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IN VITRO ANTICANCER ACTIVITY OF SELECTED INDIAN MEDICINAL PLANTS ON DIFFERENT HUMAN BREAST CANCER CELL LINES

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Abstract: Introduction: Cancer is a major public health concern in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Therefore an attempt has been made to review different medicinal plants for its anticancer properties. **Materials and Methods**: In present investigation, Aqueous and Hydroalcoholic extracts of *Abrus precatorius* L. seeds (white variety), *Aegle marmelos* Correa. leaves, *Careya arborea* Roxb. bark, *Sapindus trifoliatus* L. fruits, *Terminalia arjuna* Roxb. bark and *Withania somnifera* Dunal. leaves were evaluated for *in vitro* anticancer activity. The extracts were tested for *in vitro* anticancer activity against various human breast cancer cell lines using Sulforhodamine B (SRB) assay. **Results:** Out of the twelve extracts tested, Hydro alcoholic extract of *Withania somnifera* Dunal. leaves showed potent anticancer activity against all six human breast cancer cell lines viz. MCF7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435 whereas aqueous extract of *Withania somnifera* Dunal. leaves showed anticancer activity against MDA-MB-468. **Discussion:** The result of the present work may be useful for the development of anticancer agents of plant origin.

Keywords: *Abrus precatorius* L. seeds (white variety), *Aegle marmelos* Correa. leaves, *Careya arborea* Roxb. bark, *Sapindus trifoliatus* L. fruits, *Terminalia arjuna* Roxb. bark and *Withania somnifera* Dunal. leaves, anticancer activity

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INTRODUCTION

Breast cancer is a leading cause of death in women worldwide [1]. There has long been interest in the identification of natural products for the treatment of various diseases for thousands of years. Natural products possess immense pharmacological significance in the development of drugs [2,3,4] including cancer [5,6,7,8,9,10]. The majority of drug candidates, such as paclitaxel, etoposide, camptothecin, vinca alkaloids, indole alkaloids, podophyllotoxin derivatives, and teniposide, currently used in clinical cancer chemotherapy, were originally derived from plants. The efficacy of chemotherapy, radiotherapy, hormonal therapy or surgery, which are mainly used for the treatment of cancer, are well-known for side effects [11]; hence, the identification of novel natural products possess better effectiveness against cancer and less harmful effects [12], and therefore, natural products are continuously being explored worldwide.

Plants are being used as indigenous cure in folklore or traditional system of medicine for treatment of various kinds of illness including cancer [13]. Recently, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management [14]. In traditional medicine, plants are being used for healing purposes and are effective as they contain biologically active components which are non toxic [15]. With an understanding of cell biology, mechanism based bioassays have become increasingly important and bio-activity guided phytochemical investigation has resulted in the isolation and characterization of several new molecules possessing interesting medicinal properties [16]. Ayurveda, a traditional system of Indian system of medicine mainly based on plant drugs has been successful since very early times for preventing or suppressing ailments [17]. Early documentation about the use of medicinal plants has been mentioned in Ayurveda [18]. Epidemiological studies suggest that consumption of diets containing fruits and vegetables which are the major sources of phytochemicals and micronutrients reduce the risk of developing cancer [19]. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells [20].

Therefore we have investigated the anticancer activity of the selected plant extracts by using different cell lines.

MATERIALS AND METHODS:

Collection of the plant material and preparation of the extracts

Abrus precatorius Linn. seeds (white variety) and *Sapindus trifoliatus* L. fruits were procured and authenticated from Plant Drug Authentication Service, Agharkar Research Institute, Pune, M. S., India.

Careya arborea Roxb. bark, *Terminalia arjuna* Roxb. bark and *Aegle marmelos* Correa. leaves were collected from Badlapur, M. S., India and authenticated from Plant Drug Authentication Service, Agharkar Research Institute, Pune, M. S., India.

Withania somnifera Dunal. seeds were procured and cultivated. Herbarium was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai, M. S., India.

Bark of *Careya arborea* Roxb. and *Terminalia arjuna* Roxb. were cut into small pieces. Pieces of *Careya arborea* Roxb. and *Terminalia arjuna* Roxb., seeds of *Abrus precatorius* Linn., fruits of *Sapindus trifoliatus* L., leaves of *Aegle marmelos* Correa. and *Withania somnifera* Dunal. were kept for drying in an oven at a temperature of 40±2^{0}C for 2 days. The dried plant parts were grounded into powder and stored in air tight containers separately.

Preparation of Aqueous extracts

Extract was prepared using water as described by Anonymous [21]. Water was added to the coarse powder in a ratio of 6:1 i.e. 30 ml of Distilled water to 5 grams of powder. It was mixed thoroughly and refluxed for 2 hrs at 80 ^{0}C. The above step was repeated 3 times. Each time 30 ml of water was added. The extract was filtered and concentrated using Rotary Vacuum Evaporator. Extract prepared was stored in air tight amber coloured bottle. The percentage of yield obtained was calculated.

Preparation of Hydro alcoholic (50% Ethanolic) extract

Extract was prepared using Hydro alcohol (% Ethanol) as described by Anonymous [21]. Hydro alcohol (50% Ethanol) was added separately to the 5 gm coarse powder in a ratio of 4:1. The mixture was mixed thoroughly and macerated for 4 hrs. The mixture was refluxed for two hours. The above step was repeated 3 times. Each time 20ml of Hydro alcohol (50% Ethanol) was added. The extract was filtered and concentrated using Rotary Vacuum Evaporator. Extracts prepared were stored in air tight amber coloured bottle and kept in refrigerator. The percentage of yield obtained was calculated.

Human Breast cancer cell lines

In vitro anticancer studies were carried out on available Human Breast Cancer Cell Lines like MCF7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435 at ACTREC, Kharghar, Navi Mumbai.

***In vitro* Sulforhodamine B (SRB) Assay**

In vitro SRB Assay of the prepared extracts was carried out using various Human Breast Cancer Cell Lines at Tata Memorial Centre – Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, M. S., India.

The antiproliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye [22]. The microtitre plates were taken out after 48 hours incubation of the cells with test materials and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The tissue culture plates were incubated at 4°C for one hour to fix the cells to the bottom of the wells. The supernatant was then discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 540 nm.

RESULTS:

Hydroalcoholic (50% Ethanolic) extract of *Withania somnifera* Dunal. leaves when tested on Human Breast Cancer Cell lines showed GI50 value < 10 on all the tested Human Breast Cancer cell lines (*viz.* MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435) while Aqueous extract showed GI50 value < 10 on Human Breast Cancer cell line MDA-MB-468 indicating anticancer activity (Table 1).

Aqueous and Hydro-alcoholic extract (50% Ethanol) of *Abrus precatorius* L. seeds (White variety) showed GI50 value > 20 thereby indicating no significant anticancer activity against all Human Breast Cancer cell lines studied (Table 2).

Aqueous and Hydro-alcoholic extract (50% Ethanol) of *Sapindus trifoliatus* L. fruits showed GI50 value > 20 thereby indicating no significant anticancer activity against all Human Breast Cancer cell lines studied (Table 3).

Aqueous and Hydro-alcoholic extract (50% Ethanol) of *Careya arborea* Roxb. bark showed GI50 value > 20 thereby indicating no significant anticancer activity against all Human Breast Cancer cell lines studied (Table 4).

Aqueous and Hydro-alcoholic extract (50% Ethanol) of *Terminalia arjuna* Roxb. bark showed GI50 value > 20 thereby indicating no significant anticancer activity against all Human Breast Cancer cell lines studied (Table 5).

Aqueous and Hydro-alcoholic extract (50% Ethanol) of *Aegle marmelos* Correa. leaves showed GI50 value > 20 thereby indicating no significant anticancer activity against all Human Breast Cancer cell lines studied (Table 6).

Table 1: *In vitro* anticancer activity of *Withania somnifera* Dunal. Leaves on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	44.2	65.3	31.5	<10	>80	>80	49.3	>80	>80	52.1	>80	>80	52.1	>80	76.2	33.3
Hydro-alcoholic	77.9	32.1	<10	45.7	<10	<10	78.2	38.9	<10	70.0	25.1	<10	70.0	25.1	<10	>80	23.3	<10

Table 2: *In vitro* anticancer activity of *Abrus precatorius* L. seeds (White variety) on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	62.5	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydro-alcoholic	>80	>80	48.2	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 3: *In vitro* anticancer activity of *Sapindus trifoliatus* L. fruits, on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydro-alcoholic	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 4: *In vitro* anticancer activity of *Careya arborea* Roxb. bark, on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydro-alcoholic	>80	>80	60.5	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 5: *In vitro* anticancer activity of *Terminalia arjuna* Roxb. bark, on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydro-alcoholic	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80

Table 6: *In vitro* anticancer activity of *Aegle marmelos* Correa. leaves on human breast cancer cell lines (MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435)

	Human Breast Cancer Cell lines																	
	MCF-7			MDA-MB-468			Zr-75-1			BT-474			MDA-MB-231			MDA-MB-435		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
Aqueous	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80
Hydro-alcoholic	>80	>80	>80	>80	>80	47.1	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	>80	58.7

Key: = Values highlighted indicate positive results

Note:

GI50	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.
ADR	Adriamycin (Doxorubicin), Positive control compound.
Note: Erratic data can result due to less solubility of the compound.	
GI50 value of ≤ 10^-6 (i.e. 1 µmole) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity.	
Yellow highlighted test values under GI50 column indicate activity.	

DISCUSSION:

Cancer therapy in the form of surgery or radiotherapy is effective when the disease is detected in early stages but many cancers are diagnosed when a primary tumor have metastasized to other parts of the body. The main form of treatment at this point is chemotherapy [23]. Chemotherapy helps delivering drugs systematically so that they can reach and kill the tumor cells, but most of these drugs cause severe side effects in patients and, therefore, needs to be used at suboptimal levels. According to Jemal *et al.* [24], the low efficacy of chemotherapy in patients with advanced cancers is reflected in the low 5-year survival rates observed in these patients and the low efficacy of cancer therapy for the treatment of patients with metastasis makes the development of novel chemotherapeutic agents necessary. Denny and Wansbrough [25] reported that a major challenge is to design new drugs that will be more selective for cancer cells and have lesser side effects [26].

Integrative medicine with the approach of combining conventional western medicine with alternative or complementary treatments, such as herbal medicine, acupuncture, massages, biofeedback, yoga and stress reduction techniques, is being used to complement orthodox medicines and treatment approaches in the management of cancer patients [26]. According to Lammersfeld [27] patients may turn to integrative therapies when the disease they are battling does not respond to traditional medicinal therapies and/or to help reduce symptoms while improving overall well-being, and among cancer patients more than half use some kind of integrative therapy.

Plants have served as a rich source of therapeutic agents for many centuries, being used themselves or as the basis for synthetic drugs [28], and despite the great developments in organic synthesis, 55% of recent chemo preventive drugs [29,30]. According to Rates [31] and Jemal *et al.* [32], the interest in alternative therapies using natural products is increasing, especially those derived from plants, due to the increasingly high number of cancer cases worldwide. In order to look for new sources of therapeutic anticancer agents, many plant extracts and active principles have been studied *in vitro* and *in vivo* cancer models, and the correlation of both studies became one of the key steps for the success of this type of research [9,29].

In this study, Hydroalcoholic extract of leaves of *Withania somnifera* Dunal. leaves was significantly active (GI50value <10) against MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435 whereas aqueous extract of *Withania somnifera* Dunal. leaves showed significant anticancer activity against MDA-MB-468.

CONCLUSION:

Hydroalcoholic (50% Ethanolic) extract of *Withania somnifera* Dunal. leaves when tested on Human Breast Cancer Cell lines showed GI50 value < 10 on all the tested Human Breast Cancer cell lines (*viz.* MCF-7, MDA-MB-468, Zr-75-1, BT-474, MDA-MB-231 and MDA-MB-435) indicating anticancer activity while Aqueous extract showed GI50 value < 10 on Human Breast Cancer cell line MDA-MB-468 indicating anticancer activity. The potential anticancer activity might be due to effect of the phytomolecules present in the extract. These plants can be further explored for the discovery of novel anticancer agents.

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