In vitro anticancer activity of Cissus Repanda Root extract on MCF-7 (Breast cancer cell line).

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ABSTRACT

Aim: One of the leading causes of death among women worldwide is breast cancer.
Despite better improvements in breast cancer treatment, investigations have shown that the condition is not entirely curable. Additionally, the medical benefits of Cissus repanda are widely known in traditional folk medicine, but the benefits of the plant's roots have not been well investigated.
The purpose of this study is to ascertain whether the extract from the root of Cissus repanda inhibits breast cancer cell growth significantly.

Material & Methods: Cell lines were obtained from BIO CYTE RESEARCH AND DEVLOPMENT PVT LTD. The whole plant of *cissus repanda* for the proposed study was collected from local region of Aurangabad district of Maharashtra, India in the month of march. Authentication of the collected material was carried out at Botanical Survey of India, Pune wide letter no. BSI/WRC/IDEN.CER. /2016/ 485.

Cells were seeded at a concentration (70µl) 104cells/well in 100 µl culture medium and 100µl Cissus repanda extract (10, 40,100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells) Control wells were incubated with DMSO (0.2% in PBS) and cell line.

Result: The results obtained from MTT assay revealed that the aqueous alcoholic extract of cissus repanda (HACRR) carried significant inhibition of cells in MCF-7 cell lines. The mean IC 50 value of HACRR extract of cissus repanda was found to be 49.50 µg/ml.

Conclusion: This study illustrates that the treatment of HACRR extract of cissus repanda could inhibit cell viability, induce apoptosis, and suppress cell migration in MCF-7 breast cancer cells.

Keywords: Breast Cancer, *Cissus Repanda*, anti-cancer activity, MTT assay.

INTRODUCTION

In 2018, an estimated 9.6 million people died from cancer, which is the second highest cause of death in the world. Worldwide, cancer is responsible for around 1 in 6 fatalities. In low- and middle-income nations, cancer deaths account for over 70% of all deaths [1]. The advancements in technology and understanding of neoplastic disease are advantageous for opportunities to decrease the death rate from cancer through the creation of new medications. With the development of technology and information of neoplastic disease, there are now more chances to find new drugs that will lower the death rate from cancer**.** A multistage process, including the progression from a precancerous lesion to a malignant tumor state, results in the change of normal cells into tumor cells, which causes cancer. These alterations are the result of the interaction of a person's genetic factors with environmental elements such as ultraviolet radiation, asbestos, and cigarettes as well as biological elements such as viruses and bacteria.

The most prevalent form of cancer among women is breast cancer. A quarter of breast cancers are latent and sneaky, growing slowly but metastasizing early, despite the fact that the majority are benign and treatable by surgery. High death rates are caused by the fact that current

medicines greatly slow tumor development but that recurrence is unavoidable. Breast cancers cells' origins contain the seeds of their behavior. Cell mobility and alterations in cell contact are hallmarks of mammary development. Embryonic mammary cells are endowed with motile and invasive capabilities.

The use of medicinal plants is crucial in the fight against many ailments. Since ancient times, medicinal and fragrant herbs have been utilized to treat various illnesses. The use of natural remedies has a long history in poor nations. A significant fraction of the world's population continues to receive healthcare from medicinal plants. The importance of using herbs as a source of medicine has increased as a result of the adverse effects of many synthetic pharmaceuticals, population growth, a lack of drugs, exorbitant costs, and the emergence of drug resistance. Cissus repanda is one of the Vitaceae family's reputed medicinal plants from India. Large climber with corky bark and incredibly permeable wood. Cutting the stems of Cissus repanda produces potable water, hence its other name, Panivel (Pani-water, Vel-creeper). Over Tripura, Bihar, Orissa, Kuman to Arunachal Pradesh, and the Western Ghats region, Cissus repanda is discovered to be present, reaching heights of 1350 meters. Traditional medicine is well aware of the medicinal properties of Cissus repanda, and it is frequently utilized in folk medicine. Paste made from its roots and powder has been used healing cuts, wounds, and bone fractures for centuries. There have been reports of the Cissus genus's plant species having analgesic and anti-inflammatory properties. The attachment, penetration, and replication steps of the viral multination cycle are all inhibited by the dichloromethane and methanol extracts of Cissus repanda. The flavonoids, polyphenols, sterols, quinones, saponins, anthocyanins, and saponins found in Cissus repanda leaves are well known. The roots of the Cissus repanda are significant from a medicinal standpoint. Despite the presence of essential compounds, nothing is known about Cissus roots.

MATERIALS AND METHODS

Cell line and cell culture

Cell lines were obtained from BIO CYTE RESEARCH AND DEVLOPMENT PVT LTD. DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106

Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062 from BIO CYTE RESEARCH AND DEVLOPMENT PVT LTD.

Collection of the plant

The whole plant of *cissus repanda* for the proposed study was collected from local region of Aurangabad district of Maharashtra, India in the month of march. Authentication of the collected material was carried out at Botanical Survey of India, Pune wide letter no. BSI/WRC/IDEN.CER. /2016/ 485.

Preparation of extract of *cissus repanda*

The roots of *cissus repanda* were cut and washed properly so as to remove the soil adhered onto the roots. Later, they were shade dried and reduced into fine powder using an industrial scale grinder. Different types of extracts viz Aqueous (ACRR), Aqueous-Ethanolic (HACRR) and Ethanolic (ECRR) were prepared by cold maceration with solid to solvent ratio of 1:3 w/v. For Aqueous-Ethanolic extract, equal amounts of water (HPLC grade) and ethanol (double distilled) was used. The mixtures were stored in a round bottom flask for maceration at room temperature (RT) for 3 days with frequent shaking to the mixture. Later the extract was ultrasonicated for 15 min at RT. Extracts were initially filtered using a muslin cloth followed by using a Whatman filter paper (Merck). The filtrates were fractionated with n-Butanol at a ratio of 1:3 v/v i.e., 100ml of filtrate was fractionated with 300ml of n-butanol. The butanolic fractions were separated and concentrated on rotatory vacuum evaporator (Heidolph, Germany) with water bath temp at 55ºC and stored at 4ºC till use.

Cell Culture Preparation:

MCF-7 cell line were procured from National Center for Cell Science(NCCS), Pune, India. MCF-7 Cell was cultured in Dulbecco's Modified Eagle's Medium (DMEM) with high glucose, 10% Fetal Bovine Serum and Antibiotic-Antimycotic (100X) solution (Thermo Fisher Scientific). Cells were incubated in humidified incubator at 37 °C with 5% CO2.

In vitro cell viability assay Procedure

Cells were incubated at a concentration of 1 × 104 cells/ml in culture medium for 24 h at 37°C and 5% CO2.Cells were seeded at a concentration (70µl) 104cells/well in 100 µl culture medium and 100µl Cissus repanda extract (10, 40,100 µg/ml) into micro plates respectively (tissue culture grade, and 96 wells) Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientific BB150) After incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4 hrs at 37oC in CO2incubator.Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark colored formazan by viable cells only. Registered under Companies Act,2013 (18 of 2013)- CIN-U73100PN2021PTC198266 Registered under CPCEA 2114/PO/Re/S/20/CPCSEA After removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 370C (wrapped with aluminum foil). Triplicate samples were analyzed by measuring the absorbance of each sample by an Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm.

Result:

In vitro cell viability assay:

The cytotoxicity of aqueous-alcoholic extract of cissus repanda in MCF-7 cells was investigated using MTT. Fig. 1 depicts the percentage of viable MCF-7 cells after treatment with different concentrations of HACRR extract of cissus repanda for various concentrations displaying a dependent cytotoxicity. The mean IC 50 value of HACRR extract of cissus repanda was found to be 49.50 µg/ml.

The percentage growth inhibition was calculated using following formula,

%cell inhibition= 100-{(ODt-ODb)/(ODc*ODb)}x100

Where,

ODt= Optical Density of test compound

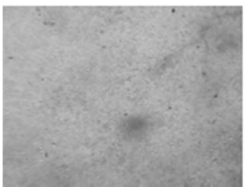
ODb= Optical Density value of blank

ODc=Optical Density value of control

Effects of compound against MCF-7 (Breast Cancer Cell line) by MTT assay:

Sr. no.	Sample	Concentration (µg/ml)	OD	Mean	%inhibition	IC 50 (µg/ml)
1	Control		1.238 1.477 1.320	1.345		
2	Std. 5 FU	10	0.223 0.315 0.259	0.265	**80.29**	**41.56**
		40	0.303 0.351 0.301	0.318	**76.35**	
		100	0.365 0.387 0.398	0.383	**71.52**	
3	Sample-HACRR	10	0.336 0.348 0.338	0.340	**74.72**	**49.50**
		40	0.244 0.442 0.524	0.403	**70.03**	
		100	0.786 0.698 0.441	0.641	**52.34**	

At the different doses (10 µg/ml to 100 µg/ml) of different synthesized compounds carried out for anticancer activity against MCF-7 cell line. The samples showed good activity as compared to standard compound.



Control

STD

Sample-HACRR

0-

Discussion:

In this study, we illustrated that the treatment of HACRR extract of cissus repanda could inhibit cell viability, induce apoptosis, and suppress cell migration in MCF-7 breast cancer cells.

In our study, HACRR exerted different inhibitory effects on cell viability in MCF-7 cells, when exposed to different concentrations of the same extract. This cell viability evaluation is based on the fact that metabolically active cells break down these tetrazolium salts into formazan crystals. To facilitate the optical density measurements, these crystals are solubilized using Dimethyl Sulfoxide (DMSO). Half-maximal inhibitory concentration (IC50) determines the appropriate concentration required to kill about 50% of cells. Considering these concentrations obtained through IC50 for HACRR extract of cissus repanda treated with MCF-7 breast cancer cell lines at 24 hr, further experiments were carried out with 49.50 µg/ml.

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