 groups with 6 animals in each group. All animals were kept under standard laboratory conditions. Animals were provided with commercial pellet diet and purified water *ad libitum.* The study protocol was approved by the institutional Animal Ethics Committee. The initial body weights of all animals in this study protocol were measured. Group I served as a normal control, for which chemical induction was not done. The remaining group animals were induced with 1, 2-dimethyle hydrazine subcutaneously. Group II served as a pathological control. Group I and II received normal saline. Group III served as a reference drug, which was treated orally with 30 mg/kg body weight of 5-flurouracil (5-FU). Group IV and V were treated with CEM fraction at 150 and 200mg/kg body weight respectively [19].

A study period of 5 week was selected for the study and it is consider being long enough to observe the formation of ACF**.** The animals' weights were recorded throughout the experimental period and the blood was collected by heart puncture for the evaluation of hematological parameters prior to sacrifice, which included hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC) [20]. All animals were placed under anaesthesia with sodium pentobarbital (45 mg/kg body weight, i.p.) and sacrificed four weeks after the after the first DMH treatment by exsanguinations [20].

Histopathological study for animals

ACF assay

After laprotomy colons were excised, flushed with 0.9% saline, cut open along the longitudinal axis, and fixed in

10% phosphate buffer formalin (pH 6.9-7.1) for 24 hours. Immediately before analysis, the colon was stain with 0.02% methylene blue for 5 mins, mounted on microscope slides with mucosal side facing upward, and observe under a light microscope at 100x magnification. ACF are easily visualised on the background on normal crypts since aberrant crypts have larger, often elongated openings and thicker lining of epithelial cells as compare to normal crypts. The number of ACF and crypt multiplicity (number of crypts in each focus) were recorded. The multiplicity of ACF is expressed as aberrant crypts (AC)/focus. Each colon specimen was examined by at least three observers in a double-blind manner [20].

Statically Analysis

The data are expressed as the mean ± SEM and analysed statically by one way ANOVA followed by Dunnet's test. P values 0.001, 0.01, 0.05 were considered as statically significant. Data were analysed using Graph pad prism 5.01.

Result

Catharanthus roseus (L.) G.Don leaves found to harbor various fungi. Seven different colony were isolated among them one fungi was selected for anticancer activity. The yield of chloroform fungal crude extract was from 60-80 mg/ litre per litre of fermented medium.

Preliminary identification of fungal colony

The leaves of *Catharanthus roseus* (L.) G.Don was divided into pieces of 1 cm and transferred to Petri dishes containing 2.5% water agar medium under aseptic conditions. Plates were observed at regular intervals. Macroscopic identification of fungle colony was appears exactly like *Rhizopus*, having loose, cottony and fluffy growth. Colonies are white or brown in color but as it ages numerous pin headed structure develops on aerial mycelium (Fig.1B). From the preliminary identification of colony the fungi was identified as *Mucor sp.* (Fig.1).

Antioxidant activity of CEM-1

DPPH assay

DPPH assay method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. Reaction with DPPH radicals of CEM-1 showed scavenging activity. The IC50 value for CEM-1 was found to104.07 µg/ml where as IC50 value for ascorbic acid was found to be 3.47µg/ml. A linear correlation coefficient (r^2 = 0.96613) was obtained (Fig.2).

Hydroxyl Radical Scavenging assay

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves *in vitro* generation of hydroxyl radicals using Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. The intensity of yellow color formed is measured at 412 nm spectrophotometrically against reagent blank Results presented indicates that phenyl hydrazine in solution generates *OH radicals as measured by 2-Deoxyribose degradation assay. It was found that activity of chloroform endophytic fractions from *Catharanthus roseus* (L.) G.Don has *OH radicals scavenging activity same as mannitol scavenges the *OH radicals and inhibited the production of TBA reactive species significantly over a period of 1 h. IC50 values was found to be 341.13 µg/ml for CEM-1, whereas, IC50 value of mannitol was found to be 4.37 mg/ml (4370 µg/ml). A linear correlation coefficient (r^2 = 0.99622) was obtained (Fig.3).

Reducing power

Results showed that as the concentration of ASC and CEM-1 (25–250 µg/ml) was increased, the absorbance increased for both standard as well as CEM-1 fraction. This depicts that fraction has reducing power activity (Fig.4).

Pharmacological studies (Anti cancer activity)

Effect on body weight of animals

Body weights of the rats for given test doses were evaluated and the treatment groups were compared to the pathological control group over the experimental period of 4 weeks. 1,2 dimethyle hydrazine induced groups did not appreciably decrease rat body weight when compared with normal control over this short time frame, nor did any treated groups reduces body weights during the experimental period (Table 1). The doses for CEM-1 tested, were find the good inhibitors of colonic ACF.

Heamatological Parameters

The initiation of the study and immediately before necropsy, blood samples were collected for haematological analysis in EDTA tubes and differentially quantified through a coulter for the following: WBC, RBC and haemoglobin (Hb) content determination (Table 3).

Histopathological study of animals

Effect of standard and test drug on ACF

Table 2 summarizes the results of the efficacy of test agents in inhibiting DMH induced foci in rat colon. At the end of study rats were sacrificed and development of preneoplastic lesions was evaluated by histopathological studies that show treatment with CEM-1 of significantly reduced the frequency of ACF numbers while comparing with pathological control but not as much effective than 5-flurouracil.

Discussion

Plants have provided mankind with sources for healing purposes for millennia. Some representative and well known medicines derived from plants are quinine, digitalin, taxol, aspirin, ipecac, reserpine, etc. Some plants have literally been threatened with extinction as a result of the enormous pressure brought upon them by virtue of their healing properties, e.g. various yew species for their taxol and taxane content. Microorganisms, especially fungi, have long been regarded as an important source of novel metabolites with promising anti-bacterial, anti-mycotic and anti-viral activity. Fungi are one of the major sources of natural bioactive compounds. Over 4000 bioactive metabolites fungal origin have been described. In some cases, plant associated fungi are able to make the same bioactive compound as the host plant itself [5]. Some compounds from endophytic fungi have been identified as producing bioactive compounds and their application in biotransformation process [5]. As *Catharanthus roseus* (L.) G.Don is well documenented for its various medicinal

attributes, such as its anticancer properties, as well as its wound healing and its antiseptic effects in the tribal areas of the Indian sub-contiment, it was selected for as a source plant to examine the population of endophytic fungi. Ultimately, this is the important step in the discovery of other biologically active compounds made by endophytes of this important medicinal plant[5]. Endophytes are the chemical synthesizers inside plants. Many of them are capable of synthesizing bioactive compounds that can be used as potential sources of pharmaceutical leads. Endophytic fungi have been proven useful for novel drug discovery as suggested by the chemical diversity of their secondary metabolites. Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds belonging to the alkaloids, steroid, flavanoid and terpenoids [10]. *Vinca rosea* is in the Apocynaceae family, well known for being rich in alkaloids. The extracts of *Vinca rosea* have demonstrated significant anticancer activity against numerous cell types [21].

In the present study, the pharmacognostic standards for the leaves of *Catharanthus roseus* (L.) G.Don is laid down. The macroscopic studies carried out to authenticate the plant *Catharanthus roseus* (L.) G.Don revealed that the characteristics of various parts were identical to those reported earlier. Plants and endophytic fungi present in the host plants show their biological activity through their secondary metabolites, those are actually the biologically active constituents of the plant. With that respect the quality as well as quantity of such phytoconstituents must be evaluated during standardization of the plant drug. In present study preliminary screening was performed by conducting qualitative chemical tests and the results of screening shows that the endophytic crude fraction of *vinca rosea* contains the major class of constituent viz., alkaloids**,** thus attempt has been made in the present study to evaluate antioxidant and anticancer activity of CEM-1 against 1,2 dimethyl hydrazine induced colon cancer. Preliminary phytochemical investigation of CEM-1also revealed the presence of flavanoids, volatile oil as secondary metabolites. CEM-1 fraction was found to be potential with minimum IC50 values for anitioxidant activity thus further selected for *in vivo* studies. The endophytic fraction was subjected to acute toxicity studies as per OECD guidelines. Since no death was observed at 5000 mg/kg, two doses 150mgkg and 200mg/kg doses were taken as effective doses for screening anticancer and antioxidant activities.

In this study the rats were treated with 1, 2 dimethyl hydrazine for the development of Aberrent crypt foci [20]. It is recognized that colon carcinogenesis is a multistep process that includes sequential selection and propagation of preneoplastic lesions. In present study, analysis of ACF demonstrated the protective effect of endophytic fraction against DMH-induced preneoplastic foci in the rat colon. *In vivo* studies have shown that DMH is metabolized to azomethane, azoxymethane, methylazoxymethanol, ethane, and carbon dioxide [22]. Furthermore, DMH has been reported to induce carcinogenesis in rats because of the high production of reactive free radicals that react with DNA, thus demonstrating its genotoxic effect [23]. Although the mechanisms underlying the protective effect against ACF formation are not clearly understood, the inhibitory action of fractions might be due to their antioxidant activity. The inhibitory effects of antioxidants on ACF were also observed in other studies. Morioka *et al*. (2004) showed that *Peucedanum japonicum*, a traditional herbal plant in the Ryukyu Islands and antioxidant, inhibited ACF formation induced by azoxymethane carcinogen [24]. ACF are present in carcinogen- treated rodent colons as well as in humans at high risk for colon cancer development and in patients with colon cancer. Several studies investigating the genotypic, morphological and growth features of ACF have supported the contention that ACF are preneoplastic lesions. The ACF system is frequently used to identify and study the modulation of colon carcinogenesis. The result of present study indicated that endophytic crude fraction significantly inhibited the formation of ACF [24]. The blood parameters evaluation support anticancer results and completion of study body weights of all animals indicates significant increase in body weights of all animals due to formation of cancer [19].

At the end of study rats were sacrificed and development of preneoplastic lesions was evaluated by histopathological study [20]. The ACF were almost restored back by both dose of endophytic crude fraction in a dose dependent manner; however it was not efficiently as 5-flurouracil. Since the preliminary phytochemical analysis of the fractions showed the presence of alkaloids compounds which have been known for their antioxidant and anticancer activities. It has been hypothesized that one of the principal of generation of free radicals in diseased state. Thus the antioxidant activity or the inhibition of the generation of free radicals is important in the protection against cancerous state. The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. Endophytic fraction CEM-1 shows significant antioxidant and anticancer activity.

Conclusion

Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. Indian system of medicine has a deep root in our cultural heritage to the medicare of large section of our population. This system mainly uses herbs. In recent time there has been a market shift towards herbal cures because of the pronounced cumulative and irreversible effects of many modern drugs. The chloroform extract of endophytic fungi *Mucor sp.* from *Catharanthus roseus* (L.) G.Don which identified as *Mucor sp*. shows potent antioxidant activity in the present study. This antioxidant effects of fraction may be responsible for their anticancer activity may be due to the secondary metabolites of endophytic fungus of *Catharanthus roseus* (L.) G.Don, needed to study further. These data supported the claim that endophytes are an alternative source for novel secondary metabolites.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Authors express his thanks to the fraternity of Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India and Shree Dhanvantary Pharmaceutical Analysis & Research Centre (SDPARC), Gujarat, India and other collaborators for financial assistance for this research work.

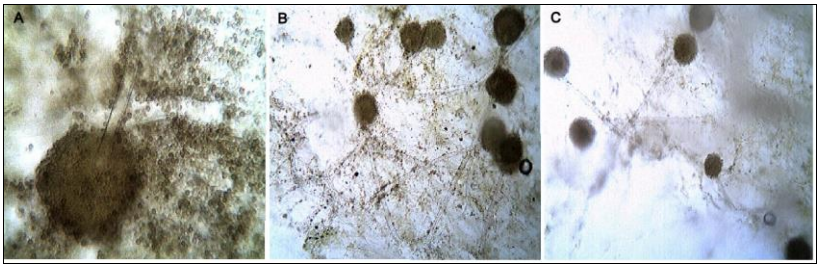

Fig 1: Macroscopically observation of *Mucor sp.*

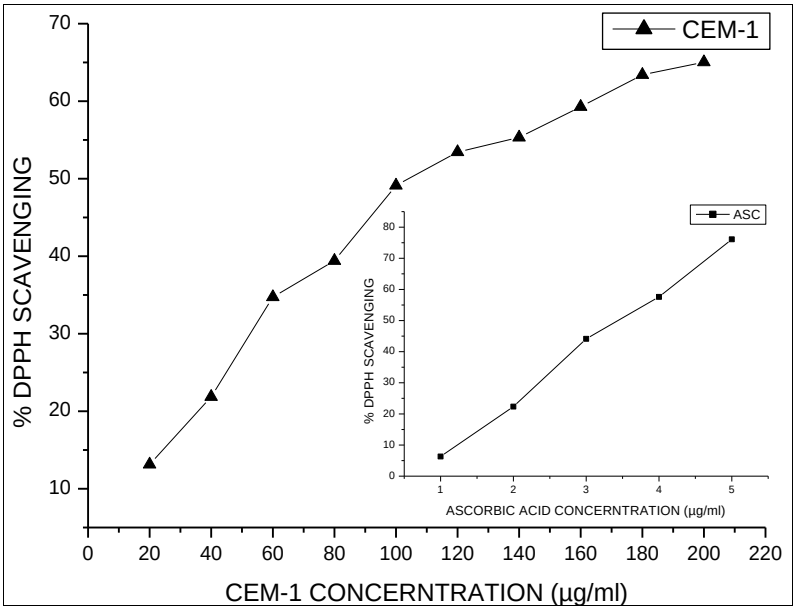

Fig 2: DPPH radicals scavenging activity of CEM-1 an endophytic fungal fraction of *Catharanthus roseus* (L.) G.Don in comparison with ascorbic acid (ASC) as a standard. All values reported as Mean±S.E.M (n=3).

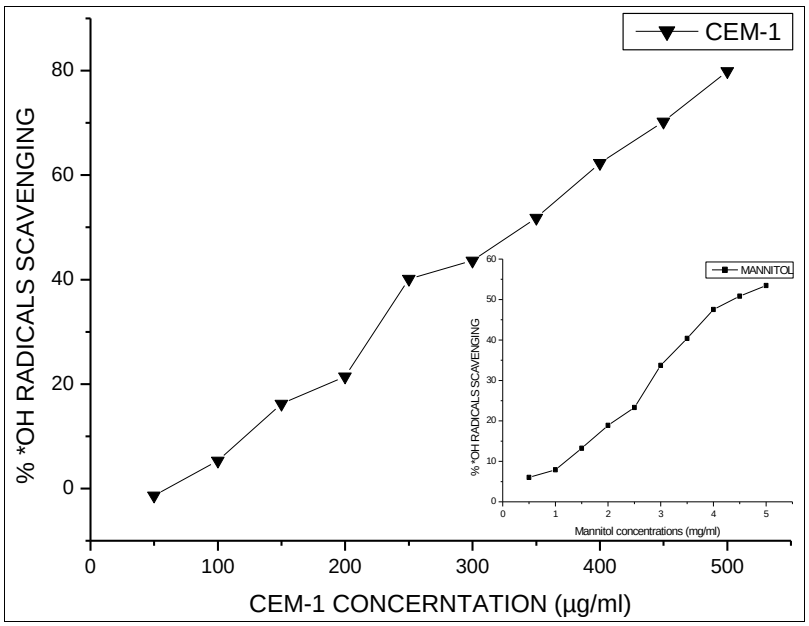

Fig 3: Hydroxyl scavenging activity of (CEM-1) an endophytic fraction of *Catharathus roseus*.

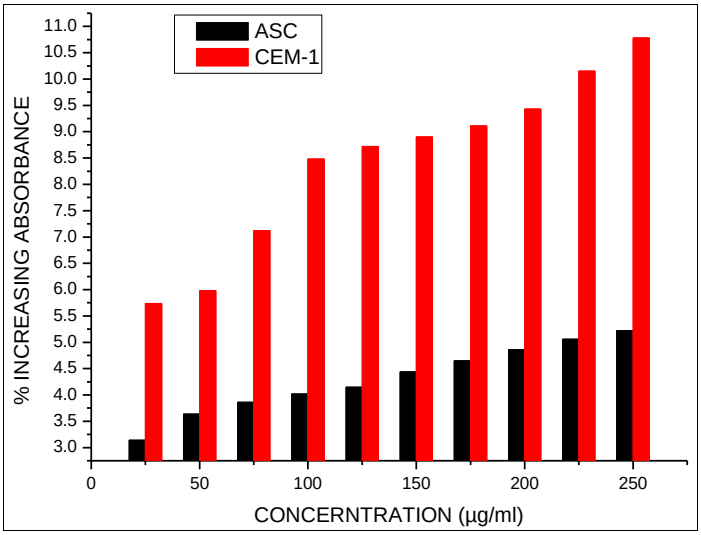

Fig 4: Reducing power activity of CME-1 an endophytic fraction of *Catharanthus roseus* (L.) G.Don in comparison with ascorbic acid (ASC). All values reported as Mean±S.E.M (n=3).

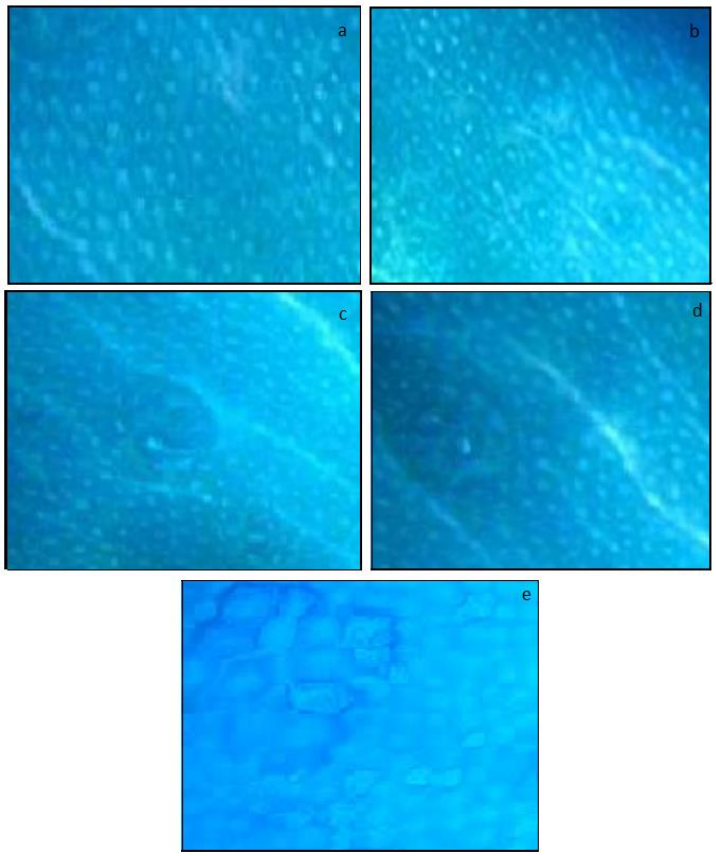

Fig 5: Microscopic observation of aberrent crypt foci (a) Normal Crypt, (b) ACF showing single Crypts, (c) ACF showing 2 crypts in whole mount, (d) ACF showing 3 crypts in whole mount and (e) ACF showing >4 crypts in whole mount.

Table 1: Body weights during the study

Groups	Before Treatments (g)	After Treatments (g)
Normal control	245.33 ± 2.73	250.66 ± 2.99
Pathological control	222.17 ± 10.64	252.5 ± 8.92#
Standard (5-flurouracil 30 mg/kg)	205.5 ± 13.89	221.17 ± 13.39*c
CEM(150 mg/kg)	221.83 ± 16.83	247.67 ± 14.52*c
CEM(200 mg/kg)	214.5 ± 8.07	227.33± 8.70*c

Values are mean ± SEM (n=6) data analysed by one way ANOVA followed by Dunnett's multiple comparison test as the post hoc test, #pathological control group versus normal control group; *all treated groups versus pathological control group; cp<0.05.

Table 2: Effect of 1, 2-Dimethylehydrazine and CEM-1 on ACF

Groups	No. of Foci Containing				%Inhibition of ACF
	Total no. of ACF	1 crypt	2 crypt	>4 crypt	
Normal control	0.00	0.00	0.00	0.00	0.00
Pathological control	53.83 ± 1.64	24.16±1.51	18.5±0.89	10.83±0.95	0.00
Standard	18.33 ±2.33*	12.00±1.65*	6.17±0.98*	1.33±0.49*	65.95
Low dose	31.83± 1.70*a	15.67±1.71*a	10.17±0.87*a	6.00±0.82*a	40.87
High dose	25.33±2.23*c	15.5±1.69*c	7.33±1.45*c	2.5±0.67*c	52.95

Values are mean ± SEM (n = 6), *all treated groups versus Pathological control group; a p <0.001; c p <0.05. All the data were analysed by one way ANOVA followed by Dunnet's test.

Table 3: Hematological parameters

Groups	Hb content (g%)	RBC (cells ×10^6/mm^3)	WBC (cells ×10^6/mm^3)
Normal saline (5 ml/kg)	15.13 ±0.15	7.28 ± 0.08	8.66 ± 0.09
Pathological Control	9.95 ±0.13#	4.08 ± 0.05#	19.36 ±0.19#
Standard (5-flurouracil 30 mg/kg)	14.04±0.14a	6.21 ±0.06a	8.66± 0.09a
CEM-1 (150 mg/kg)	10.55.±0.18c	4.77. ± 0.03a	11.43± 0.29a
CEM-1 (200 mg/kg)	11.53.±0.13a	5.51 ± 0.08a	9.06 ± 0.06a

Values are mean ± SEM (n = 6), #Pathological control group versus normal control group; *all treated groups versus Pathological control group; ap <0.001; bp <0.01. All the data were analysed by one way ANOVA followed by Dunnet's test.

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