ORIGINAL ARTICLE

In vitro cytotoxicity studies on *Carissa congesta*, *Polyalthia longifolia*, and *Benincasa hispida* extracts by Sulforhodamine B assay method

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Background: Indian medicinal plants have contributed to the growth of world's ethnopharmacological heritage. Roots of *Carissa congesta* (CC) powder are mixed with horse urine, lime juice, and camphor and used as remedies for relieving itching conditions, *Polyalthia longifolia* (PL) leaves are aromatic and used for decoration in festivals as sonamukhi and *Benincasa hispida* (BH) seeds provide treatment for cough and vitiated conditions of *pitta*. **Aims of the Study:** In the current studies, crude petroleum ether extracts (BH and CC) and ethanolic extract of (PL) were screened for *in vitro* cytotoxicity activity using different cell lines. **Settings and Design:** In the experiment, human colon cancer HCT15, human breast cancer MCF7 and human leukemia MOLT4 cell lines were studied on the extracts. **Materials and Methods:** The method used was Sulforhodamine B (SRB) assay method in which growth inhibition of 50% (GI_{50}) was analyzed by comparing it with standard drug Adriamycin (ADR) (doxorubicin). **Results:** The CC and PL extracts showed equivalent activity to ADR (doxorubicin) for human breast cancer cell line MCF7 and human leukemia cell line MOLT4 respectively. BH extract did not show satisfactory activity on selected cell lines. **Conclusion:** In the future, new cell lines may be screened in order to check the potency of CC, PL, and BH extracts.

Key words: *Carissa congesta*, human breast cancer cell line MCF7, human leukemia cell line MOLT4, *Polyalthia longifolia*, Sulforhodamine B assay method

INTRODUCTION

Among various diseases, cancer has become a big threat to humans globally. As per Indian population census data, the rate of mortality due to cancer in India was high and alarming with about 806,000 existing cases by the end of the last century. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year. This is owing to the poor availability of prevention, diagnosis, and treatment of the disease.[1]

In view of these facts, to warrant immediate action and to scale-up the interventions for chronic diseases through private and public sectors, improved public health and primary health-care systems are essential for the implementation of cost-effective interventions. Some of the lead molecules isolated from different medicinal plants are already in use to treat cancer and chemotherapeutic side effects. These potential and successful anticancer molecules include vincristine, vinblastine, taxol, camptothecin, and podophyllotoxin.[2]

According to a report by World Health Organization, the use of herbal medicine is increasing in both developing and industrialized countries.[3] Considering the fact that over one-third of the population in developing countries lack access to essential medicines, the provision of safe and effective traditional therapies could become a critical tool to increase access to healthcare.[4] Recent reports have cited many plants and their components acting as a tumor suppressor and apoptotic inducers in cancer cells.[5]

Carissa congesta (CC) herbs are used for major and minor ailments, *Polyalthia longifolia* (PL) is used for increasing the flora and *Benincasa hispida* (BH) (Thunb) Cogn.

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a rasayana herb have been reported for anticonvulsant, cardiotonic, hepatoprotective, antimicrobial, antidiabetic, antiviral, anticancer, spasmolytic agent, antimicrobial, anti-inflammatory, antiulcer, analgesic, and hepatoprotective.[6-20]

The sulforhodamine B (SRB) assay method is rapid, sensitive, reliable and inexpensive method for analyzing the cellular protein content of adherent and suspension cultures in 96-well microtiter plates. It is an ideal method for ordinary laboratory purposes and large-scale applications as in disease-oriented studies in which *in vitro* anticancer-drug discovery screening is to be undertaken. It gives a colorimetric end that is nondestructive, indefinitely stable and visible to the naked eye. It provides a sensitive measure of drug-induced cytotoxicity useful in quantitating clonogenicity as well-suited to high volume, automated drug screening.[21]

In this study, an attempt has been made to study the effect of crude plant extracts on human colon cancer cell line HCT15, human breast cancer cell line MCF7 and human leukemia cell line MOLT4 by SRB assay method as depicted in graphical abstract [Figure 1].

MATERIALS AND METHODS

We have previously reported all the studies undertaken in part A related to CC, PL and BH extracts.[22-24] All the cell cultures and media used in studies comprising of human colon cancer cell line HCT15, human breast cancer cell line MCF7 and human leukemia cell line MOLT4 and standard drug Adriamycin (ADR) (doxorubicin) were procured and maintained at Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Kharghar, Mumbai during the studies. All the chemicals were procured from local suppliers throughout the experimentation with the help of ACTREC, Kharghar, Navi Mumbai.

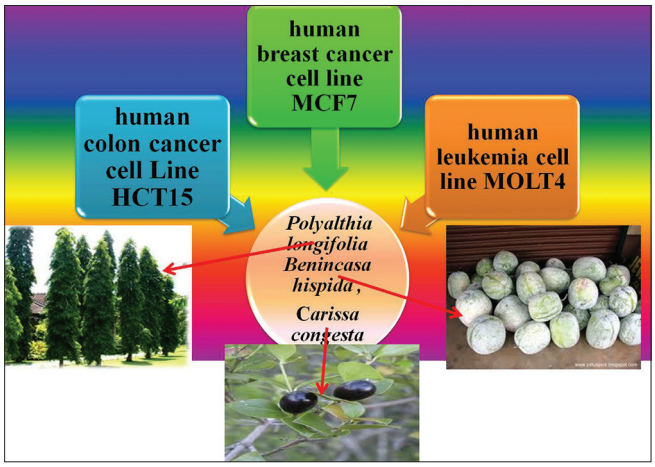

Figure 1: Graphical abstract

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 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 the addition of experimental drug and extracts. Extracts of CC, PL, and BH were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test extracts. 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 that is, 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml. The positive control used in experimentation was ADR (doxorubicin) and each experiment were repeated thrice.

After extracts and standard drug addition, plates were incubated at standard conditions for 48 h and the assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by gentle addition of 50 µL of cold 30% w/v TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air-dried. SRB solution (50 µL) at 0.4% (w/v) in 1% acetic acid was added to each of the wells and the plates were incubated for 20 min at room temperature. After staining, the 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 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 six absorbance measurements (time zero, 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 extracts and standard drug concentration levels. Percentage growth inhibition was calculated as [Ti/C] ×100%.[25,26]

RESULTS

The results of extraction yield and preliminary analysis of the extracts have been already reported by us.[22-24] The

extracts throughout the studies showed negative activity on cell lines except PL and CC extract that showed equivalent activity comparable to the standard compound ADR for human leukemia cell line MOLT4 and human breast cancer cell line MCF7, respectively. Further, CC extract showed mild progressive activity on other two selected cell lines [Tables 1-6 and Figures 2-6].

Table 1: Human colon cancer cell line HCT15 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR

Human colon cancer cell line HCT15								
Percentage of control growth and drug concentrations (µg/ml)								
Details	Experiment 1				Experiment 2			
Concentrations	10	20	40	80	10	20	40	80
PL (A2)	99.9	100.0	76.5	28.8	99.2	97.8	73.2	26.3
BH (A1)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.6
CC (A3)	98.8	78.4	52.4	37.9	90.7	76.4	47.7	33.7
ADR	18.1	18.2	18.6	10.9	18.3	17.4	17.9	13.2
Details	Experiment 3				Average values			
PL (A2)	100.0	99.3	80.1	29.5	99.7	99.0	76.6	28.2
BH (A1)	100.0	100.0	100.0	99.4	100.0	100.0	100.0	99.3
CC (A3)	84.0	76.2	53.4	38.2	91.2	77.0	51.2	36.6
ADR	18.8	17.5	17.0	16.3	18.4	17.7	17.9	13.5

Results in triplicate with average for A1 (CC), A2 (PL) and A3 (BH) extracts and ADR. CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

Table 2: Drug concentrations (µg/ml) calculated from graph of human colon cancer cell line HCT 15 of A1 (BH), A2 (PL) and A3 (CC) extracts ADR

Drug concentrations (µg/ml) calculated from graph			
HCT15	LC50	TGI	GI50
A2 (PL)	>80	>80	63.3
A1 (BH)	>80	>80	>80
A3 (CC)	>80	>80	57.1
ADR	>80	>80	<10

GI50 value of ≤10 µg/ml is considered to demonstrate activity in case of pure compounds whereas GI50 value ≤20 µg/ml in extracts, TGI. TGI – Total growth inhibition; CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

Table 3: Human breast cancer cell line MCF7 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR

Human breast cancer cell line MCF7								
Percentage of control growth and drug concentrations (µg/ml)								
Details	Experiment 1				Experiment 2			
Concentrations	10	20	40	80	10	20	40	80
PL (A2)	74.8	42.4	17.0	6.2	69.8	46.4	16.8	5.9
BH (A1)	100.0	96.8	90.8	84.9	96.9	94.0	91.8	80.3
CC (A3)	49.0	26.8	18.6	14.3	43.6	26.5	15.1	11.3
ADR	−7.6	−15.2	−37.7	−64.5	−12.7	−21.1	−41.0	−63.7
Details	Experiment 3				Average values			
PL (A2)	60.2	43.3	15.5	3.1	68.3	44.0	16.4	5.1
BH (A1)	94.8	91.3	84.3	74.5	97.3	94.1	88.9	79.9
CC (A3)	34.3	23.7	13.9	10.5	42.3	25.7	15.9	12.0
ADR	−15.3	−19.8	−45.2	−65.8	−11.9	−18.7	−41.3	−64.7

Results in triplicate with average for A1 (CC), A2 (PL) and A3 (BH) extracts and ADR. CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

DISCUSSION

All types of cancers have been reported in Indian population including the cancers of skin, lungs, breast, rectum, stomach, prostate, liver, cervix, esophagus, bladder, blood, mouth, etc., The causes of such high incidence rates of these cancers may be both internal (genetic, mutations, hormonal, poor immune conditions) and external or environmental factors (food habits, industrialization, overgrowth of population, social, etc).[1,2] In the current anticancer studies, PL and CC extract showed good activity on human leukemia cell line MOLT4 and human breast cancer cell line MCF7, respectively.

Table 4: Drug concentrations (µg/ml) calculated from graph of human breast cancer cell line MCF7 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR

Drug concentrations (µg/ml) calculated from graph			
MCF7	LC50	TGI	GI50
A2 (PL)	>80	73.6	28.0
A1 (BH)	>80	>80	>80
A3 (CC)	>80	77.9	18.1
ADR	58.4	26.3	<10

GI50 value of ≤10 µg/ml is considered to demonstrate activity in case of pure compounds whereas GI50 value ≤20 µg/ml in extracts, TGI. TGI – Total growth inhibition; CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

Table 5: Human leukemia cell line MOLT4 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR

Human leukemia cell line MOLT4								
Percentage of control growth and drug concentrations (µg/ml)								
Details	Experiment 1				Experiment 2			
Concentrations	10	20	40	80	10	20	40	80
PL (A2)	7.0	−18.4	−23.4	−27.9	33.1	12.0	0.8	−20.6
BH (A1)	42.7	40.0	28.0	25.1	94.3	82.5	69.8	49.5
CC (A3)	48.4	39.8	17.3	7.2	76.4	74.0	63.5	30.4
ADR	−12.4	−19.8	−30.3	−37.9	−27.1	−27.4	−27.5	−30.7
Details	Experiment 3				Average values			
PL (A2)	2.8	−3.8	−11.6	−22.7	14.3	−3.4	−11.4	−23.7
BH (A1)	74.7	68.9	65.3	62.8	70.6	63.8	54.4	45.8
CC (A3)	60.5	55.5	50.1	15.8	61.8	56.4	43.6	17.8
ADR	−29.6	−36.9	−37.7	−40.2	−23.1	−28.0	−31.8	−36.2

Results in triplicate with average for A1 (CC), A2 (PL) and A3 (BH) extracts and ADR. CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

Table 6: Drug concentrations (µg/ml) calculated from graph of human leukemia cell line MOLT4 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR

Drug concentrations (µg/ml) calculated from graph			
MOLT4	LC50	TGI	GI50
A2 (PL)	>80	44.3	<10
A3 (CC)	>80	>80	37.8
A1 (BH)	>80	>80	61.5
ADR	73.1	27.5	<10

GI50 value of ≤10 µg/ml is considered to demonstrate activity in case of pure compounds whereas GI50 value ≤20 µg/ml in extracts, TGI. TGI – Total growth inhibition; CC – *Carissa congesta*; PL – *Polyalthia longifolia*; BH – *Benincasa hispida*; ADR – Adriamycin

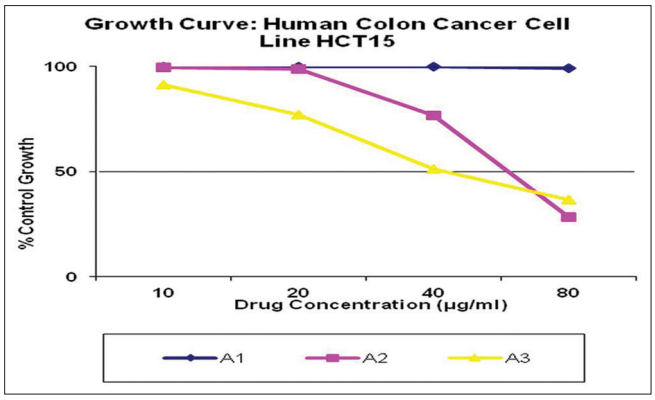

Figure 2: Growth Curve: Human Colon cancer cell line HCT 15 of A1 (BH), A2 (PL) and A3 (CC) extracts

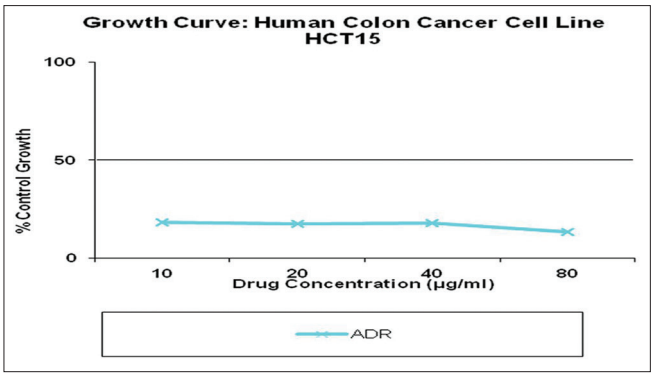

Figure 3: Growth Curve: Human Colon cancer cell line HCT 15 of ADR (Adriamycin)

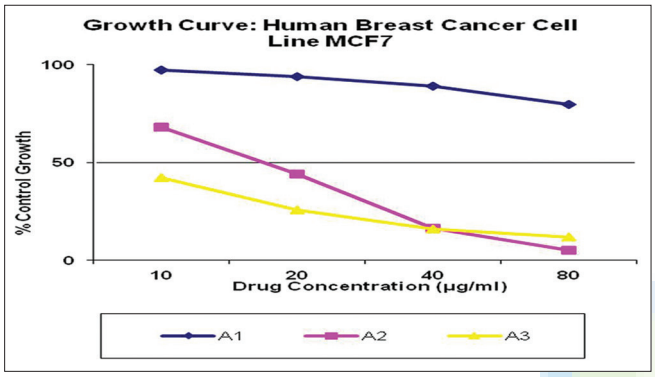

Figure 4: Growth Curve: Human breast cancer cell line MCF7 of A1 (BH), A2 (PL) and A3 (CC) extracts

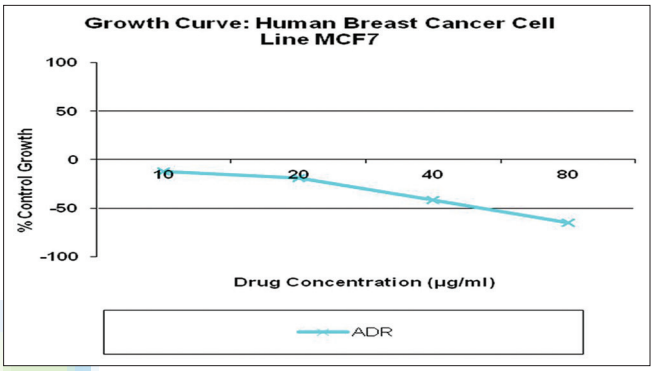

Figure 5: Growth Curve: Human breast cancer cell line MCF7 of ADR (Adriamycin)

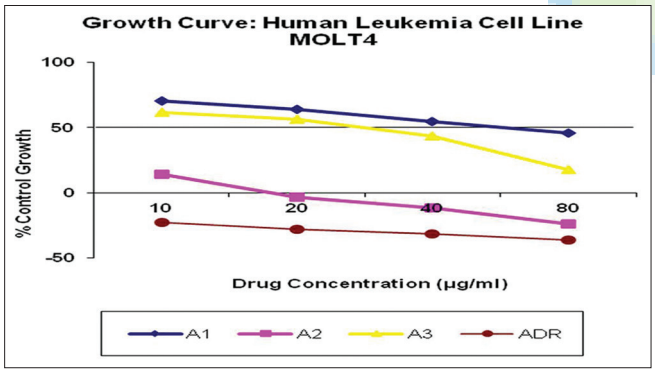

Figure 6: Growth Curve: Human Leukemia cell line MOLT4 of A1 (BH), A2 (PL) and A3 (CC) extracts and ADR (Adriamycin)

CONCLUSION

CC, PL and BH extracts with different parts of the plant could be studied for enormous cell lines having various concentrations. In addition, probable identified constituents in the extracts may be correlated to anticancer activity while undertaking *in vitro* and *in vivo* studies.

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Conflicts of Interest

There are no conflicts of interest.

REFERENCES

1. Imran A, Waseem AW, Kishwar S. Cancer scenario in India with future perspectives. Cancer Ther 2011;8:56-70.
2. Oliver K, Wim Q. Medicinal Plant Biotechnology: From Basic Research to Industrial Applications. New York: Wiley-VCH; 2007.
3. World Health Report; 2006. Available from: http://www.who.int/whr/2006/en. [Last accessed on 2015 Jan 24].
4. Vidhya R, Neethu V, Ancy S. An investigation on cytotoxic and antioxidant properties of *Clitoria Ternatea* L. Int J Drug Discov 2011;3:74-7.
5. Sunila ES, Kuttan G. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. J Ethnopharmacol 2004;90:339-46.
6. Devmurari K, Shivanand P, Jivani P. A review on Carissa Congesta: Phytochemical constituents, traditional use and pharmacological properties. Int J Chem Sci 2010;8:81-7.
7. Harwansh RK, Garabadu D, Rahman AM, Garabadu P. *In vitro* anthelmintic activity of different extracts of root of *Carissa Spinarum*. Int J Pharm Sci Res 2010;1:84-8.

8. Hegde K, Joshi AB. Hepatoprotective effect of *Carissa carandas* Linn root extract against CCl4 and paracetamol induced hepatic oxidative stress. Indian J Exp Biol 2009;47:660-7.
9. Hedge K, Thakker S, Joshi AB, Shastry CS, Chandrasekhar KS. Anticonvulsant activity of *Carissa carandas* linn. Root extract in experimental mice. Trop J Pharm Res 2012;8:117-25.
10. Mandana B, Russly AR, Farah ST, Noranizan MA, Zaidul IS, Ali G. Antioxidant activity of winter melon (*Benincasa hispida*) seeds using conventional soxhlet extraction technique. Int Food Res J 2012;19:229-34.
11. Mukhtar HM, Vashishth D, Ali B, Kaur R. A study for quality assessment of the dried seeds of *Benincasa hispida*. Res J Pharmacogn Phytochem 2012;4:197-201.
12. Murthy KS, Kiran BR. Medicinal plants usage in cardiovascular diseases: A review. Int J Adv Sci Technol 2012;2:264-80.
13. Rachchh MA, Jain SM. Gastroprotective effect of *Benincasa hispida* fruit extract. Indian J Pharmacol 2008;40:271-5.
14. Singh B, Sangwan P. Taxonomy, ethobotany and antimicrobial activity of *Alstonia scholaris* (L.) R. Br. *Carissa carandas* L. and *Catharanthus roseus* (L.) G. Don. Int J Biotechnol Biosci 2011;1:102-12.
15. Gupta SM, Shivaprasad HN, Kharya MD, Rana AC. Immunomodulatory activity of the ayurvedic formulation "Ashwagandha Churna". Pharm Biol 2006;44:263-5.
16. Malairajan P, Gopalakrishnan G, Narasimhan S, Veni KJ. Analgesic activity of some Indian medicinal plants. J Ethnopharmacol 2006;106:425-8.
17. Katkar KV, Suthar AC, Chauhan VS. The chemistry, pharmacologic, and therapeutic applications of *Polyalthia longifolia*. Pharmacogn Rev 2010;4:62-8.
18. Sampath MM, Vasanthi M. Isolation, structural elucidation of flavonoids from *Polyalthia longifolia* (sonn.) Thawaites and evaluation of antibacterial, antioxidant and anticancer potential. Int J Pharm Pharm Sci 2013;5:336-41.
19. Jothy SL, Aziz A, Chen Y, Sasidharan S. Antioxidant activity and hepatoprotective potential of *Polyalthia longifolia* and *Cassia spectabilis* leaves against paracetamol-induced liver injury. Evidence-Based Complement. Altern. Med. 2012; 1-10. DOI:10.1155/2012/56128.
20. Sharma RK, Mandal S, Rajani GP, Gupta N, Srivastava DP. Antiulcer and antiinflammatory activity of fresh leaves extracts of *Polyalthia longifolia* in rats. Int J Drug Dev Res 2011;3:351-9.
21. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al*. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107-12.
22. Doshi GM, Une HD. Chromatographic studies on *Benincasa hispida* (thunb.) cogn seed extract scrutinized by HPLC and HPTLC. Pharmacogn J 2014;6:42-8.
23. Doshi GM, Chaskar PR, Zine SP, Une HD. Cold extraction of *Carissa congesta* wight monitored by a comparative revision of HPLC and HPTLC. Pharmacogn Commun 2014;4:29-33.
24. Doshi GM, Chaskar PK, Zine SP, Une HD. Solicitation of HPLC and HPTLC for determination of rutin from *Polyalthia longifolia*. Pharmacogn Res 2014;2:29-33.
25. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006;1:1112-6.
26. Skehn P, Storeng R, Scudiero A, Monks J, McMohan D, Vistica D, *et al*. New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst 1990;82:1107.