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IN-VITRO SCREENING OF SOME MEDICINAL PLANTS ON BREAST, OVARY AND COLON CANCER CELL LINES.

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ABSTRACT

In the present study, the aqueous extracts of *Curcum caesia*, *Curcuma longa*, *Curcuma zedoaria* and *Gloriosa superba* was studied for their *in vitro* cytotoxic activity against human breast cancer (MCF-7), colon cancer (HCT-116) and ovarian cancer (PA-1) cell lines using sulphorhodamine B (SRB) assay. The results showed that aqueous extract of *Curcuma zedoaria* and *Gloriosa superba* exhibited the prominent inhibitory effect whereas *Curcuma longa* and *Curcuma caesia* have moderate to weak effects in *in vitro* conditions as compared with standard drug 5-fluorouracil.

KEYWORDS: **Human cancer cell lines; Invitro Cytotoxicity test; SRB assay; Indian medicinal plants.**

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INTRODUCTION

Cancer, a progressive uncontrolled proliferation of cells, is a serious worldwide life- threatening disease killing about seven million people per year. The human cancer comprises more than 200 different diseases, accounting for about one fifth of all dead worldwide.[1] The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in the treatment of cancer, indicates that there is an imperative need of alternative strategies in cancer management. Investigational cellular growth for control mechanism has contributed in understanding the carcinogenesis and identification of compound with specific antiproliferative activity.[2] In last few decades, human immortal cancer cell lines have aggregated an accessible, easily usable set of biological models to examine cancer biology and to analyze the anticancer properties as a natural or synthetic drugs. Plants have long history of use in the treatment of cancer.[3] Many natural products discovered from medicinal plants, or secondary metabolites such as terpenoids, phenolic acids, lignans, tannins, flavonoids, quinones, coumarins, alkaloids, which exhibit significant antioxidant and other activities, have played an important role in treatment of cancer.[4] Many of the anticancer agents derived from natural sources such as *Catharanthus roseus, Angelica gigas, Podophyllum peltatum, Taxus brevifolia, Podophyllum emodii, Ocrosia elliptica,* and *Campototheca acuminate* have been used in treatment of malignancies.[5] Till date, there are many plants unexplored for their medicinal values comprising as anticancer agents. In the present study, we have explored four aqueous rhizome extracts from selected plants *Curcuma longa* (Family: Zingiberaceae), *Curcuma caesia* (Family: Zingiberaceae), *Curcuma zedoaria* (Family: Zingiberaceae) and *Gloriosa superba* (Family: Liliaceae) for cytotoxic activity on human breast cancer MCF-7,[6]ovarian PA-1 [7] and colon HCT- 116,[8] cell lines and compared the antiproliferative activity with the 5-Fluorouracil as a standard anticancer drug in the market.

MATERIALS AND METHODS

Plants material

The rhizomes of four plants [*Curcuma caesia* (D-1), *Curcuma longa* (D-2), *Curcuma zedoaria* (D-3), *Gloriosa superba* (D-4)] was collected during the months of March and April 2014. The plants were identified and authenticated by Agharkar research institute, Pune, Maharashtra, India (Authentication No. D1-APTRF/2014/017). and Botanical Survey of India, Pune (Authentication No. D2-AMS2, D3-AMS3, D4-AMS002).

Cancer cell lines and reagents

Human Breast cancer-MCF-7, Colon cancer-HCT-116 and Ovarian cancer-PA-1 were obtained from National Centre for Cell Sciences (NCCS), Ganeshkhind, Pune, India. MCF-7 and PA-1 cells were maintained in minimum essential medium (MEM) (Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 100µg/ ml penicillin and 100µg/ ml streptomycin/gentamicin (Sigma-Aldrich, St. Louis, MO, USA)) in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C. HCT-116 cells were cultured in McCoy's 5a medium (Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS), 100µg/ ml penicillin and 100 µg/ ml streptomycin and 50µg/ml gentamicin (Sigma-Aldrich, St. Louis, MO, USA)) in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C. Stock solutions of plant extracts were prepared in media of respective cell lines, filter sterilized and stored at -20˚C. The final concentration of the different extracts used in the culture were 0, 25, 50, 100, 150, 200 and 250µg/ml. The standard control used was 5-Fluorouracil. Sulforhodamine B dye (SRB), Trypsin- EDTA, Trichloroacetic acid (TCA) were purchased from Sigma-Aldrich.

Preparation of extract

Plant materials (2.0 kg) were dried in shade, coarse powdered and extracted with aqueous solvent using Soxhlet apparatus for 24 hr at 60^0C. The resulting aqueous residue (yield 250 gm) was evaporated at 60 °C using rotary evaporator and stored at -20 °C until further use.

In vitro Assay for cytotoxic activity

The growth inhibitory activity of the four aqueous plant extracts was evaluated in three human cancer cell lines - Human Breast cancer-MCF-7, Colon cancer-HCT-116 and Ovarian cancer-PA-1-using the SRB assay.[9] The cell lines were routinely maintained as monolayer cell cultures containing 10% fetal bovine serum (FBS), 100µg/ ml penicillin and 100 µg/ ml streptomycin/gentamicin. The viability of the cells was determined by the trypan blue exclusion method with haemocytometer. Exponentially growing cells were harvested and plated in 96 well plates at a concentration of 1×10^4 cells/well and incubated for 24 h at 37 °C in a humidified CO_2 (5%) incubator. The final concentrations (0, 25, 50, 100, 150, 200 and 250µg/ml) of different aqueous extracts and 5-Fluorouracil were added to each well for 48 hrs. After 48 h of incubation, cells were fixed with ice cold TCA (TCA 40% w/v) for 1hr at 4^oC, followed by five times washing with distilled water. To each well, 50µl of SRB solution (4.0% w/v in 1% v/v acetic acid) was added and kept at room temperature for 30 minutes. The unbound stain was washed off with acetic acid (1% v/v). The protein bound stain was solubilized by adding 100µl of 10mM Tris base (pH 10.5) in each well, followed by the absorbance measurement at 492 nm on ELISA Reader.[9] Cell viability and growth in presence of test material was calculated as follows

% Growth in presence of test material = (Growth in presence of test material / Growth in absence of test material) X 100.

Statistical analysis

The data from biological assays were analyzed using One-way analysis of variance (ANOVA) procedures which were presented as mean ± SEM. The Dunnett's test was used to compare means. $P<0.05$ was considered the lowest limit of significant.

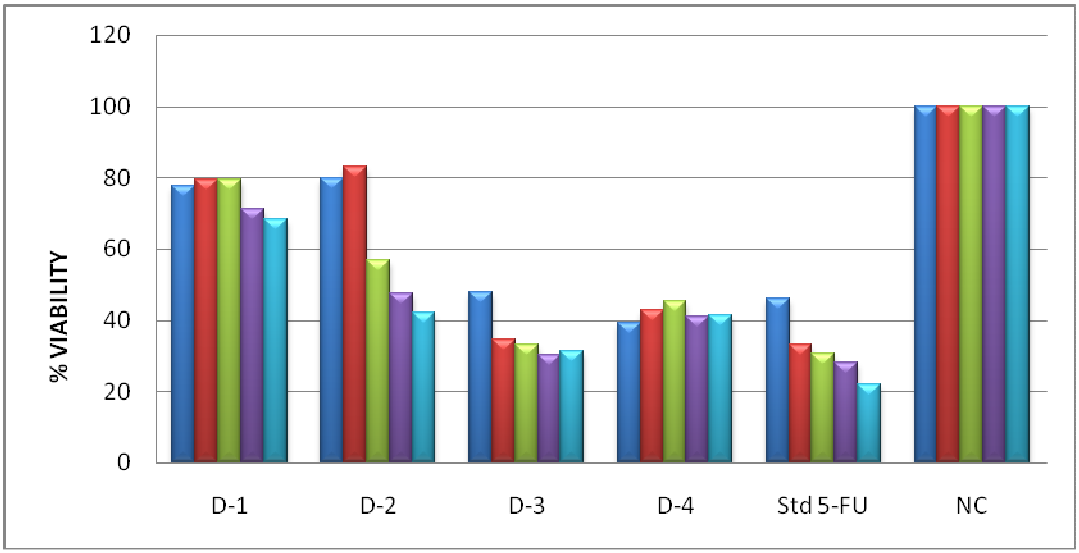
RESULTS

The results of the phytochemical screening of all the plants were shows the presence of flavonoids, terpenoids and phenolics in plants having cytotoxic activity. The screening of aqueous extracts of four plants was done for their antiproliferative activity on three human cancer cell lines - Human Breast cancer-MCF-7, Colon cancer-HCT-116 and Ovarian cancer-PA-1 using the SRB assay. The study was conducted with appropriate positive control (5-Fluorouracil). After 48hr of treatment, some plant extracts exhibited higher inhibitory effect against cancer cells, with varying efficiencies and selectivity's while others have shown moderate to weak growth inhibition on cancer cells. From the results, D-3 *(Curcuma Zedoaria)* and D-4 (*Gloriosa superba*) plant extracts have shown remarkable (***p<0.001) cytotoxic effect (34.6±0.01% and 42.7±0.00%) at 100µg/ml on MCF-7 cell line (Table-1), whereas the cytotoxicity of the D-2 and D-3 extract ranged from moderate to weak effects. The D-3 *(Curcuma Zedoaria)* and D-4 (*Gloriosa superba*) plant extracts exhibit significant (***p<0.001) cytotoxic effect (47.3±0.00% at 150µg/ml and 38.4±0.00% at 200µg/ml concentration) on HCT-116 cell line **(**Table-2), whereas the cytotoxicity of the other plant extract ranged from moderate to weak effects. D-3 *(Curcuma Zedoaria)* plant extract significantly (**p<0.01) inhibit the growth of PA-1 cell line (47.9±0.00%) at 100µg/ml concentration (Table-3), whereas the cytotoxicity of the other plant extract ranged from moderate to weak effects**.**

Table 1
Effect of various herbal extracts on breast cancer cell line MCF-7 (% viability) with respect to control

Conc.	D-1	D-2	D-3	D-4	Std. 5-FU	NC
50µg/ml	77.8±0.04	80.0±0.05	47.9±0.03***	39.2±0.00***	46.0 ± 0.07***	100.0
100µg/ml	79.4±0.04	83.0±0.06	34.6±0.01***	42.7±0.00***	33.3± 0.07***	100.0
150µg/ml	79.6±0.04	56.7±0.01*	33.3±0.01***	45.4±0.01***	30.7± 0.06***	100.0
200µg/ml	71.1±0.02	47.5±0.00**	30.2±0.00***	40.9±0.00***	28.0± 0.03***	100.0
250µg/ml	68.5±0.04	42.0±0.00**	31.5±0.00***	41.5±0.00***	21.9± 0.03***	100.0

Values are Mean ± S.E.M., n=6 in each group, *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group (One way ANOVA fallowed by Dunnett's test).



Graph 1
Percentage viability of MCF-7 cell line at different concentrations of plant extracts

Figure 1
Images of % viability of MCF-7cell-line

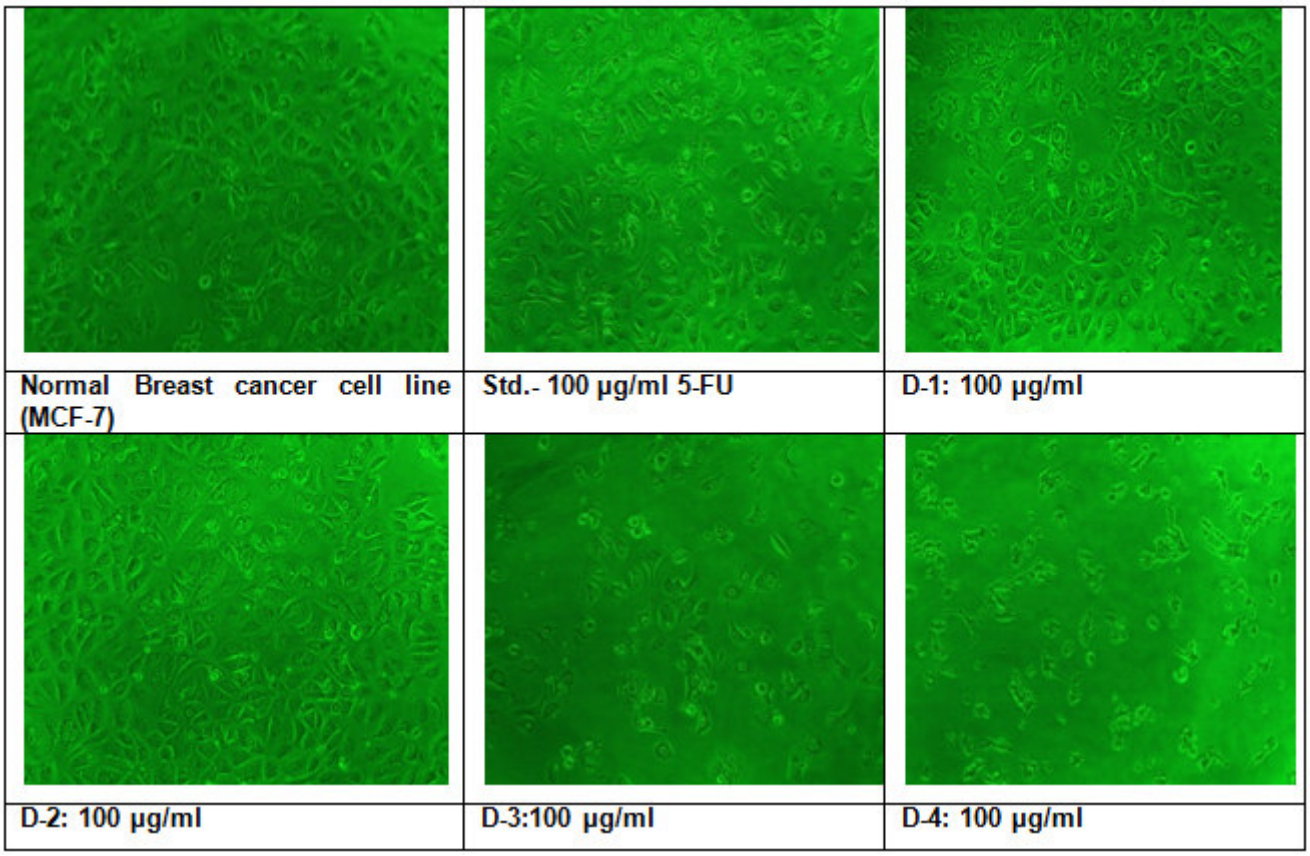
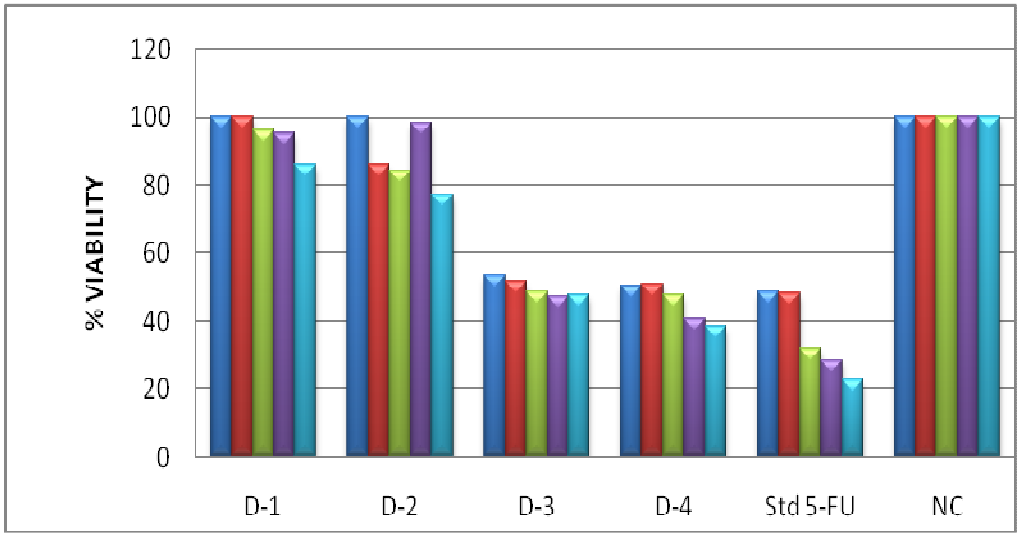


Table 2
Effect of various herbal extracts on Colon cancer cell line HCT-116 (%viability) with respect to control.

Conc.	D-1	D-2	D-3	D-4	Std. 5-FU	NC
25µg/ml	100±0.03	100 ±0.00	53.6±0.01*	50.2±0.00*	48.5±0.00**	100.0
50µg/ml	100±0.04	86.0±0.00	51.7±0.01*	50.6±0.01*	48.0±0.01**	100.0
100µg/ml	95.9±0.02	84.1±0.00	48.6±0.01**	47.5±0.00**	32.0±0.00***	100.0
150µg/ml	95.2±0.00	98.5±0.02	47.3±0.00**	40.5±0.00**	28.3±0.01***	100.0
200µg/ml	86.0±0.03	77.3±0.00	47.6±0.00**	38.4±0.00***	22.4±0.00***	100.0

Values are Mean ± S.E.M., n=6 in each group, *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group (One way ANOVA fallowed by Dunnett's test).



Graph 2
Percentage viability of HCT-116_cell line at different concentrations of plant extracts

Figure 2
Images of % viability of HCT-116 cell-line

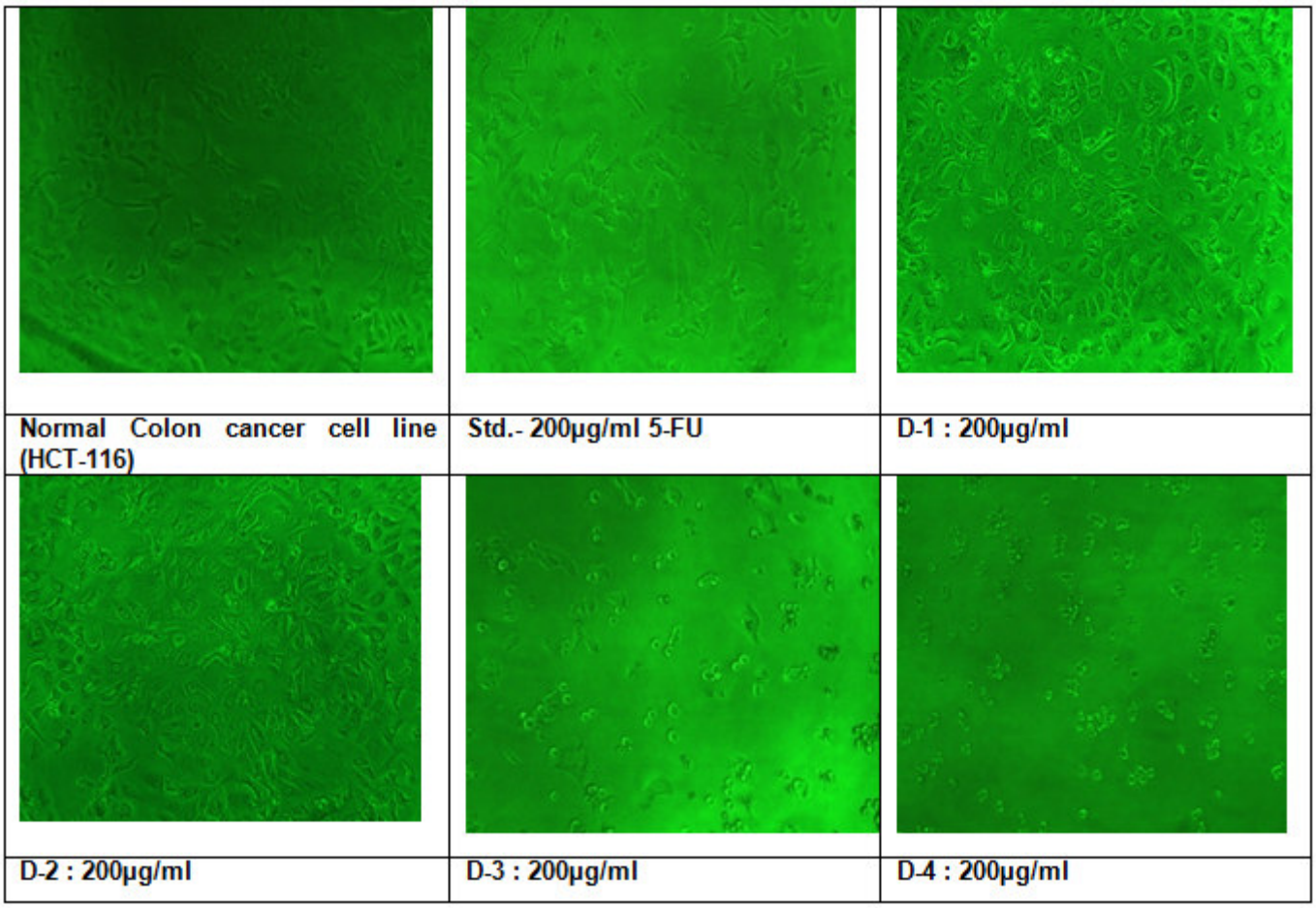
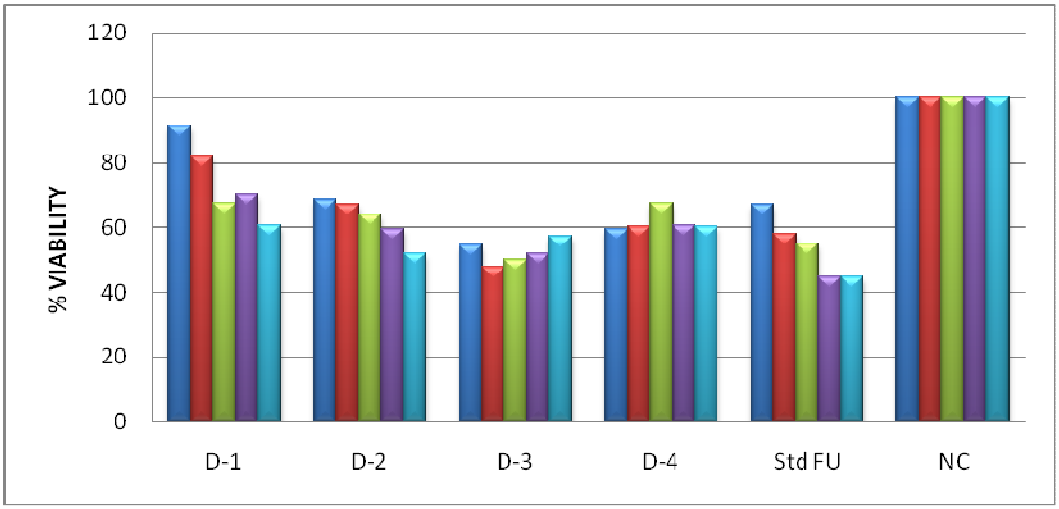


Table 3
Effect of various herbal extracts on ovarian cancer cell line PA-1. (% viability) with respect to control

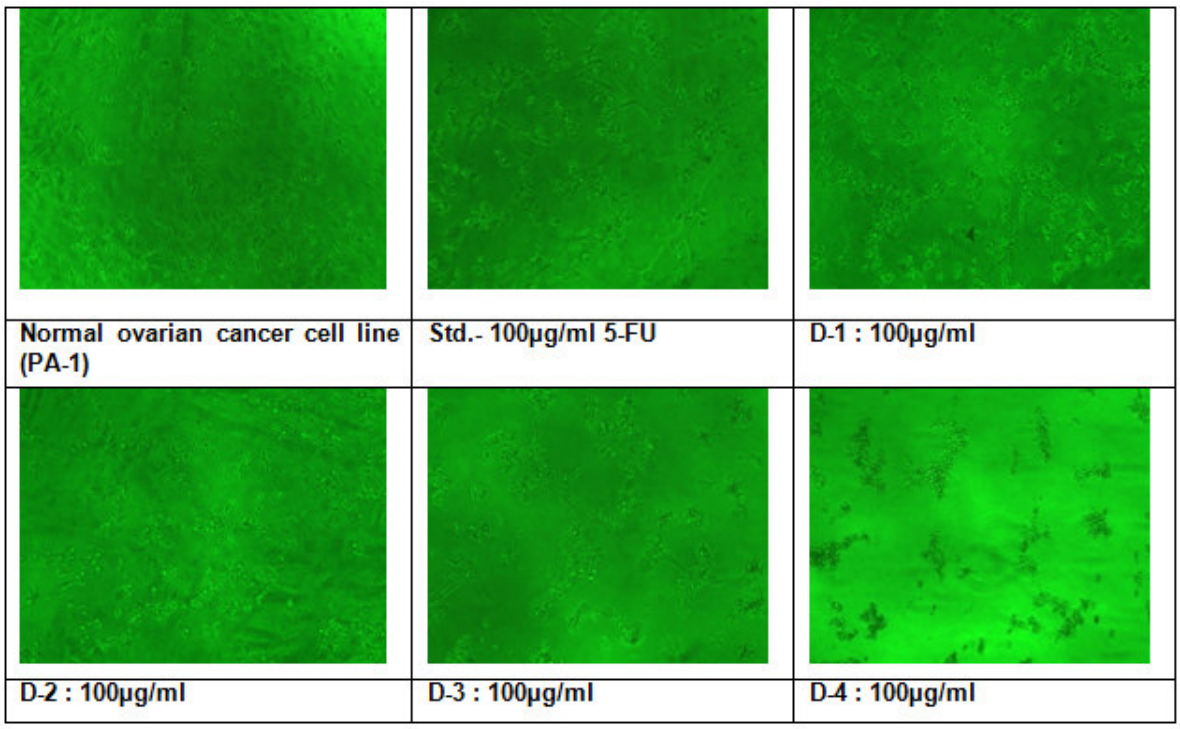
Conc.	D-1	D-2	D-3	D-4	Std 5-FU	NC
50µg/ml	91.3±0.00	68.5±0.00	54.5±0.00*	59.3±0.00	67.0±0.00	100.0
100µg/ml	81.9±0.01	66.9±0.00	47.9±0.00**	60.1±0.00	58.0±0.00	100.0
150µg/ml	67.4±0.01	64.0±0.00	50.2±0.00*	67.5±0.00	55.0±0.00*	100.0
200µg/ml	70.0±0.00	59.3±0.00	52.1±0.00*	60.7±0.00	44.7±0.00**	100.0
250µg/ml	60.7±0.00	52.1±0.00*	57.1±0.00	60.1±0.00	44.8±0.00**	100.0

Values are Mean ± S.E.M., n=6 in each group, *p<0.05, **p<0.01, ***p<0.001 when compared with normal control group (One way ANOVA fallowed by Dunnett's test).



Graph 3
Percentage viability of PA-1 cell line at different concentrations of plant extracts

Figure 3
Images of % viability of HCT-116 cell-line



DISCUSSION

Natural products from plants used either alone or with combinatorial synthetic methodologies constitutes a multidisciplinary approach in search of recent anticancer drugs. Some drugs from plants in clinical use include Vincristine and Vinblastine from *Catharanthus roseus*, Paclitaxel (Taxol) and Taxotere from species of *Yew (Taxus)*, Etoposide drived from lignans of *Podophyllum spp.* and Campothecin analogues such as topotecan, from *Camptotheca acuminate*. All of these are fundamentally cytotoxic and act principally by inhibiting cell proliferation but by different mechanism. Two major techniques are used to assess the cell cytotoxicity or cell proliferation. The first one uses either 3-(4, 5 – dimethylthiazol -2-yl) -2,5-diphenyltetrazolium bromide (MTT) or 2,3 –bis(2-methoxy-4-nitro-5-sulphophenyl) -2H-tetrazolium- 5-Carbonanilide sodium salt (XTT). These reagents are metabolically reduced by the mitochondria in viable cells to a coloured product(formazan). The intensity of the formazan product is measured spectrophotometrically in a plate reader. The colour formation relies on the activity of the mitochondria, so if the function of these reagents is inhibited by variation in cellular levels of NADH, glucose and other factors, variable results are obtained and indicating that the cells were not alive or not proliferating. The Sulphorhodamine B (SRB) assay is more preferred assay for testing cytotoxicity, which relies on uptake of the negatively charged pink aminoxanthine dye SRB, by basic amino acids in the cells. Increase in cell number, results in more SRB uptake, after fixing, when the cells are lysed, released dye will give more intensed color and greater absorbance. In present study, three species of *Curcuma* and *Gloriosa superba* were tested against three human cancer cell lines - breast (MCF-7), colon (HCT-116) and Ovary (PA-1). Aqueous plant extract of *Curcuma zedoaria* and *Gloriosa superba* exhibited higher activity of anti cancer growth potential. *Curcuma zedoaria* and *Gloriosa superba* shows definite cytotoxic effect against breast cell line (MCF-7) at 100µg/ ml concentration when compared to standard 5-FU. Whereas *Curcuma caesia* and *Curcuma longa* show moderate cytotoxicity at 100 µg/ml concentration as compared to standard drug. Cytotoxic activity recorded in the present study is in accordance with this finding that, since the phytochemical evaluation indicated the presence of flavonoids and steroids in all of the four plant species and may have a key role in mediating the cytotoxic potential in these plants. However exact mechanism underlying cytotoxic potential of these herbal extract still needs to be elucidated.

CONCLUSION

The aqueous plant extracts from rhizomes of *Curcuma zedoaria* and *Gloriosa superba* shows definite cytotoxic effect against breast (MCF-7), Ovary (PA-1) and Colon (HCT-116) cancer cell-lines when compared to standard 5-FU.

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REFERENCES

1. Padmavathi P.P., Panneerselvam T., Shastry C.S. Synthesis and anticancer evaluation of 2-Phenyl thiaolidinone substituted 2-phenyl benzothiazole-carboxylic acid derivatives. Journal of Saudi Chemical Society,19(2):181-185,(2015).
2. Kashyap C.P., Bandana T., Shikha S. Human cancer cell lines-A brief communication. J Chem Pharm. Res,3(6):514-520,(2011).
3. Ritesh J., Sanmati K.J. Screening of in vitro cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh state,India. Asian Pacific Journal of Tropical Biomedicine,147-150,(2011).
4. Rajandeep K., Karan K., Harpreet K. Plants as a source of anticancer agents. J Nat Prod Plant Resour, 1(1):119-124,(2011).
5. Sanjay P., Nirav G., Ashok S. In-Vitro Cytotoxicity Activity Of Solanum Nigrum Extratct Against Hela Cell Line And Vero Cell Line. International Journal of Pharmacy and Pharmaceutical Science,1(1):38-46,(2009).
6. Sheng C.C., Chein T.H., Ssu M.H. Experimental Study on Effect of Compounds in Inhibiting HCT-116 Human Colon Cancer: The Preliminary Results. J Soc Colon Rectal Surgeon (Taiwan),21(3):121-128,(2010).
7. Ansil P.N., Wills P.J., Varun R. Cytotoxic and apoptic activities of Amorphophallus campnulatus (Roxb.) Bl.tuber extract against human colon carcinoma cell line HCT-115. Saudi Journal of Biological Sciences, 21:524-531,(2014).
8. Sundaram S., Verma S. K., Priyanka D. In-Vitro Cytotoxic Activity Of Indian Medicinal Plants Used Traditionally To Treat Cancer. Asian Journal of Pharmaceutical and Clinical Research,4(1):27-29,(2011).
9. Houghton P., Fanq R.,Techatanawat I. The Sulphordamine (SRB) assay and other approaches to testing plant extract and derived compounds for activities related to reputed anticancer activity. Methods, 42(4):377-387,(2007).
10. Aljaiyash A.A., Mariam H.G., Mojahidul I. Antibacterial and cytotoxic activities of some Libyan medicinal plants. J.Nat.Prod plant Resour,4(2):43-51,(2014).
11. Abhishek B., Kunal N., Sanjna K. Antimicrobial and Cytotoxic Activities of Fungal Isolates of Medicinal Plant *Gloriosa superb.* International Journal of Recent Advances in Pharmaceutical Research,2(1): 37-45,(2012).
12. Ayesh B., Matouk I., Ohana P. Inhibition of tumor growth by DT-A expressed under the control of IGF2 P3 and P4 promoter sequences. Molecular Therapy,7(4):535-41,(2003).
13. Boyle P., Langman J.S. ABC of colorectal cancer:Epidemiology. *BMJ*,321:805–808, (2000).
14. Carvalho F.R., Vassao R.C. Effect of *Curcuma zedoaria* crude extract against tumor progression and immunomodulation. J Venom Anim Toxins incl Trop Dis,16(2):324-341,(2010).
15. Fan W., Johnson K.R., Miller M.C. In vitro evaluation of combination Chemotherapy against human tumor cells. Oncology Report, 5 (5):1035-1042,(1998).
16. Gyanendra S., Jaya D., Sarvesh P. Evaluation of radio protective properties of Curcuma longa rhizome extract:A cytogenetic analysis in cancer. Pharmacognosy Communication,2(2):44-48,(2012).
17. Geetanjali S. B., Poonam D. Review on *Gloriosa superba*: important medicinal plant. International Journal of Natural Product Science,1:144,(2012).
18. Goh K.L., Quek K.F., Yeo G.T. Colorectal cancer in Asians: a demographic and anatomic survey in Malaysian patients undergoing colonoscopy. Aliment Pharmacol Ther;22:859–64,(2005).
19. Hemaiswarya S., Raja R., Anbazhagan C. Antibacterial and Mutagenic properties of the root tubers of *Gloriosa superba* Linn. Pak J Bot,41(1):293-99,(2009).
20. Kavitha R., ChittiBabu C.V., Subha T.S. In vitro studies on antimicrobial and antioxidant properties of indigenous medicinal plants–Diplocyclos palmatus, Ctenolopis garcinii, and Kedrostis rostrata. American J of Bio-pharm Biochem and Life Sci,1(1):58-62,(2012).
21. Muthu K.T., Maryviolet C.A., Anusha M. Anticancer and antioxidant activity of *Curcuma zedoaria* and *Curcuma amada* rhizome extracts. J Acad Indus Res,1(2): 91-96,(2012).
22. Said O., Khalil K., Fulder S. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West bank region. Journal of Ethnopharmacology,83:251-265,(2002).
23. Muthu K.T., Maryviolet C.A., Anusha M. Anticancer and Antioxidant activity of Curcuma zedoaria and Curcuma amada rhizome extracts. J Acad Indus Res,2(1):91-96,(2012).
24. Alaa A. G., Khaled M.M., Zienab A.S. Antioxidant Potential Activity and Cytotoxicity Effects Of Different Parts Of Peanuts (*Arachis Hypogaea* L.). Int J Pharm Bio Sci,6(3):19–32,(2015).