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IN VITRO ANTICANCER ACTIVITY OF *CALLICARPA ARBOREA* ROXB. AND *BUETTNERIA ASPERA* COLEBR., A TRADITIONAL MEDICINAL PLANTS FROM MIZORAM, NORTHEAST INDIA

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ABSTRACT

Cancer is a dreadful disease and it is one of the major causes of deaths in humans. Any practical solution in combating this disease is of importance to public health. In recent times, the trend in cancer research is shifting towards identifying new medicine from natural resources for management of cancer. Many herbs have been evaluated and are currently being investigated phytochemically to understand their antitumor actions against various types of cancers. In present investigation, the methanolic leaves extract of *Calicarpa arborea* Roxb and *Buettneria aspera* Colebr which are being used by the mizo people of Northeast India for the treatment of ulcers and other diseases of patients were evaluated for its anticancer activity. The SRB assay protocol was followed to observe the activity of the extracts against five human cancer cell lines such as breast cancer cell line (MCF-7), colon cancer cell lines (HT-29), cervical cancer cell line (HeLa), leukemia cell line (MOLT-4) and ovarian cancer cell line (OVCAR-3). The study drug was tested in 96 well plates with its 4 dilutions in triplicates at 4 dose levels. The growth curve graphs were plotted and LC50, GI50, TGI values were calculated. Adriamycin was used as positive control. The results showed that the methanolic extracts of two plants exhibited a high degree of *in vitro* anticancer activity against the different cancer cell lines. The result of the present work may be useful for the development of anticancer agents of plant origin.

KEY WORDS: *Calicarpa arborea* Roxb, *Buettneria aspera* Colebr, Human cancer cell lines, *In vitro* anticancer activity, SRB assay.

INTRODUCTION

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. About 80 percent of the world populations rely on the use of traditional medicines, which are predominantly based on plant materials. [1] Numerous research papers and reviews indicate that medicinal plants exhibit a variety of therapeutic properties and also described the importance of compounds derived from natural products, especially plants and microorganism to treat human diseases. [2-4]

Cancer is one of the most life threatening diseases and possess many health hazard in both developed and developing countries. [5] Indeed, the struggle to combat cancer is one of the greatest challenges of mankind. [6] There are several medicines available in the market. However, the emerging problem of drug associated toxicity and drug resistance necessitates the development of newer improved anti cancer drugs. Plants have long history used in the treatment of cancer. Active constitutes of *Catharanthus roseus*, *Angelica gigas*, *Podophyllum peltatum*, *Taxus brevifolia*, *Podophyllum emodii*, *Ocrosia elliptica*, and *Campototheca acuminata* have been used in the treatment of advanced stages of various malignancies. [7] The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. About 60 % to 75 % of FDA approved anticancer and anti-infectious drugs are of natural origin. [3] So, the potential of natural products cannot be ignored and natural plant derived molecules may serve as templates for discovery of new anticancer drugs. [8] *In vitro* anticancer screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work. Screening and isolation of active compounds from plants possessing anticancer potential appears to be a promising way of discovering novel therapeutic compound. [9,10] Since many modern allopathic medicines have been developed from the traditional knowledge, the ethnomedicinal use and hence,

the probable pharmacological and medicinal activity of *Callicarpa arborea* Roxb. and *Buettneria aspera* Colebr. cannot be ignored.

Callicarpa arborea Roxb. is a middle-sized evergreen tree belonging to the family *Verbenaceae*. It is commonly known as Hnahkiah in Mizoram, Northeast India. Traditionally a decoction prepared by boiling the leaves is used for various ailments such as cholera, dysentery, diarrhoea, internal bleeding, colic and stomach ulcer. [11] The bark juice is applied on cuts and wounds as hemostatic. [12] *Buettneria aspera* Colebr *is* a large woody climber with grooved branches belonging to the family Sterculiaceae. It is commonly known as Zawngluanghrui in Mizoram. Traditionally Juice of the stem is retained in the mouth for a while to cure children mouth sore, stomach ache etc. [11]

MATERIALS AND METHODS

Plant material

The leaves of *Callicarpa arborea* Roxb. and *Buettneria aspera* Coleb.r was collected from Southern part of Mizoram, Northeast India during the month of March. Herbarium sheet was prepared and authentication was done at the Botanical Survey of India, Kolkata (Reference No: CHN/60/2012/Tech.II/942 Dated: 17-01-2013). The plants were properly documented and the specimens were preserved in the Department of Pharmacy, RIPANS, Aizawl, Mizoram, Northeast India for future references.

Preparation of plant extract

The dried powdered leaves of the plant were subjected to successive extraction first by cold maceration process at room temperature (27±2 °C) using petroleum ether and then followed by soxhlet apparatus using chloroform and methanol as solvent respectively for 48 hours. The extracts was evaporated under vacuum in a rotary evaporator, kept in air tight container and further used for the experiment.

Cell lines

Various human cancer cell lines used for in vitro SRB Assay are Human breast cancer cell line (MCF-7), colon cancer cell lines (HT-29), cervical cancer cell line (HeLa), leukemia cell line (MOLT-4) and ovarian cancer cell line (OVCAR-3). The stock cultures were grown in T-75 flasks containing 50 mL of RPMI-1640 medium with glutamine, bicarbonate and 5 % 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). Experimental cultures were plated in microtiter plates containing 0.2 mL of growth medium per well at densities of 1,000-200,000 cells per well. The cell lines were maintained at 37 ºC in a 5 % CO_2 atmosphere with 95 % humidity. [13]

Sulforhodamine B (SRB) assay

The invitro SRB assay for anticancer activity evaluation of drugs were done at Anti-cancer Drug screening facility (ACDSF) at ACTREC, Tata Memorial Centre, Navi Mumbai where cell lines were maintained in ideal laboratory conditions.

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 Skehan. [14] The cell lines were 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 90 µL/well at appropriate plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, in 5 % CO_2, 95 % air and 100 % relative humidity for 24 hrs prior to addition of experimental drugs. After 24 hrs, cells from one plate of each cell line were fixed in-situ with TCA (trichloro acetic acid), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental extracts were solubilised in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration of 100, 200, 400 and 800 µg/ml. Aliquots of 10 µl of these different dilutions were added to the appropriate microtiter wells already containing 90 µl of cell suspension, resulting in the required final drug concentrations of 10, 20, 40 and 80 µg/ml.

Positive control

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

Endpoint measurement

After compound addition, plates were incubated at standard conditions for 48 hrs 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 discarded; the plates were washed 5 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 staining, unbound dye was recovered and the residual dye was removed by washing 5 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 an Elisa 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. Using the 6 absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the 4 concentration levels (Ti)]; the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: [(Ti-Tz)/(C-Tz)] 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

The results showing anti-cancer activity of extracts of *Callicarpa arborea* Roxb and *Buettneria aspera* Colebr. against human cancer cell lines such as human breast cancer cell line (MCF-7), colon cancer cell lines (HT-29), cervical cancer cell line (HeLa), leukemia cell line (MOLT-4) and ovarian cancer cell line (OVCAR-3) are presented in table no. 1 and 2 and the growth curve of different cancer cell lines are given in figure no 1, 2, 3, 4 and 5. Experimentally the test samples showing growth inhibition of 50 % at 20 µg/ml were considered to be active. Out of the 5 cell lines used for studying anticancer activity, methanolic extract of *Calicarpa arborea* Roxb showed activity against HeLa, HT-29 and MOLT 4 while *Buettneria aspera* Colebr were active against MOLT-4, and OVCAR-3.

Table 1: *In vitro* anticancer activity of extracts of *Calicarpa arborea* and *Buettneria aspera* using SRB Assay on MCF-7, HeLa and HT-29 cancer cell lines

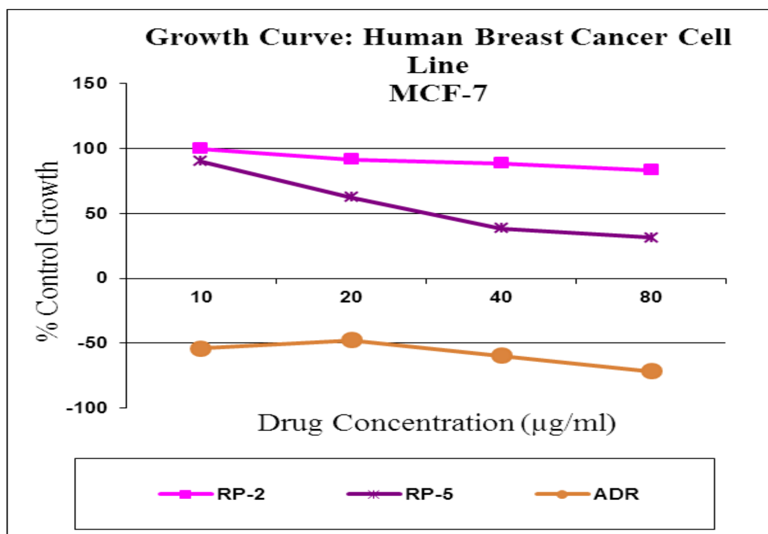
Extracts	Cell Lines								
	MCF-7			HeLa			HT-29		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	LC50
RP-2	>80	>80	>80	>80	62.0	36.5	>80	>80	>80
RP-5	>80	>80	45.0	>80	33.1	<10	NE	NE	<10
ADM	11.0	<10	<10	5.5	<10	<10	<10	<10	<10

RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Table 2: *In vitro* anticancer activity of extracts of *Calicarpa arborea* and *Buettneria aspera* using SRB Assay on MOLT-4 and OVCAR-3 cancer cell lines

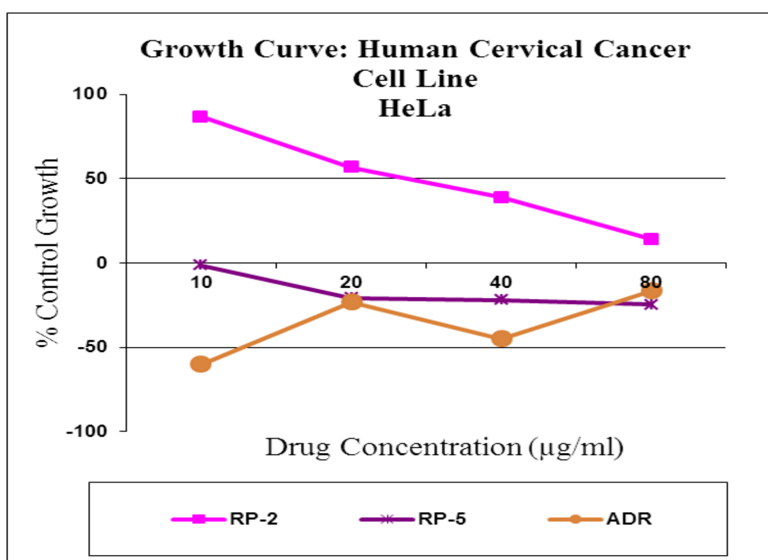
Extract	Cell Lines					
	MOLT-4			OVCAR-3		
	LC50	TGI	GI50	LC50	TGI	GI50
RP-2	NE	NE	<10	>80	>80	<10
RP-5	NE	NE	<10	NE	NE	13.7
ADM	NE	NE	<10	66.9	<10	<10

RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin



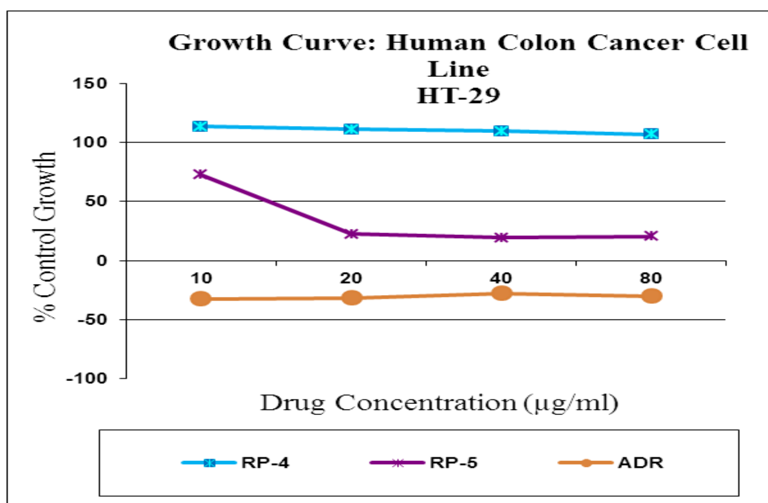
RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Fig. 1: Percentage control growth of Human Breast Cancer cell line MCF-7



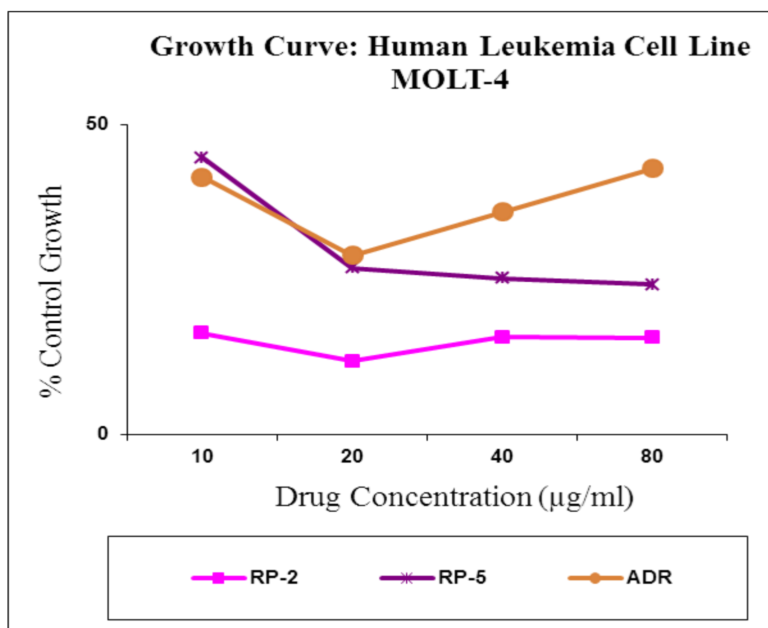
RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Fig. 2: Percentage control growth of Human Cervical Cancer cell line HeLa



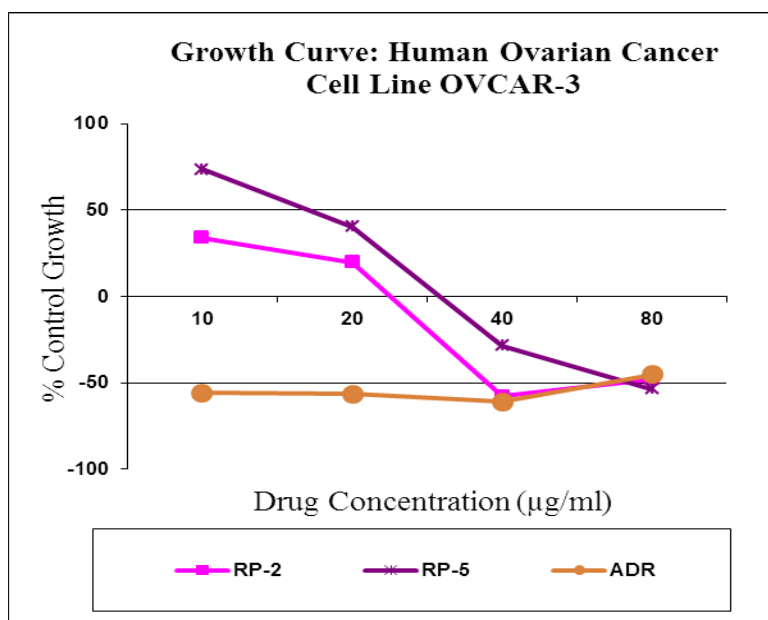
RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Fig 3: Percentage control growth of Human Colon Cancer cell line HT-29



RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Fig. 4: Percentage control growth of Human Leukemia Cancer cell line MOLT-4



RP- 2: Buettneria aspera RP-5: Calicarpa arborea ADM: Adriamycin

Fig. 5: Percentage control growth of Human Ovarian Cancer cell line OVCAR-3

CONCLUSION

In the present study, we concluded that the plant extracts showed selective *in vitro* anticancer activities against some human cancer cell lines. The activity might be depended upon the morphology of cell lines and mechanism of action of the plant extract. However, further studies are required to assess the molecular mechanism of anticancer activity of these two plants. Further, all these plants extract need to be screened against different cell lines apart from the selected cell lines to confirm the activity. The results of the study will also need to be confirmed using in vivo models and the result of the present work may be useful for the development of anticancer agents of plant origin.

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REFERENCES

1. Patil A, Vadera K, Patil D, Phatak A, Juvekar A, Chandra N. *In vitro* anticancer activity of *Argemone mexicana* Seeds and *Alstonia scholaris* R. Br. bark on different human cancer cell lines. World Journal

of Pharmacy and Pharmaceutical Sciences, 2014; 3(11): 706-22.
2. Lam KS. New aspects of natural products in drug discovery. Trends Microbiol, 2007; 15(6): 279-89.
3. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. J Nat Prod, 2003; 66(7): 1022-37.
4. Butler MS. The role of natural product chemistry in drug discovery. J Nat Prod, 2004; 67(12): 2141-53.
5. Houghton P. The role of plants in traditional medicine and current therapy. J Altern Complem Med, 1995; 1(3): 131-43.
6. Divisi D, Di TS, Salvemini S, Garramone M, Crisci R. Diet and Cancer, Acta Biomed, 2006; 77: 118-23.
7. Eva JM, Angel GL, Laura P, Ignacio A, Antonia C, Federico G. A New Extract of the Plant *Calendula Officinalis* produces a dual *in-vitro* effect: Cytotoxic anti-tumor activity and lymphocyte activation. BMC Cancer, 2006; 6(1): 119.
8. Kim J, Park EJ. Cytotoxic anticancer candidates from natural resources. Curr Med Chem Anti-Canc Agents, 2002; 2: 485–537.
9. Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Canc, 2002; 2: 143–48.
10. Cardellina. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV inhibitory natural products extracts. In: Bohlin L and Bruhn JG (eds.). Bioassaay Methods in Natural Product Research and Development, Dordrecht; Kluwer Academic Publishers: 1999, pp. 25–36.
11. Rozika R. Ramhmul Damdawite (Medicinal Plants). 1st ed., Aizawl; Medicinal Plant Board Mizoram: 2005.
12. Sharma HK, Chhangte L, Dolui AK. Traditional medicinal plants in Mizoram, India. Fitoterapia, 2001; 72(2): 146-61.
13. Kenneth G. Macleod PL, Simon PL. Essential Techniques of Cancer Cell Culture, Methods in Molecular Medicine, vol. 88. In: Langdon SP (eds.). Cancer Cell Culture: Methods and Protocols, Totowa, NJ; Humana Press Inc: 2004.
14. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst, 1990; 82(13): 1107-12.