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EVALUATION OF IN VITRO ANTI CANCER ACTIVITY OF PLANT *KALANCHOE PIANNATA* AGAINST MCF 7 AND HOP 62 CELL LINES

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ABSTRACT

*Kalanchoe pinnata is a succulent perennial plant that grows 3-5 feet tall, commonly known as "Air plant". It contain alkaloids, glycosides, flavonoids, steroids etc. leaves and leaf juice are used for kidney stone, diabetics, diaoohea, headache etc. the objective of study is to investigate extract of roots of Kalanchoe pinnata for anticancer activity on human breast cells (MCF7) and lung cancer cells (Hop6). The sulphorhudamine B assay was done to evaluate in vitro anticancer activity; Adriamycin was used as a reference standard. The Growth inhibition of 50 % (**GI50**), Concentration resulting in total growth inhibition (**TGI**), Concentration of drug causing lethality to 50% of the cells **(LC 50)** was calculated. The result shows both methanolic and chloroform extract shows significant anticancer activity as compare with Adriamycin.*

KEY WORDS

Adriamycin, Anticancer activity, Kalanchoe pinnata, Srb assay.

INTRODUCTION

Cancer is group of disease characterized by uncontrolled growth (division beyond the normal limits) and spread of abnormal cells (metastasis). If the spread is not controlled it can result in death. Cancer is caused by both external factor (tobacco, chemicals, radiation and infectious organism) and internal factor (inherited mutations, hormones, immune conditions and mutation that occur due to metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. The development of cancer requires multi steps that occur over many years. Certain types of cancer can be prevented by eliminating exposure to tobacco and other factors that accelerates this process. Other potential malignancies can be detected before cells become cancerous, when the disease is most treatable. Cancer is treated by surgery, radiation, chemotherapy, hormones and immunotherapy.[1]

Plant also have a long history of use in the treatment of cancer, Hartwell lists more than 3000 plant species that have reportedly been used in the treatment of cancer. Plant have played an important role as a source of effective anticancer agents, and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organism and micro-organism.[2]

The majority of evidence has indicated that flavonoids by their antioxidant nature found to inhibit membrane lipid peroxidation (LPO) and intake of flavonoid containing drugs reduces the tumour burden which suggests a protective antioxidant action imparted by flavonoids.[3] Interaction between flavonoids and ascorbic acid has also been documented. Ascorbate is reported to have flavonoid protective and flavonoid enhancing activities. In turn, vitamin C present in sub optimal concentration is stabilized by flavonoids. As flavonoids and vitamin C coexist in *Kalanchoe pinnata,* they could be a useful strategy to improve anticancer activity. [4]

MATERIAL AND METHODS

Collection of plant material

The roots of *Kalanchoe pinnata* was collected from Satpuda hills near Akkalkuwa, Dist: Nandurbar, Maharashtra, India, cleaned and dried at room temperature in shade and away from direct sunlight. The plant authenticated by T. Chakraborthy, Deputy Director Botanical Survey of India, Koregaon Road Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number QMAKP1).

Preparation of extract

The root of *Kalanchoe pinnata* was collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in Mukherjee was used for extraction of powdered plant. Extraction was done by Pet. Ether, Chloroform, Methanol, and Aqueous. [5]

Preliminary phytochemical screening

The extracts were then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituent. The results show presence that petroleum ether extract contain steroids, the chloroform extract contain steroids and alkaloids, the methanolic extract contain Steroids, Saponins, Alkaloids, Glycosides, Flavonoids, Tannins, Carbohydrates, Proteins. and aqueous extract contain Saponins, Glycosides, Flavonoids, Tannins, Carbohydrates, Amino acids. [6]

Evaluation of anticancer activity by Sulforhodamine B assay [7, 8]

Cell culture

Human Breast Cancer Cell Line (MCF7) and Human Lung Cancer Cell Line (Hop62) were purchased from NCI, USA. 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 % CO2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs

Assay procedure

After 24 h, one 96 well plate containing $5*10^3$cells/well was fixed *in situ* with TCA, to represent a measurement of the cell population at the time of drug addiction (Tz). Experimental drugs were initially solubilized in dimethyl sulfoxide 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 100µg/ml, 200µg/ml, 400µg/ml and 800µg/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.

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 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 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 (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-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. Growth inhibition of 50 % (**GI50**) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (**TGI**) was calculated from Ti = Tz. The **LC50** (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.

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.

Identification of possible phytoconstituents

Pharmacological screening of various extract of root of *Kalanchoe pinnata* revealed that out of these methanolic and chloroform extract was found to be more significant on both cell lines i.e. MCF7 and Hop6. Thus, it was found worthy to characterize these extract with the help of HPLC. [9, 10]

RESULTS

The all extract of plant kalanchoe pinnata was evaluated for anticancer activity against human lung cancer cell line (Hop 62) and human breast cancer cell line (MCF 7). SRB assay was used for evaluation and absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The 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. The % Growth of Hop 62 and MCF 7 cell lines against various extract are given in Table 1 and Table 2 respectively.

Table 1 % Growth of Hop 62 cell line against various extract of *Kalanchoe pinnata*.

	Drug Concentration (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
P	74.66	29.05	21.97	23.91	74.4	28.22	22.02	18.3	78.91	40.47	34.26	29.13	75.99	32.58	26.08	23.78
C	12.79	16.97	22.3	20.25	16	14.62	13.97	7.46	12.45	7.22	16.71	10.61	13.75	12.94	17.66	12.77
M	13.41	8.41	10.65	11.69	10.26	3.82	-16.7	-19.8	16.21	6.11	5.05	4.41	13.29	6.11	-0.32	-1.25
Aq	102.8	78.01	36.09	18.68	106.1	77.44	27.72	10.21	109	75.4	26.4	9.23	106	76.95	30.07	12.71
AD	9.47	7.9	2.99	-0.69	6.18	6.08	2.09	-3.77	6.91	4.27	1.88	-3.99	7.52	6.08	2.32	-2.81

Graph 1: % Growth curve of various extract on Hop6 Cell line

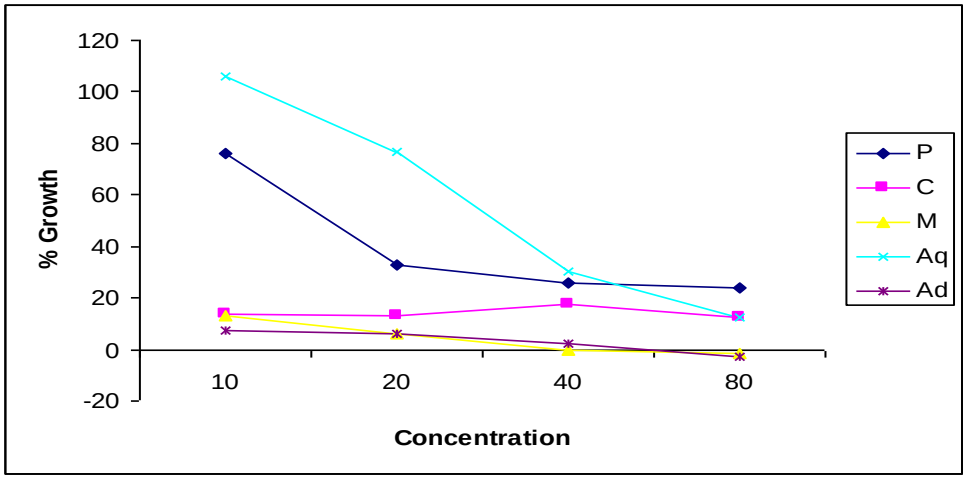


Table 2: % Growth of MCF 7 cell line against various extract of *Kalanchoe pinnata*.

	Drug Concentration (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
P	106.8	75.9	46.8	25.5	101.6	74	43.6	16.6	99.8	85.5	39.1	16.4	102.7	78.5	43.2	19.5
C	13.7	3.3	-5.6	-6.7	15.8	4.5	-1.8	-14.1	9.6	5.5	-8.2	-22.9	13	4.4	-5.2	-14.6
M	58	17.2	1	-16.7	53.5	14	-1.3	-18.3	42.5	12.5	-12.8	-30.7	51.3	14.6	-4.4	-21.9
Aq	119.7	126.3	105	53.3	121.4	127.3	101.6	52.7	128.2	122.5	97.1	37.9	123.1	125.4	101.2	48
AD	-41.7	-56.6	-67.5	-63.8	-38	-43.4	-61.2	-55.1	-25.1	-24.4	-48.3	-56.8	-34.9	-41.5	-59	-58.6

Graph 2: % Growth curve of various extract on MCF 7 Cell line

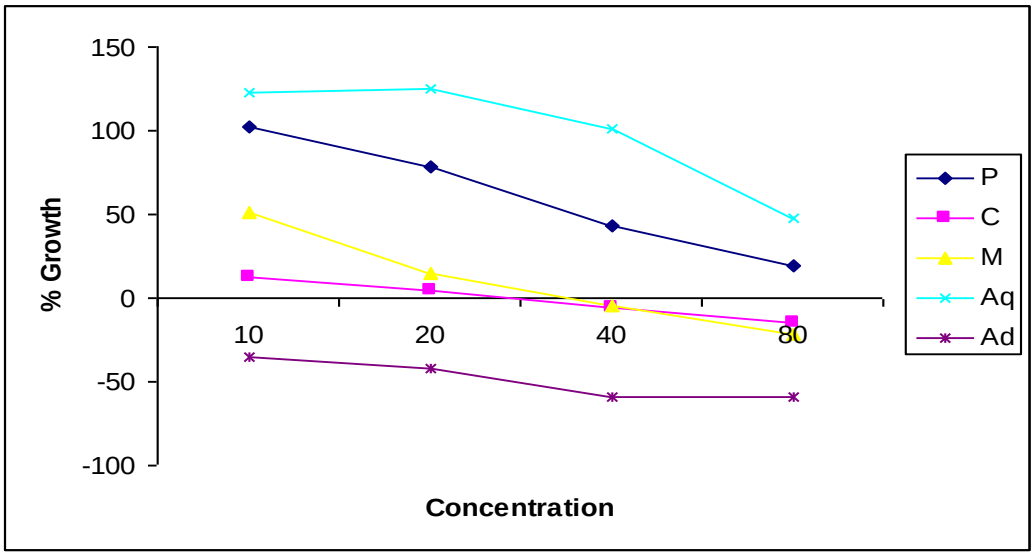
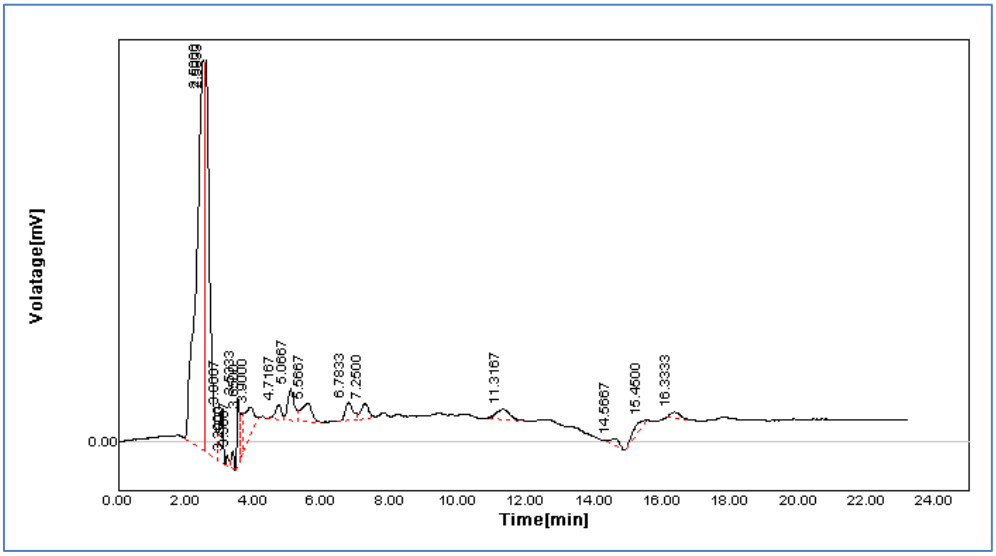


Table 3: Results of SRB assay against Hop 62 and MCF 7 cell line

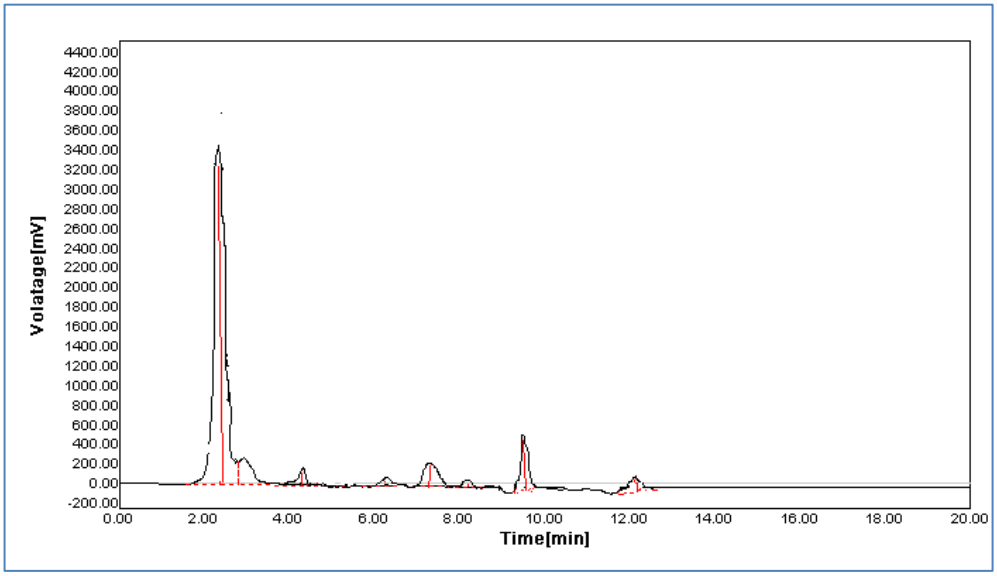
	Hop 62 cell line			MCF 7 cell line		
herbal extract	LC50	TG	GI50	LC50	TGI	GI50
P	>80	>80	33.0	>80	>80	47.9
C	> 80	79.6	<10	> 80	52.0	<10
M	>80	58.8	<10	>80	50.1	14.4
Aq	> 80	>80	43	> 80	>80	>80
ADR	54.1	58.2	<10	54.1	17.1	<10

As the Pharmacological screening of various extract of root of *Kalanchoe pinnata* revealed that out of these methanolic and chloroform extract was found to be more significant on both cell lines i.e. MCF7 and Hop6. Thus, it was found worthy to characterize these extract with the help of HPLC. The data obtained is as follows.

Graph 3: Chromatogram of HPLC for methanolic extract for flavonoids

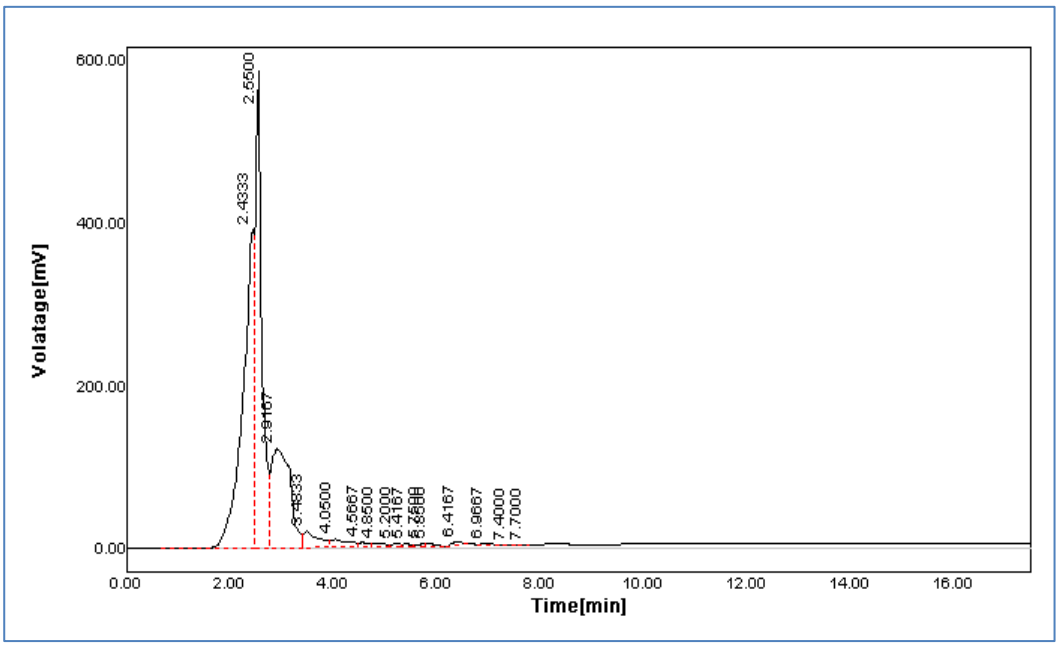


Graph 4: Chromatogram of HPLC for methanolic extract for steroids

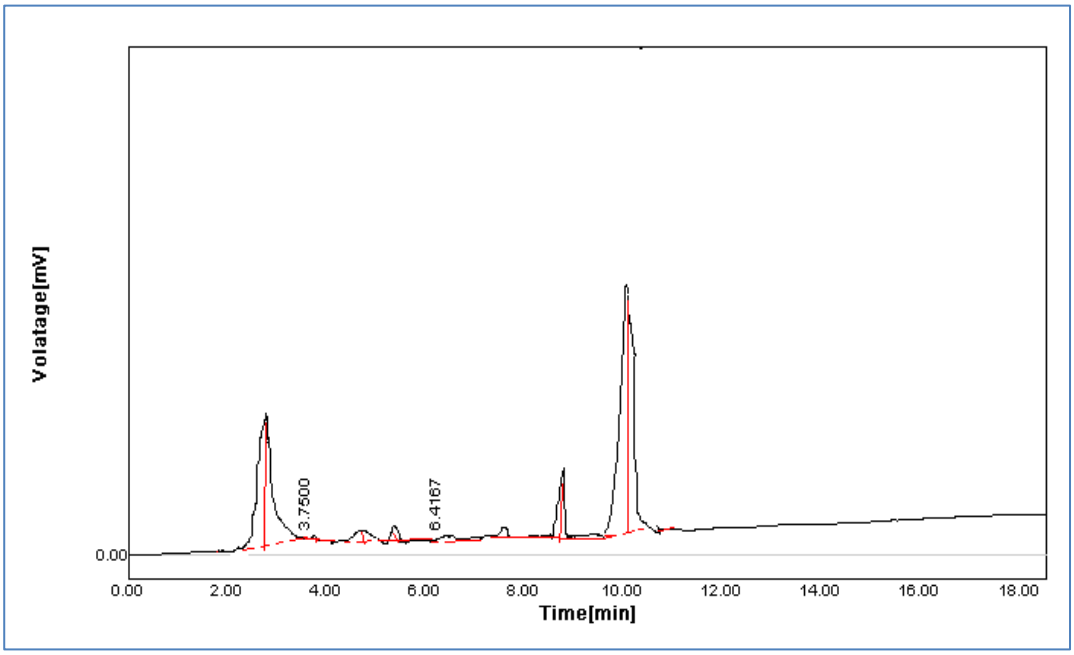


The HPLC data of methanolic extract for flavonoids shows peak at 7.2500 and 14.5667 which is nearer to myrcetin and quercetin respectively. And for steroids shows peak at 7.5890 and 9.7583 which is nearer to cholesterol and beta sitosterol respectively.

Graph 5: Chromatogram of HPLC for chloroform extract for flavonoids



Graph 6: Chromatogram of HPLC for chloroform extract for steroids



The HPLC data of chloroform extract for flavonoids shows peak at 7.4000which is nearer to myrcetin. The hplc data of chloroform extract for steroids shows peak at 7.8920 and 10.1563 which is nearer to cholesterol and beta sitosterol respectively.

CONCLUSION

From above results it was concluded that kalanchoe pinnata shows the presence of steroids, saponins, alkaloids, glycosides, flavonoids, tannins, carbohydrates, proteins, and amino acids by Preliminary phytochemical screening of various extracts. The chloroform and methanolic extract shows the significant activity against MCF 7 cell line (Breast cancer) and HOP 6 cell line (Lung cancer). The results of SRB assay were compared with ADR (Adriamycin), a known drug available in the market as anti-cancer agent. The HPLC data shows the presence of flavonoids and steroids in methanolic and Chloroform extract.

FUTURE SCOPE

As per the results obtained, it was found that Methanolic and Chloroform extracts have significant anticancer activity, as compare to Adriamycin. Hence the extract can further be subjected for isolation of flavonoids, steroids and other phytochemicals and their structural elucidation thereof which may be a lead molecule for the above proven activities. And further formulation of suitable dosage form, will be done.

REFERENCES

1. American Cancer Society. Global Cancer Facts and Figures 2007.
2. S.Khurana, M.L.Dubey, et al . Association of parasitic infection and cancers. Indian Journal of Medical Microbiology, 23(2): 74-79, (2005).
3. Premalatha B, Sachdanandam P. *Semecarpus anacardium* L. nut extract administration induces the in vivo antioxidant defence system in aflatoxin B1 mediated hepatocellular carcinoma. Journal of Ethanopharmacology, 66:131-139, (1999).
4. Shanumugam Arunkumaran, Vannu Ramkumar Ramprasath, et al. Alteration of DMBA- induced oxidative stress by additive action of a modified indigenous prepration- Kalpaamurtha. Chemo-Biological Interactions, 167: 99-106, (2007).
5. Mukherjee P K. Quality control of herbal drugs: An approach to evaluation of botanicals. 1st edition. New Delhi. Business Horizons Publication; 133-384, (2002).
6. Khandelwal, K.R, Practical Pharmacognosy Techniques and Experiments, 19th edition, Nirali Prakashan; 149-156, (2005).
7. Vanicha Vichai and Kanyawim Kirtikara. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 1 (3):1112-1116, (2006).
8. Peter Houghton, Rui Fang, et al, the sulphorhodamine (SRB) assay and other approaches to testing plant extracts and derived compounds for activities related to reputed anticancer activity. Methods, 42 (4): 377-387, (2007).
9. O.Tokusoglu, M.K. Unal, et al. HPLC-UV& GC-MS characterization of flavonol aglycon quercetin, kaempferol and myrcetin in tomato pastes and other tomato based product. Acta Chromatographoca, 13:196-207, (2003).
10. V.D.P. Nair, I. Kanfer, et al. Determination of stigmasterol, β sitosterol and stigmastanol in oral dosage forms using high performance liquid chromatography with evaporative light scattering detection. Journal of Pharmaceutical and Biomedical Analysis41: 731–737, (2006).

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