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Screening of Anticancer Potential and DNA Damage Ability of *Syringodium isoetifolium* against HeLa Cell Line

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ABSTRACT

Cervical cancer is a kind of cancer, which mainly occurs in the lower portion of uterus. Human papilloma virus plays a major role in the occurrence of cervical cancer. Major symptoms are vaginal bleeding, foul odour in vagina, pelvic pain, *etc.* It is mostly diagnosed by biopsy. Chemotherapy, radiation and surgery are the widely used treatments for cervical cancer, but the main drawback was their side effects. To avoid these conditions people might follow the ancient methods of treatment. Medicinal plants got wide attention in the current society to cure various diseases. The seagrass obtained from the seashores possess a wide range of medicinal properties like anticancer activity. In this study, the anticancer potentials of the seagrass, *Syringodium isoetifolium* was checked with the help of MTT and Comet assays. The various functional compounds present in the seagrass were also detected by FT-IR analysis. From the results obtained from MTT assay, the least inhibitory concentration of the sample was found to be 35.63 µg/mL. Further results obtained from the Comet assay prove that the sample has the ability to kill the cancer cells. This study concludes that the methanolic extract of *Syringodium isoetifolium* can be used as an alternative and marine based source for the treatment of cervical cancer.

KEYWORDS

Syringodium isoetifolium, DNA damage, Cervical cancer, Anticancer, Comet assay, MTT assay.

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INTRODUCTION

Cervical cancer could even be a big condition, with nearly 5 lakhs women were affected by the disease annually throughout the world, in which most of the cases were found in less developed countries due to the unavailability of proper screening systems. In undeveloped countries, it is the foremost reason for death occurs due to cancer. Cervical cancer is regarded as the second most harmful disease among women worldwide [1]. The development of cervical cancer is mainly mediated by human papillomavirus (HPV), where HPV DNA was found in 90% of the squamous cervical cancers [2]. The virus is acquired mainly through gender. Patients who administrated with immunosuppressive agents and folk who are affected by HIV are at high risk of developing cervical cancer [3]. About

80% of cervical cancers were developing from the previously infected squamous cells. In developing countries, Adenocarcinoma occurs in cervix is responsible for 20% of invasive cervical cancers [4].

Seagrasses are group of marine flowering plants, which is usually seen in underwater and it provide shelter or food for the marine organisms. They are necessary factors for the nearshore ecosystem and it acts as an indicator for determining the environmental quality [5]. Seagrass beds were spread from the tropics to boreal margins of the ocean [6]. The leaves of the seagrass act as a substratum for the attachment of epiphytic microalgae and gives shelter for invertebrates and fishes that reside in large densities [7,8]. When compared to the terrestrial ecosystems, Seagrasses possess only a low biomass, but they have a high biomass than the plankton-type communities. Exceedingly productive seagrass ecosystems provide a mixture of food and shelter that allows great biomass and productivity of commercially vital fish species [6,9]. Seagrasses gives a major nursery site for plentiful species that helps offshore fisheries and for adjacent habitats like salt marshes, shellfish beds, coral reefs and mangrove forests. Throughout the world, people around the coastal areas consume majority of the protein from seagrass [10].

Syringodium isoetifolium is otherwise called as Noodle grass and are often found in tropical and subtropical marine environments [11]. It is rarely found in shallow intertidal areas and the places with high nutrient availability. It is commonly seen in Western Pacific shores including sea, South China, Gulf, Maldives, Andaman & Nicobar and Lakshadweep islands (India). The seagrass contains high phenolic, tannin and antioxidant content. *Syringodium isoetifolium* has the potential to act as an antioxidant agent and it also shows promising antimicrobial and antitumor activity [12].

EXPERIMENTAL

Sample collection: The seagrass, *Syringodium isoetifolium* were collected aseptically from the shores of Pamban, Rameshwaram city, India and it was transferred in to a sterile container. The collected sample was shade dried for few days to remove the moisture content present in the sample. Dried samples were further grinded to get the powdered form and stored in an air tight container.

Extraction of sample: The sample was extracted by percolation method. In this method, the powdered form of *Syringodium isoetifolium* was incubated along with the solvent methanol. It was kept for 15 days and mixed at a regular interval of time to extract the compounds present in the sample. After 15 days, the extract was transferred in a petri-plate and allows the solvent to evaporate. The complete evaporation of solvent left the powdered form of sample extract, which was further stored in a container and then completely covered with an aluminium foil to reduce the contact with light.

Fourier transform infrared analysis: Extract (1 mL) was loaded and examined using infrared in the transmittance range of 4000-750 cm^{-1} and the presence of chemical bonds are interpreted using the acquired peaks.

MTT assay: The methanolic extract of *Syringodium isoetifolium* was tested for *in vitro* cytotoxicity, using Hela cells by MTT assay. In this assay, HeLa cells were cultured and collected by the process called trypsinization. Then, the cells have been plated in a 96-well plate along with DMEM medium including 10% FBS and 1% antibiotic solution and it was incubated for 24-48 h at 37 ºC. After incubation the wells have been washed using sterile PBS and again incubated through different concentrations of the extract along with serum free DMEM medium. The above protocol was repeated for three times and the cells were finally incubated at 37 ºC in a humidified 5% CO_2 incubator for 24 h. After 24 h, 20 µL of 5 mg/mL MTT dye was poured into the wells and again incubated for 2-4 h until purple precipitates have been evidently perceptible underneath an inverted microscope. Lastly, the medium beside with MTT have been draw out from the wells and washed with PBS of 1X concentration. DMSO has been added in the wells to dissolve the formazan crystals. The absorbance has been measured at 570 nm by using a microplate reader and the percentage (%) of viable cells was observed. Finally, IC_{50} value has been determined using GraphPad Prism 6.0 software (USA).

Comet assay: HeLa cells were seeded in a six well plate at a density of 10,000 cells/well and incubated for 24 h at 37 ºC in a humidified 5% CO_2 incubator. The wells have been washed properly with sterile PBS and treated with 35.63 µg/mL of methanolic extract of *Syringodium isoetifolium* in a serum free DMEM medium and incubated for 24 h at 37 ºC. The cells were collected by trypsinization and transfer the cells into a 1.5 mL tube and comet assay was carried out based on the procedure of Nadhakumar *et al.* [13] with slight modifications. The microscopic slides were layered with 0.75 % normal melting agarose as the first layer and 0.5% low melting agarose as the second layer. Then, mixed 20 µL cell suspensions with 60 µL of 0.5% low melting agarose and it was coated as the third layer. The slides were incubated in cell lysis buffer for overnight at 4 ºC. After that, the slides were immersed in double distilled water and again incubated for 20 min in unwinding solution. After incubation the slides were put in a gel electrophoresis tank containing electrophoresis buffer. The electrophoresis was carried out at 25 volts for 25 min. After that the slides were placed in neutralization buffer for 10 min. Finally, the slides were washed and stained with 50 µL of ethidium bromide dye and then the cells were observed with the help of a fluorescent microscope. To reduce the DNA damage, all the steps were carried out under the absence of light.

RESULTS AND DISCUSSION

FT-IR analysis: The different type of chemical bonds and functional groups present in the methanolic extract of *Syringodium isoetifolium* are shown in Fig. 1 and their vibrational frequencies are summarized in Table-1. The FT-IR spectrum shows a band at 658 and 600 cm^{-1} which confirmed the presence of the halo compound present in the extraction [14]. The band at 717 cm^{-1} is due to the stretching vibration of alkene or aromatic C-H and amine [15-17]. The bands at 876 and 1657 cm^{-1} are assigned to the C-C/C-O (alcohol/ether) [16,18,19] and C=C/C=O (cyclic alkene/carbonyl) [14,17,20], respectively. The alcohol/phenolic C-H/C-O exhibit peaks at 1096 [17] and 1194 cm^{-1} [16,17,19]. The C-N/C-H/C-O exhibits peak at 1141 cm^{-1}, which is due to amine/aliphatic ether compound present in the

TABLE-1
DIFFERENT CHEMICAL BONDS AND FUNCTIONAL GROUPS PRESENT IN THE METHANOLIC EXTRACT OF *Syringodium isoetifolium*

S. No.	Vibrational frequency (cm^{-1})	Vibrational assignment(s)	Functional group(s)	Ref.
1	600	C-Br	Halo compound	[14]
2	658	C-Br	Halo compound	[14]
3	717	C=C/C-H/N-H	Alkene/aromatic/amine	[15-17]
4	876	C-C/C-O	Alcohol/ether	[16,18,19]
5	1096	C-H/C-O	Alcohol	[17]
6	1141	C-N/C-H/C-O	Aliphatic ether	[16,17,19]
7	1194	C-H/C-O	Alcohol/phenolic	[16,17,19]
8	1321	C-N	Aromatic amine	[14]
9	1417	C=C/CH_2	Alkene/methylene	[14]
10	1538	N-O	Nitro compound	[17,20]
11	1657	C=C/C=O	Cyclic alkene/carbonyl	[14,17,20]
12	2318	C-H	Alkane	[14]
13	2851	C-H	Aromatic/alkane/alkene	[21-23]
14	2921	C-H	Aromatic/alkane/alkene	[21-23]
15	3399	O-H & N-H	Alcohol/aliphatic primary amine	[21-23]

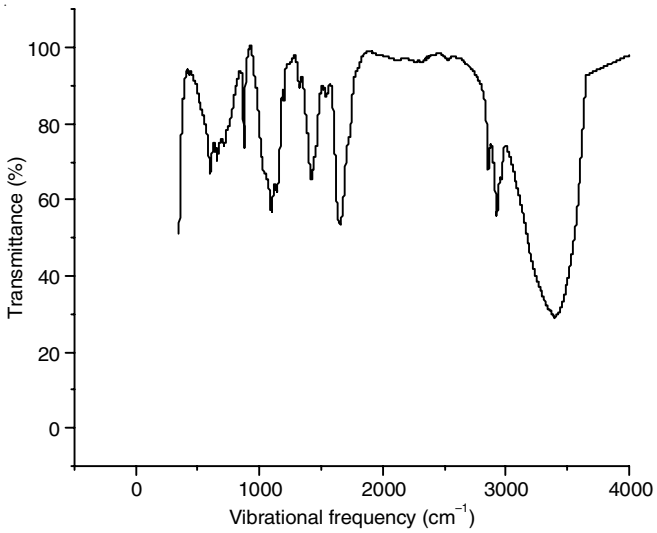

Fig. 1. Various peaks representing the different functional groups present in the seagrass extract

extraction [16,17,19]. The peaks at 1321 and 1538 cm^{-1} are assigned to C-N [14] and N-O groups [17,20], respectively. A peak at 1417 cm^{-1} is due to the alkene and methylene groups. The aromatic and aliphatic C-H group shows at 2318, 2851 and 2921 cm^{-1}. The alcoholic or phenolic O-H and amine N-H groups exhibit peak at 3399 cm^{-1} [21-23]. From the results, it clearly shows the presence of various functional groups like alcohol, ether, carbonyl, amine and halo compounds in large amount.

MTT assay: In this study, the methanolic extract of *S. isoetifolium* will inhibit the growth of growth of HeLa cells at a least inhibitory concentration of 35.63 µg/mL. Fig. 2 shows that the viability of the cells become high at least concentration of the extract and when increased the concentration of the extract, the number of viable cells will become decreases.

Comet assay: The main aim of the comet assay is to find the DNA Damage present inside the cell. The assay is worked under the presence of an electric field. When the electric field is supplied the cell will move from one end to another end like the electrophoresis.

In comet assay, The class 1 cells show least DNA damage whereas the class 4 cells show maximum DNA damage. In this study, class 3 level of DNA damage is observed, which is high (Fig. 3). Comet assay clearly shows that the extract can able to break the DNA of cancer cells into small fragments and 67.5% of DNA damage was observed in the HeLa cells treated with methanolic extract of *Syringodium isoetifolium.* The study concludes that the methanolic extract of *Syringodium isoetifolium* can be used as an alternative source of therapeutics for the treatment of cervical cancer.

Conclusion

The study investigates the anticancer activity of the seagrass, *Syringodium isoetifolium.* The aquatic plants got a wide attention in the current scenario due to their wide range of medicinal properties. The seagrass is low-cost, pollution free and it doesn't produce any side effects, so it can be used as a replacement for the current treatments like chemotherapy. A better drug candidate should produce good results even with a least drug concentration. In this study, the methanolic extract of *S. isoetifolium* is highly effective at the concentration of 35.63 µg/mL.

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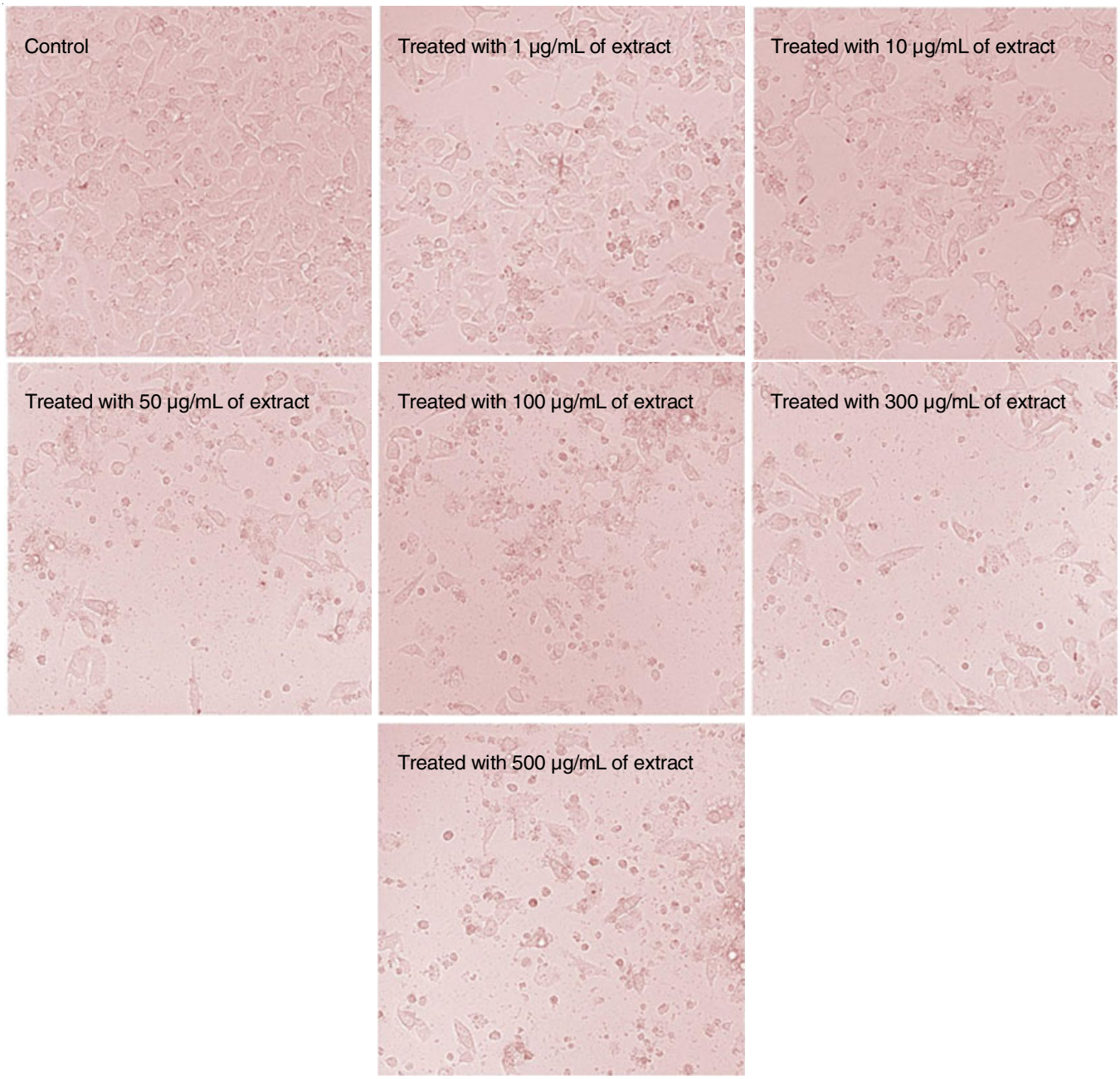

Fig. 2. Viability of the cells treated with various concentrations of Methanolic extract of *Syringodium isoetifolium*

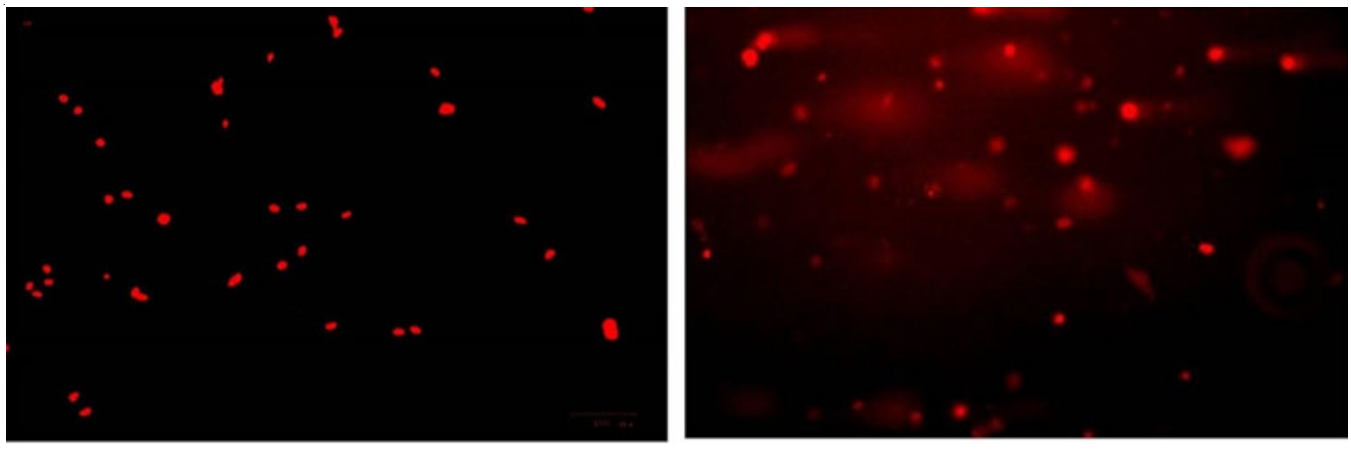

Fig. 3. Control cells and DNA Damage occur in the HeLa cell line after the treatment with methanolic extract of *Syringodium isoetifolium*

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