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ORIGINAL ARTICLE **OPEN ACCESS**

Cytotoxic, antioxidant and phytochemical analysis of *Tecoma gaudichaudi* DC (Bignoniaceae)

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ABSTRACT
Bignonia Linn (Bignoniaceae) is a monotypic genus of woody climbers, native to North America and mostly grown for ornament in the tropics of the old world. In the present work, invitro cytotoxic (SRB) assay was carry out against five melanoma cell lines such as MCF 7, B16F10, B16F1, SK-MEL-2, MDA-MB-231 for determining the cytotoxic effects in cells in response to plant extracts. Initially Tecoma gaudichaudiDC were first sequentially extracted with pet ether, ethanol, ethyl acetate respectively by soxhlet extraction and subjected to phytochemical analysis. Preliminary phytochemical investigation of extracts of Tecoma gaudichaudi DC species was carried out by chemical test it reveals that plant contains triterpenoids, steroids, tannins, flavonoids. The ethyl acetate, ethanol, pet ether extract of Tecoma gaudichaudiDC along with Ursolic acid was not found effective on these five cancer cell lines at concentrations 10-80µg/ml by in-vitro cytototoxic assay.
***Keywords**: Tecoma gaudichaudi dc, bignoniaceae, cytotoxic activity, melanoma cell lines.*

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INTRODUCTION

Bignonia Linn (Bignoniaceae) is a monotypic genus of woody climbers, native to North America and mostly grown for ornament in the tropics of the old world [1]. Bignoniaceae family was having 100 genera and more than 750 plant species observed in various tropical regions of India. Known numbers of this family are Bignonia, Tecoma, Catalpa, Tabebuia and Jacaranda. These are succulent herbs, shrubs, stem sometimes reduced to a rhizome or tuber. Numerous species of this family are observed as poisonous to leeches [2]. In Charak, Sushruta, the root, bark, stem and leaf of some species of Bignoniaceae family is useful for snake bite, the stem and wood for scorpion sting. Previous reports state that species of Bignoniaceae family show presence of promising active constituents such as tannins, flavonoids, triterpenes, alkaloids, carbohydrates, etc. [3]. The phytochemical analysis of various species of Bignoniaceae family was not studied so far hence; the following research deals with to carry an out phytochemical analysis of various extracts of leaves of *Tecoma gaudichaudi* DC. However, no previous biological activities have been reported for *Tecoma gaudichaudi* DC leaf powder except some ethnomedicinal claims were reported such as in Bangladesh whole plant of *Tecoma gaudichaudi* DC use of a remedy for diabetes and infertility problems [3]. The present study aims to evaluate the in vitro cytotoxic activity of ethanol, ethyl acetate, pet ether extract of *Tecoma gaudichaudi* DC against five cancer cell lines, such as human breast cancer cell line MDA-MB-231, MCF 7, mouse melanoma cell line B16F10 and B16F1, human melanoma cell line SK-MEL-2 and study also focus on analysis of various chemical moiety by preliminary phytochemical analysis. This study is considered to be the first report on the *Tecoma gaudichaudi* DC use against these cancer cell line.

MATERIAL AND METHODS

Plant Collection

The stems with leaves of *Tecoma gaudichaudi* DC (Family Bignoniaceae) were collected from Moshi area of Pune district (Maharashtra) in the month of September 2011 and the plant were authenticated at Botanical Survey of India, Pune. The specimen voucher number is KALKTEG1.

Extraction

Extracts of powder of leaf were prepared by successive extraction methodology by using Soxhlet apparatus [4, 5]. The leaves were shade dried and 500gm coarse powder of leaves were subjected to various solvents by increasing polarity such as pet ether, ethyl acetate, ethanol, in soxhlet apparatus for 6hrs. Then the resultant extract was keeping in desiccators and stored until further use.

Phytochemical Analysis

Preliminary chemical analysis was performed according to the established protocol mentioned in Rangari V.D., 2002 and Khandelwal K.R., 2005. [6,7]

Cell culture and in vitro cytotoxic activity

Cancer cell lines, such as human breast cancer cell line MDA-MB-231 and MCF 7, mouse melanoma cell line B16F10 and B16F1, human melanoma cell line SK-MEL-2 were used in the study and the cell lines were preserved at the Tata memorial center (ACTREC) Kharghar, Navi Mumbai. All these cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mm L-glutamine. For the present screening experiment, cells were inoculated into 96 well 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 hrs prior to addition of experimental drugs. The cytotoxicity activity of ethyl acetate was carried out by using sulforhodamine-B (SRB) assay according to the reported method [8,9]. Ethyl acetate and Adriamycin solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use and finally drug concentration was prepared as 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and the 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 discarded; the plates were washed five times with tap 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 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 mMtrizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. 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. The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 x 105 cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37^{o}C for 72 hours in 5% CO_2 incubator and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form an overall concentration 10%. The plates were incubated at 4^{o}C for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air dried. The air-dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100µl of 10mm Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using a microplate reader at a wavelength of 540nm [8,9]The percentage growth inhibition was calculated using following formula,

% Growth inhibition = 100 - (Mean OD of individual Test Group/ Mean OD of control Group) X 100

Evaluation of in-vitro antioxidant activity

DPPH scavenging activity

The percentage radical scavenging activity was investigated by using reported method [11]. 1 ml of extract solution, 5ml of methanolic solution of DPPH were mixed and incubated at 37^{0}C for 20min. The absorbance was measured against methanol as a blank at 517nm. The absorbance of DPPH was taken as a control. The percent antiradical activity was calculated by using following formula [11]

% antiradical activity = (A Control. – A sample) / A Control X 100

Where A Control: Control absorbance (DPPH); A sample: Sample/standard absorbance.

RESULTS AND DISCUSSION

Phytochemical analysis

The detailed phytochemical analysis of the arial parts of *Tecoma gaudichaudi*DCreveals that it contains adequate quantity of tannins, steroids and terpenoids, flavonoids which shows tremendous pharmacological potential of further studies. (Table 1)

In vitro cytotoxic activity

In the current study, Sulphorhodamine B assay was carried out against five cancer cell lines for example MDA-MB-231, MCF 7, B16F10, B16F1, SK-MEL-2 for measure the cytotoxicity in cell in response to plant extracts. Thus, here study accomplished that ethyl acetate, ethanol, pet ether extract and ursolic acid of *Tecoma gaudichaudi* DCdid not shows significant effect on these five cancer cell lines at concentrations 10-80µg/ml by in-vitro cytototoxic assay. (Results are noted in Table 3.1 to 3.10 and fig. 1 to 5)

In vitro antioxidant activity

For *in-vitro* antioxidant activity ethyl acetate extracts of *Tecoma gaudichaudi* DC were screened by DPPH radical scavenging and they showed significant antioxidant activity. inhibitory concentration (IC50) 33.15, 40.72 of ethyl acetate and ethanol extracts of *Tecoma gaudichaudi* DC respectively as compared with ascorbic acid. (Table 2)

Table 1. Phytochemical analysis of ethyl acetate extract of bark of *Tecoma gaudichaudi DC*

Phytoconstituents	*Tecoma gaudichaudi*DC
Carbohydrates	-
Proteins	-
Alkaloids	-
Glycosides	-
Steroids	++
Triterpenoids	++
Flavonoids	+
Tannins	+
Volatile oil	-

Table no. 2. Evaluation of antioxidant activity, DPPH radical scavenging activity of *Tecoma gaudichaudi DC*

Sr. no.	Test sample	Concentration (µg/ml)	Percentage inhibition	IC_{50} (µg/ml)
1	EATG	10 20 40 60 80	37.17 43.2 54.2 67.36 70.32	33.15
2	ETG	10 20 40 60 80	13.6 23.59 57.02 78.23 84.45	40.72
3	Ascorbic acid	10 20 40 60 80	23.61 39.66 74.52 91.42 94.86	28.03

Table 3. IN-VITRO CYTOTOXICITY ASSAY

Table 3.1 Cytotoxicity assay on MDA-MB-231 (Human origin breast cancer Cell line)

	MDA-MB-231			
	Percentage Control Growth			
	Drug Concentrations in µg/ml			
	Average Values			
	10	20	40	80
EATG	69.66±1.6	72.62±1.6	46.56±10.7	21.07±10.8
ETG	77.07±4.1	80.20±4.7	70.75±1.8	65.29±4.3
PETG	80.19±4.9	85.18±4.6	74.23±4.8	55.59±5.1
UA	67.09±2.4	61.27±4.3	19.51±2.7	-43.1±1.7
ADR	-73.7±1.8	-76.3±0.8	-82.7±0.8	-84.4±0.7

Table 3.2 Cytotoxicity assay on MDA-MB-231 (Human origin breast cancer cells)

Sample names	Cytotoxicity assay on cancer cell line MDA-MB-231(Human origin breast cancer cells) (concentration in microgram per millilitre)		
	LC50	TGI	GI50*
EATG	NE	>80	40.8
ETG	NE	>80	>80
PETG	NE	>80	>80
UA	NE	53.5	22.9
ADR	<10	<10	<10

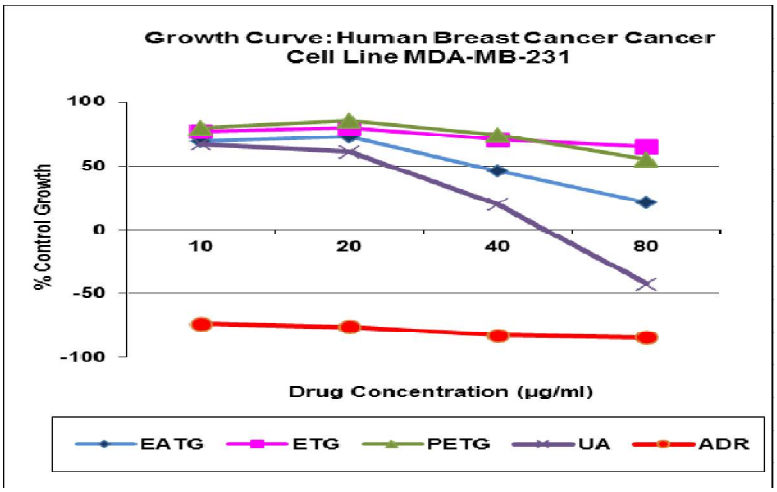

Fig. 1 Growth Curve: MDA-MB-231(Human origin breast cancer cells)

Table 3.3Cytotoxicity assay on MCF-7 cancer cell line (Human origin breast cancer cell line)

	MCF-7 (Human origin breast cancer cell line)			
	Percentage of Control Growth			
	Drug Concentrations in microgram per millilitre			
	Average Values			
	10	**20**	**40**	**80**
EATG	**86.70±2.7**	**90.67±5.8**	**62.51±5.6**	**41.55±1.0**
ETG	**98.61±5.7**	**101.70±5.8**	**84.34±5.4**	**39.30±0.5**
PETG	**94.40±5.5**	**90.71±4.9**	**54.87±3.6**	**19.76±1.7**
UA	**94.25±5.7**	**83.28±6.0**	**49.66±4.0**	**54.49±2.6**
ADR	**-47.7±5.4**	**-31.1±7.3**	**-38.1±6.5**	**-29.2±2.4**

Sample names	Cytotoxicity assay on MCF-7 (Human origin breast cancer cell line) Parameters		
	LC50	**TGI**	**GI50***
EATG	**NE**	**>80**	**66.1**
ETG	**NE**	**>80**	**71.6**
PETG	**NE**	**>80**	**50.9**
UA	**NE**	**>80**	**74.0**
ADR	**<10**	**<10**	**<10**

Table 3.4 Cytotoxicity assay on MCF-7 cancer cell line (Human origin breast cancer cell)

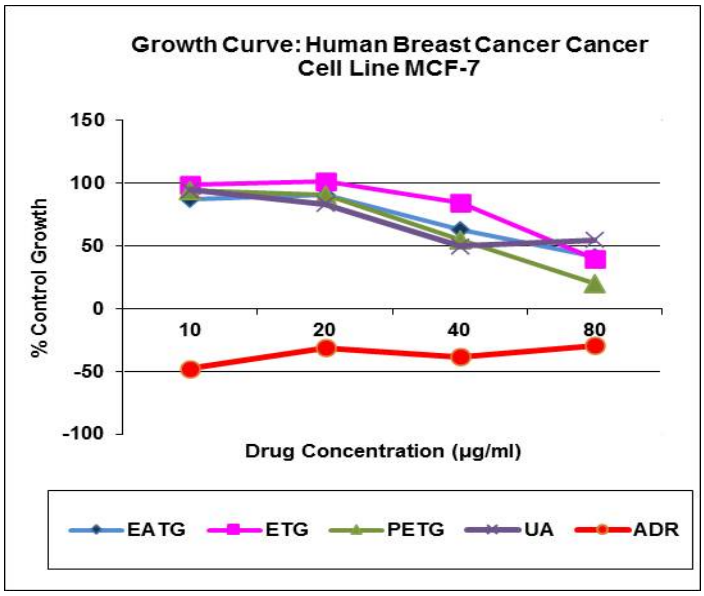

Fig. 2 Growth Curve: MCF-7 (Human origin breast cancer cell line)

Table 3.5 Cytotoxicity assayon cell line B16F10 (Mouse Melanoma origin)

	MOUSE MELANOMA B16F10 CANCER CELL LINE			
	Percentage of Control Growth			
	Drug concentration in microgram per milliliter			
	Average Values			
	10	**20**	**40**	**80**
EATG	**68.37±2.5**	**61.43±1.5**	**46.8±6.2**	**27.54±12.4**
ETG	**74.29±0.6**	**73.29±1.3**	**69.63±1.8**	**60.70±5.9**
PETG	**70.37±0.8**	**71.23±1.9**	**61.52±2.2**	**55.19±4.8**
UA	**87.57±6.0**	**73.48±4.15**	**9.05±2.81**	**81.46±3.6**
ADR	**-73.9±1.8**	**-79.0±1.3**	**-80.7±1.0**	**-72.7±3.2**

Table 3.6 Cytotoxicity assay on cell line B16F10 (Mouse Melanoma origin)

Sample names	Cytotoxicity assay on MOUSE MELANOMA B16F10 CANCER CELL LINE		
	LC50	**TGI**	**GI50***
EATG	**NE**	**>80**	**39.3**
ETG	**NE**	**>80**	**>80**
PETG	**NE**	**>80**	**>80**
UA	**NE**	**53.5**	**31.4**
ADR	**<10**	**<10**	**<10**

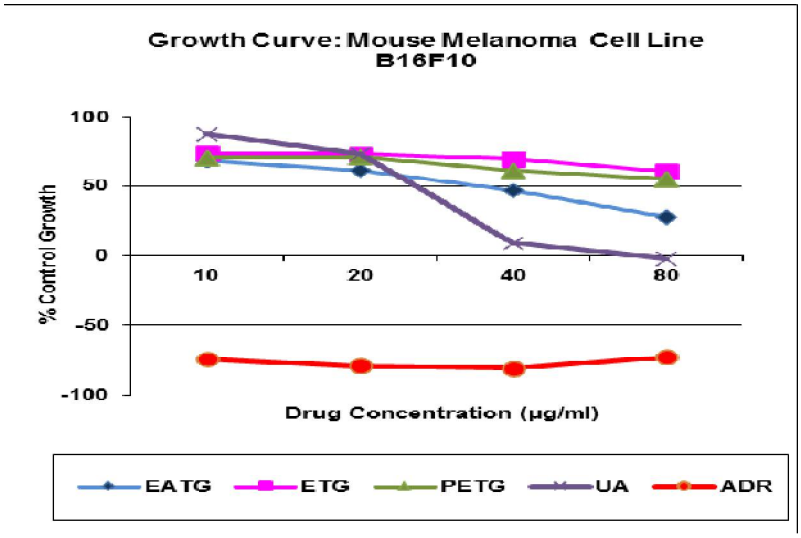

Fig 3. Growth curve: MOUSE MELANOMA

B16F10 CANCER CELL LINE

Table 3.7 Cytotoxicity assay on Cell Line B16F1 (Mouse Melanoma origin)

	MOUSE MELANOMA B16F1 CANCER CELL LINE			
	Percentage of Control Growth			
	Drug concentration in microgram per milliliter			
	Average Values			
	10	**20**	**40**	**80**
EATG	**103.6±2.8**	**96.4±1.7**	**79.6±2.7**	**39.2±1.7**
ETG	**106.8±3.3**	**99.1±2.7**	**92±0.9**	**73.1±0.3**
PETG	**107.3±4.2**	**95.1±3.3**	**71.5±4.7**	**24.5±7.9**
UA	**93.5±4.8**	**67.4±1.3**	**40.7±2.7**	**23.4±1.3**
ADR	**-53.9±1.7**	**-53.9±0.5**	**-52.5±0.2**	**-25.9±4.6**

Table 3.8 Cytotoxicity assay on Cell Line B16F1 (Mouse Melanoma origin)

Sample names	Cytotoxicity assay on Human Melanoma cell line B16F1 (Mouse Melanoma origin) (concentration in µg/ml)		
	LC50	**TGI**	**GI50***
EATG	**NE**	**>80**	**69.4**
ETG	**NE**	**>80**	**>80**
PETG	**NE**	**>80**	**58.3**
UA	**NE**	**>80**	**44.3**
ADR	**<10**	**<10**	**<10**

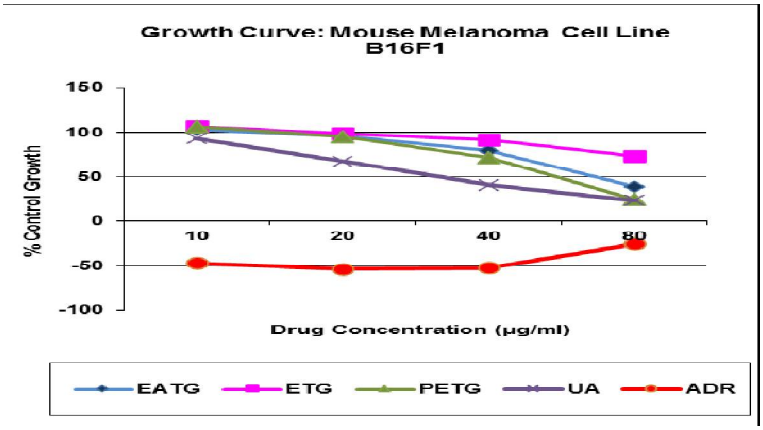

Fig 4. Growth curve Human Melanoma cell line

B16F1 (Mouse Melanoma origin)

Table 3.9 Cytotoxicity assay on cancer cell line SK-MEL-2 (Human Melanoma origin)

	SK-MEL-2 (HUMAN MELANOMA CANCER CELL LINE)			
	% Control Growth			
	Drug Concentrations (µg/ml)			
	Average Values			
	10	**20**	**40**	**80**
EATG	**120.12±7.0**	**129.76±9.6**	**126.2±14.3**	**68.70±4.8**
ETG	**114.39±5.8**	**136.30±10.6**	**135.869.3**	**114.435.6**
PETG	**133.48±13.2**	**144.81±16.31**	**134.96±19.44**	**126.89±9.81**
UA	**142.03±16.1**	**123.03±23.5**	**93.45±17.9**	**23.41±19.2**
ADR	**-60.4±1.0**	**-64.7±1.2**	**-73.9±1.5**	**-75.9±3.0**

	Cytotoxicity assay on Human Melanoma cell line SK-MEL-2 (concentration in µg/ml)		
	LC50	**TGI**	**GI50***
EATG	**NE**	**>80**	**>80**
ETG	**NE**	**>80**	**>80**
PETG	**NE**	**>80**	**>80**
UA	**NE**	**>80**	**64.6**
ADR	**<10**	**<10**	**<10**

Table 3.10 Cytotoxicity assay on Human Melanoma cell line SK-MEL-2

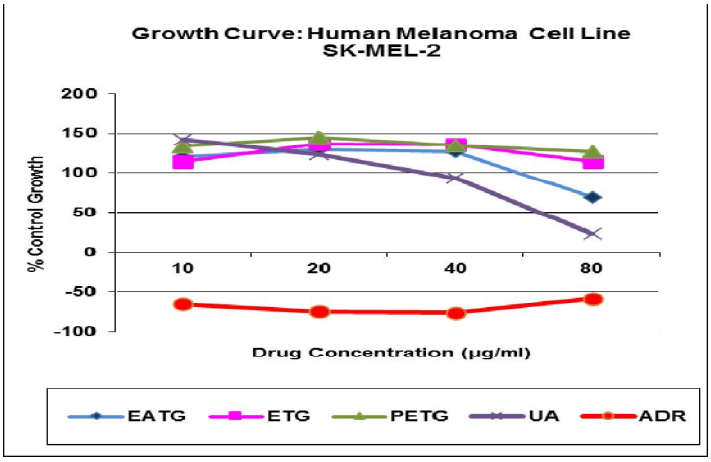

Fig. 5 Growth curve: Human Melanoma cell line SK-MEL-2

CONCLUSION

Medicinal plants having various phytocostituents, many of which biologically active compounds are responsible for exhibiting diverse pharmacological activities [12]. The simplest antiproliferative assay is to measure the concentration of sample needed to inhibit cell growth by 50% against a single cell line. The major advantage of antiproliferative assay is that all potential mechanisms concerning cellular proliferation are monitored simultaneously. In the present study, SRB assay was carried out against five cancer cell lines such as MDA-MB-231, MCF 7, B16F10, B16F1, SK-MEL-2 for measuring the cytotoxicity in cells in response to plant extracts. So, the present study concluded that ethyl acetate, ethanol, pet ether extract of *Tecoma gaudichaudi* DC along with ursolic acid was not found effective on these five cancer cell lines at concentrations 10-80µg/ml by in-vitro cytototoxic assay. Further, the potent antioxidant potential of ethyl acetate extract was investigated shows significant antioxidant activity further helps in pharmacological evaluation of plant species.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

CONSENT (WHEREEVER APPLICABLE)
It is not applicable.

ETHICAL APPROVAL (WHEREEVER APPLICABLE)
It is not applicable.

ABRIVIATION
TG: *Tecoma gaudichaudi* DC; EATG: Ethyl acetate extract of *Tecoma gaudichaudi* DC; ET: Ethanol extract of *Tecoma gaudichaudi* DC; PETG: Pet ether extract of *Tecoma gaudichaudi* DC, UA: Ursolic acid; ADR: Adriamycin; SRB;Sulforhodamine B assay; BSI: Botanical Survey of India; ACDSF: Anticancer Drug Screening Facility.

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