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EVALUATION OF IN VITRO ANTICANCER ACTIVITY OF STEMBARK EXTRACTS OF PLANT *MAYTENUS EMARGINATA*

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ABSTRACT

Cancer is a major public health problem in all over the world. Medicinal herbs have been always on the forefront whenever we talk about anticancer properties. Many herbal plants have been evaluated and are currently being investigated phytochemically to understand their anticancer actions against various types of cancers. The aim of the present study is to evaluate the effect of in-vitro anticancer activity of extracts. The petroleum ether, chloroform, methanolic, aqueous and hydroalcoholic extract of stembark of plant *Maytenus emarginata* against five human cancer cell lines such as Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205), Lung cancer cell lines (A-549), Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7) and it was compared with Adriamycin as Positive control compound by using Sulforhodamine B (SRB) assay. Anticancer activity of Chloroform and Methanolic extract of plant *Maytenus emarginata* on Hela, Colo-205, A-549, PC-3 and MCF-7 cancer cell lines showed potent cytotoxic activity as compared to other extracts. The results of the present work may be beneficial for the development of anticancer agents of plant origin.

KEYWORDS: Adriamycin, *Maytenus emarginata*, In-vitro anticancer activity, Human cancer cell lines, Sulforhodamine B (SRB) assay etc.

INTRODUCTION

Medicinal plants have been used since ancient times as medicines for the treatment of a range of diseases. Herbal plants have played a key role in world health. An increasing number of research papers and reviews clearly indicate that medicinal plants exhibit a variety of therapeutic properties and provide health security to rural people in primary health care.[1,2] Cancer is group of disease characterized by uncontrolled growth and spread of abnormal

cells. If the spread is not controlled it can leads to death. Cancer is majorly caused by both external factor like as tobacco, chemicals, radiation and infectious organism and internal factors like as inherited mutations, hormones, immune conditions and mutation that occur due to metabolism. These factors may acts together or in sequence to initiate or promote carcinogenesis. The development of cancer in human body requires multi steps that occur over many years. Certain types of cancer can be prevented by eliminating exposure to tobacco, chemicals and other factors that accelerates this process. Any other potential malignancies can be detected before cells become cancerous, when the disease is most treatable. Cancer is mainly treated by various way like as surgery, radiation, chemotherapy, hormones and immunotherapy. Cancer is one of the most life threatening diseases and possess many health hazard in developed as well as developing countries. [3] It can confirm that struggle to combat cancer is one of the greatest challenges of mankind. [4] From the traditional times, herbal plants have been prized for their pain-relieving and healing abilities and today upto we rely largely on the curative properties of plants. According to World Health Organization (WHO), 85% of the population living in rural areas depend on medicinal herbs as primary healthcare system. The available synthetic anticancer remedies are beyond the reach of common population because of cost factor. Herbal medicines have played a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical.[5] Screening models for In-vitro anticancer activity may provide the important preliminary data to help in selection of plant extracts with potential anticancer properties for future work. Screening and isolation of active chemical compounds from plants which possess potential anticancer activity appears to be a promising way of discovering novel therapeutic compound.[6,7] Phytoconstituents which are obtained from flowering plants play a significant role in cancer chemotherapy. Anticancer drugs like as vincristine and vinblastine from Vinca plant, palitaxel (Taxol) and taxotere from species of yew (Taxus), etoposide derived from lignans of Podophyllum plant and camptothecin analogues, such as topotecan, from *Camptotheca acuminate* all of these are fundamentally shows cytotoxic and act principally by inhibiting cell proliferation, but by different mechanisms. Some natural substances from plants have been found to act by novel mechanisms and so have enabled to get novel targets to developed for screening, it can be explained by the discovery that paclitaxel inhibited mitosis by stabilising microtubules and so preventing their depolymerisation back to tubulin, in contrast to many other anticancer agents which inhibit the formation of microtubules in the first place.[8]

Maytenus emarginata (Willd.) Hou belongs to family Celastraceae, is an evergreen tree that tolerates various types of stresses of the desert, locally known as "Kankero", "Baikal" in Hindi,and "Thorny staff tree" in English. Traditionally species of Maytenus has been used for fever, anthelmentics, ucer, asthama, rheumatism and gastrointestinal disorders worldwide. Recently some biomolecules from Maytenus species has been reported to be active against HIV-Protease[9] and MDR (Multi Drug Resistance).[10] Roots are used in gastrointestinal troubles, especially in flatulence and dysentery. Stem of the plant help for treating ulcer.[11] The bark of plant is ground to make a paste and applied with mustard oil to kill lice in the hair. Pulverized leaves of *Maytenus emarginata* are given with milk to children as a Anthelmentic. A decoction of the leaf is used as a mouthwash to relieve toothache. The leaves are used to heal sores.[12] The tender of leaves are chewed raw in the treatment of jaundice.

MATERIALS AND METHODS

Plant Collection

The fresh stembarks of plant *Maytenus emarginata* were collected from Haripura and Manudevi region of Taluka Yawal, District Jalgaon, India. The selected plants were authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L. K. Dr. P. R. Ghogrey Science College, Dhule, Maharashtra. Stembarks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Preparation of Plant extract[13]

The stembark of plant were thoroughly washed with tap water, dried at room temperature and transformed to coarse powder. The stembark powder was extracted with solvents i.e Petroleum ether, chloroform, methanol, water and water-ethanol separately by Soxhlet extraction method. Finally, the extracts were evaporated and dried under vacuum and tray dryer to obtain thick sticky extract.

Cell lines

Various human cancer cell lines used for in vitro SRB Assay are Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205), Lung cancer cell lines (A-549), Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7). The stock cultures were grown in T-75 flasks containing 50 mL of RPMI-1640 medium with 2 mM L-glutamine, bicarbonate and

10 % fetal calf serum. Medium was changed at 48 hours intervals. Cell were dissociated with 0.25 % trypsin and 3 mM 1,2-cyclohexanediaminetetraacetic acid in NKT buffer (137 mM Nacl, 5.4 mM Kcl and 10 mM Tris; pH 7.4). For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.[14]

Sulforhodamine B (SRB) Assay procedure[15,16]

The anticancer activities of extracts were studied at Advanced Center for Treatment, Research and Education in Cancer (ACTREC), Mumbai where all cell lines were maintained in ideal laboratory conditions. All cell lines were selected grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs (Extracts and Positive control). Experimental drugs (Extracts and Positive control) were initially solubilized in dimethyl sulfoxide (DMSO) at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml.

Positive control

Adriamycin (Doxorubicin) a known anticancer was used as a positive control for each of the experiments.

Endpoint measurement

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was then discarded and the plates were washed 5 to 6 times with water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic

acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After making the staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm wavelength kept as reference.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [Ti/C] x 100% for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero [(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz. (Ti-Tz) negative. The dose response parameters were calculated for each test article. The experiment data were estimated using linear regression method of plots of the cell viability against the molar drug concentration of tested compounds and results were given in terms of LC50, TGI and GI50 values. The summary of the parameters is as follows

GI50- Concentration of drugs causing 50 % inhibition of cell growth, calculated from [(Ti-Tz)/(C-Tz)] X 100= 50

LC50- Concentration of drugs causing 50 % cell kill, calculated from [(Ti-Tz)/Tz] X 100= 50

TGI- Concentration of drugs causing total inhibition of cell growth, calculated from Ti=Tz

GI50 value of ≤ 20 µg/ml is considered to demonstrate activity.

Statistical analysis

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

RESULTS AND DISCUSSION

The results showing anti-cancer activity of various extracts of *Maytenus emarginata* against human cancer cell lines such as Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205), Lung cancer cell lines (A-549), Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7) are presented in table No. 1 and 2 with LC50, TGI and GI50 valuses, the percentage control growth were calculated at each of drug concentration level are

presented in table No. 3 and 4, the growth curve of different cancer cell lines are given in figure No. 1 to 5. Out of the five Human cancer cell lines used for studying anticancer activity, chloroform extract showed prominent anticancer activity against Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205), Lung cancer cell lines (A-549), Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7) whereas methanolic extract showed prominent anticancer activity against Cervical cancer cell lines (Hela) and Colon cancer cell lines (Colo-205).

Table 1: *In vitro* anticancer activity of extracts of *Maytenus emarginata* using SRB Assay on Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205) and Lung cancer cell lines (A-549).

Extracts	Cell Lines								
	Cervical cancer cell lines (Hela)			Colon cancer cell lines (Colo-205)			Lung cancer cell lines (A-549)		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
PET-I	NE	NE	NE	NE	NE	NE	NE	NE	NE
CHL-I	>80	15	<10	NE	NE	<10	NE	38.3	<10
MET-I	>80	56	<10	71.8	<10	<10	>80	65.1	22
AQ-I	NE	NE	NE	NE	NE	NE	NE	NE	NE
HAL-1	NE	NE	NE	NE	NE	27	NE	NE	NE
ADR	NE	<10	<10	NE	<10	<10	NE	<10	<10

Table 2: *In vitro* anticancer activity of extracts of *Maytenus emarginata* using SRB Assay on Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7).

Extracts	Cell Lines					
	Prostate cancer cell lines (PC-3)			Breast cancer cell lines (MCF-7)		
	LC50	TGI	GI50	LC50	TGI	GI50
PET-I	NE	NE	NE	NE	NE	NE
CHL-I	NE	38.3	<10	NE	15	<10
MET-I	>80	65.1	22	NE	72.8	20.9
AQ-I	NE	NE	NE	NE	NE	NE
HAL-1	NE	NE	NE	NE	NE	NE
ADR	<10	<10	<10	NE	NE	<10

Table 3: % Control Growth of extracts of plant *Maytenus emarginata* against Cervical cancer cell lines (Hela), Colon cancer cell lines (Colo-205) and Lung cancer cell lines (A-549).

Test Component	% Control growth											
	Drug Concentrations (ug/ml)											
	Cervical cancer cell lines (Hela)				Colon cancer cell lines (Colo-205)				Lung cancer cell lines (A-549)			
	10	20	40	80	10	20	40	80	10	20	40	80
PET-I	98.7	104.3	107.3	110.6	79.3	68.9	101.7	88.7	79.5	81.5	80.8	86.4
CHL-I	13.9	-1.1	-40.2	-38.7	-19.2	-34.1	-32.8	-45.5	12.2	10.5	6.8	-27.6
MET-I	71.1	10.7	12.2	-17.4	10.3	-20.6	-38.0	-49.8	67.4	64.4	5.0	-8.6
AQ-I	100.1	102.3	115.5	108.4	62.0	60.4	47.8	57.8	78.2	79.5	84.9	92.3
HAL-1	95.2	101.2	92.4	72.4	56.2	52.2	45.3	31.8	70.1	74.0	71.3	65.4
ADR	-39.9	-40.7	-44.7	-22.8	-57.9	-51.6	-65.2	-66.5	-7.6	-7.6	-11.3	-10.9

Table 4: % Control Growth of extracts of plant *Maytenus emarginata* against on Prostate cancer cell lines (PC-3) and Breast cancer cell lines (MCF-7).

Test Component	% Control growth							
	Drug Concentrations (ug/ml)							
	Prostate cancer cell lines (PC-3)				Breast cancer cell lines (MCF-7)			
	10	20	40	80	10	20	40	80
PET-I	105.5	120.0	123.5	119.1	89.7	90.2	83.5	85.9
CHL-I	45.3	56.3	42.7	-29.3	5.4	-2.0	-14.5	-19.6
MET-I	111.1	112.0	61.0	32.0	58.6	46.1	42.1	-10.7
AQ-I	121.1	142.2	146.8	133.9	96.3	105.1	104.0	108.1
HAL-1	120.8	136.8	139.1	114.3	86.4	87.8	92.8	77.1
ADR	-54.6	-61.2	-65.1	-54.0	-19.3	-24.7	-18.4	27.3

Where, PET-I- Petroleum ether extract, CHL-I- Chloroform extract, MET-I Methanolic extract, AQ-I Aqueous extract, HAL-1 Hydroalcoholic extract and ADR Adriamycin

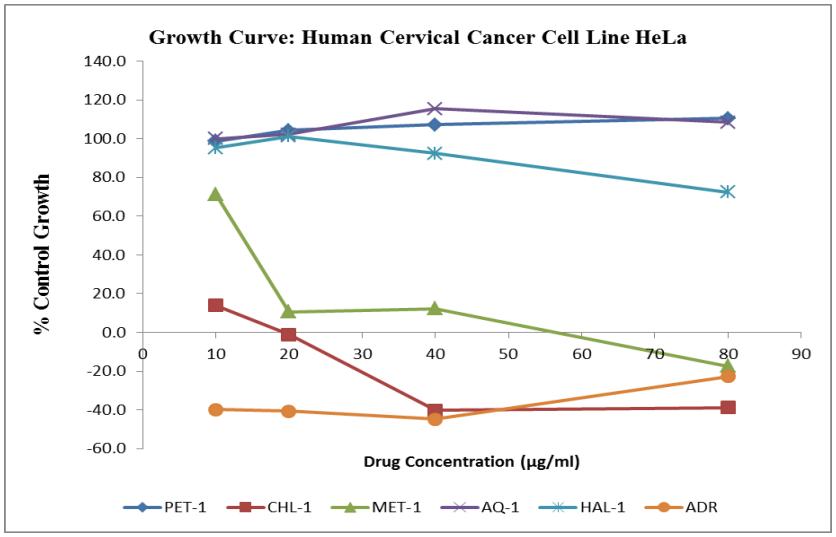

Fig. 1: Percentage control growth of Human Cervical Cancer Cell Line HeLa.

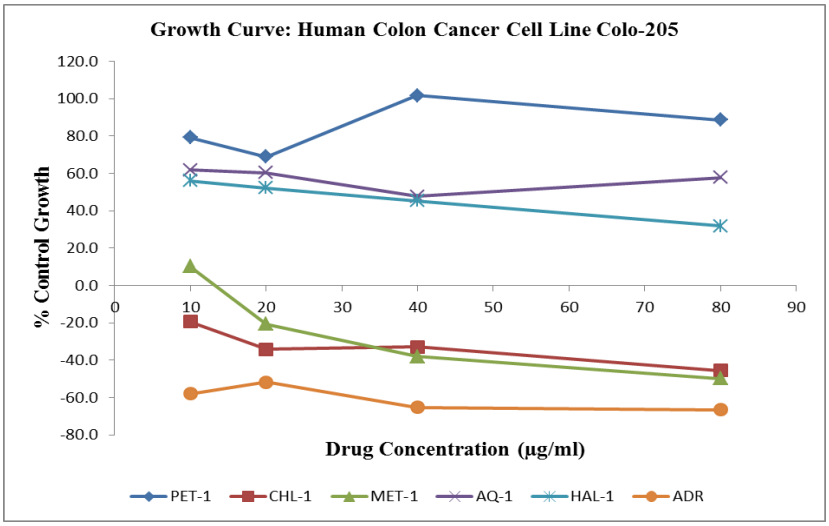

Fig. 2: Percentage control growth of Human Colon Cancer Cell Line Colo-205.

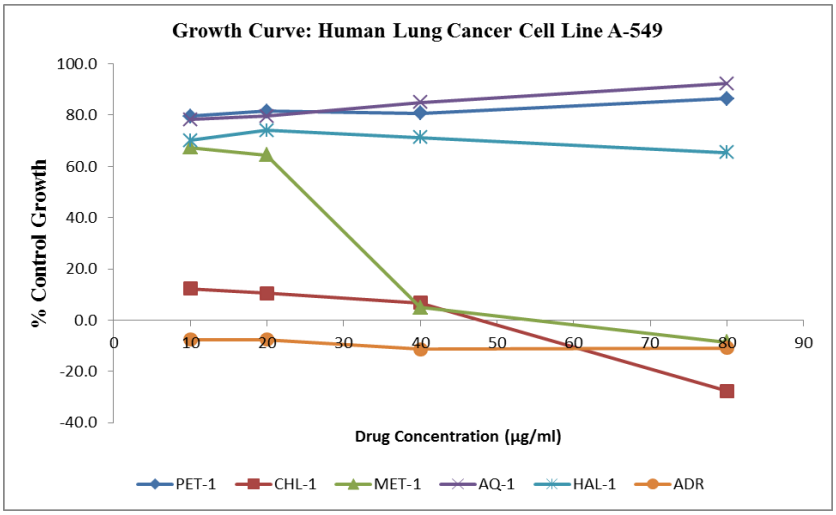

Fig. 3: Percentage control growth of Human Lung Cancer Cell Line A-549.

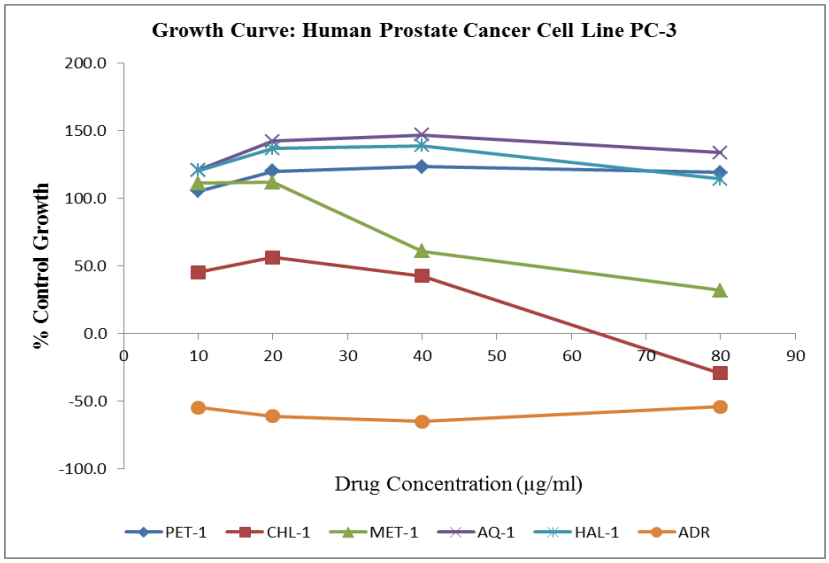

Fig. 4: Percentage control growth of Human Prostate Cancer Cell Line PC-3.

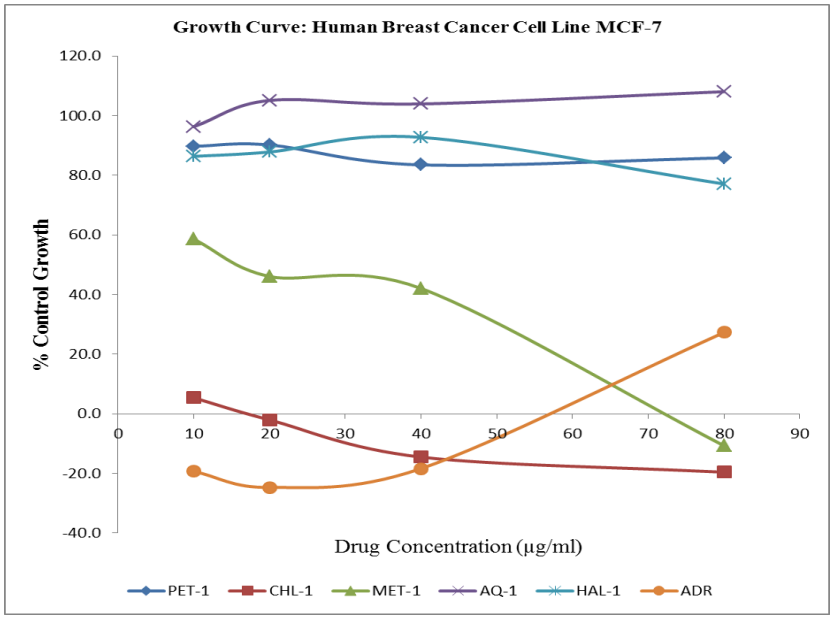

Fig. 5: Percentage control growth of Human Breast Cancer Cell Line MCF-7.

Where, PET-I- Petroleum ether extract, CHL-I- Chloroform extract, MET-I Methanolic extract, AQ-I Aqueous extract, HAL-1 Hydroalcoholic extract and ADR Adriamycin

CONCLUSION

Cancer is one of the most leading causes of mortality worldwide. There are two in vitro screening techniques like SRB and MTT assays are the reliable techniques used to carry out evaluation of anticancer activity on the human cancer cell lines. The SRB assay which provides a good linearity with cell number and with higher sensitivity and its staining is not dependent on cell. It is seen that, in contrast to the MTT assay the SRB assay stains recently lysed cells. In the present study, we concluded that the various plant extracts of *Maytenus ematginata* showed prominent *in vitro* anticancer activities against some selected human cancer cell lines. The activity may be depended upon the morphology of cell lines and mechanism of action of the plant extract. Further, all these plants extracts of plant *Maytenus ematginata* need to be screened against different cell lines apart from the selected cell lines to confirm the activity. So finally it can be concluded that both plant parts can be promising candidates in future anticancer therapy.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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