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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of *In vitro* Anti-Cancer Activity of Kaempferol Buccal film

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ABSTRACT
The aim of the present study was to investigate an anticancer profile of Kaempferol formulation in the form of buccal film. Kaempferol has been proposed as a potential drug for cancer chemoprevention and treatment but its therapeutic use is limited by its low aqueous solubility. Here, a buccal film was formulated to improve its dissolution property and anticancer activity. The anticancer activity of Kaempferol buccal film was determined using the SRB assay. Optimised batch of buccal film was selected for the activity.
Key-words: *Kaempferol Buccal film, SRB assay, Human Squamous Cell Carcinoma (SCC-40), Human Ovarian Cancer Cell Line (SK-OV-3).*

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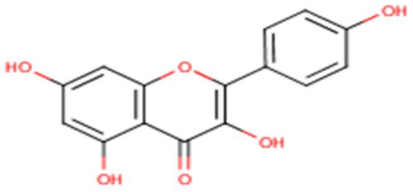
INTRODUCTION

The World Health Organization (WHO) has estimated that approximately 80% of the world's population depends on traditional medicines for meeting their primary health care needs. As an important component of complementary and alternative medicine, traditional Ayurvedic medicine may be a useful model for scientific research because of its standardized system of therapies and long-time practices. Due to the high costs and time-consuming nature of animal cancer model studies, the initial screening of anticancer compounds from natural products is best accomplished using *in vitro* cancer cell methods [1-4]. Cancer is a genetic disease that is caused by cellular genomic alterations which include insertions, deletions and chromosomal aberrations. These cellular alterations can lead to uncontrolled cell growth that is evident in tumors [5]. Cultured human cancer cell lines are commonly used to screen chemicals and natural product extracts *in vitro* to speed the discovery of new anticancer drugs. The formulation of Kaempferol Buccal film contains anticancer potential having phytochemical like kaempferol and hence it was decided to explore their anticancer potential. SRB assay was more preferred assay among *in-vitro* anticancer screening [6-7]. The cell lines preferred for finding this potential were Human Squamous Cell Carcinoma (SCC-40), Human Ovarian Cancer Cell Line (SK-OV-3).
The Kaempferol (3, 5, 7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), a yellow compound with a low molecular weight (MW: 286.2 g/mol) and molecular formula ($C_{15}H_{10}O_{6)}$ is a common natural flavonoid which representative of the subcategory of flavonol that commonly found in many plant-derived foods and in plants used in traditional medicine [8].



Kaempferol

MATERIAL AND METHODS

Kaempferol sample was purchased from Yucca Laboratories Pvt. Ltd, Mumbai.

In vitro anticancer activity by SRB assay

Less number of anticancer drugs and their unpredictable cure enforces need of screening of medicinal plants to get more potent anticancer drugs. In vivo methods of anticancer screening require animal and thus ethical permissions. Again due to the high costs and time-consuming nature of animal cancer model studies, the initial screening of anticancer compounds from natural products is best accomplished using in vitro cancer cell methods. Cultured human cancer cell lines are preferred to screen chemicals and natural product extracts in vitro to speed the discovery of new anticancer drugs [9].

Present study has undertaken to carry out in vitro anticancer effects of Kaempferol buccal film on selected human cancer cell lines. The samples of optimized batch of Kaempferol buccal film were used for the anticancer activity. Adriamycin (ADR) was used as a standard anticancer drug.

SRB Assay procedure

The anticancer activities of extracts were studied at Advanced Center for Treatment, Research and Education in Cancer (ACTREC), Mumbai where 14 cell lines were maintained in ideal laboratory conditions. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2mM 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% CO2, 95% air and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, cells from one plate of each cell line were fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental samples were solubilized 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 micro-titer wells already containing 90 µl of cell suspension, resulting in the required final drug concentrations of 10, 20, 40 and 80 µg/ml. For each of the experiments a known anticancer drug was used as a positive control. 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 mMTrizma 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. The summary of parameters is as follows.

- GI50 Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] × 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
- TGI Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
- 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] × 100 = 7minus; 50.
- GI50 value of = 20 µg/ml is considered to demonstrate activity.

RESULTS AND DISCUSSION

Sulforhodamine B (SRB) assay

Table 1: % Control Growth of Buccal film against Human Squamous Cell Carcinoma SCC-40

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	20	40	80
BF1	125.6	117.5	96.4	56.5
BF2	130.1	119.8	99.3	53.4
ADR	-52.2	-58.2	-69.9	-62.5

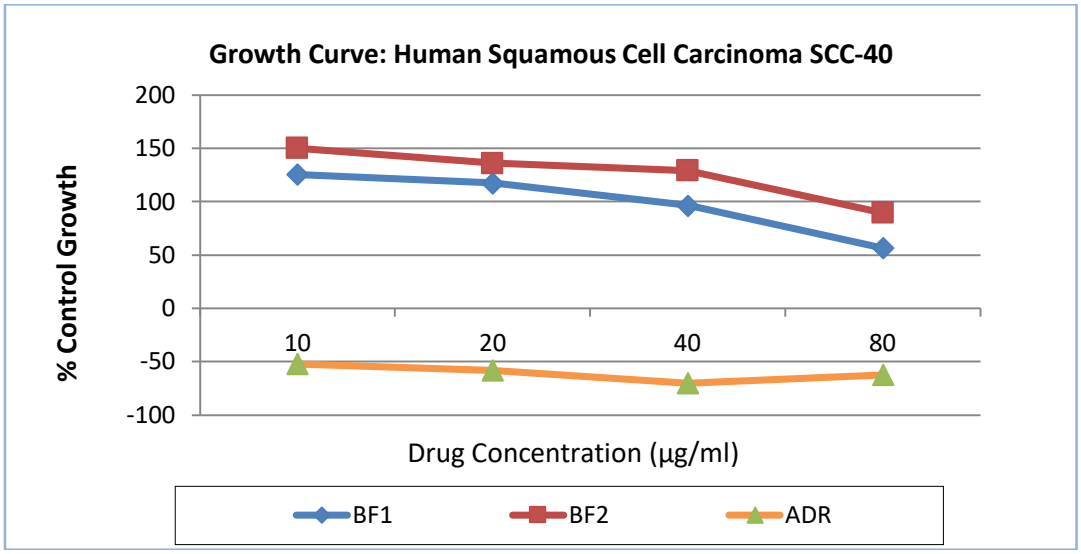


Figure 1 :Growth Curve of Buccal film against Human Squamous Cell Carcinoma SCC-40

In vitro anticancer screening against human cancer cell lines by SRB assay was carried out. The sample used for testing was buccal film 1 and buccal film 2. The cell lines used for this experimentation was Human Squamous Cell Carcinoma SCC-40. The obtained results indicates buccal film 1 shows less potency and buccal film 2 shows very less potency as compared with the standard drug.

Table 2 % Control Growth of Buccal film against Human Ovarian Cancer Cell Line SK-OV-3

Test component	% Control Growth			
	Drug Concentrations (µg/ml)			
	10	**20**	**40**	**80**
BF1	79.4	70.0	47.4	37.8
BF2	80.2	75.4	57.4	42.1
ADR	-21.8	-27.4	-5.3	-30.1

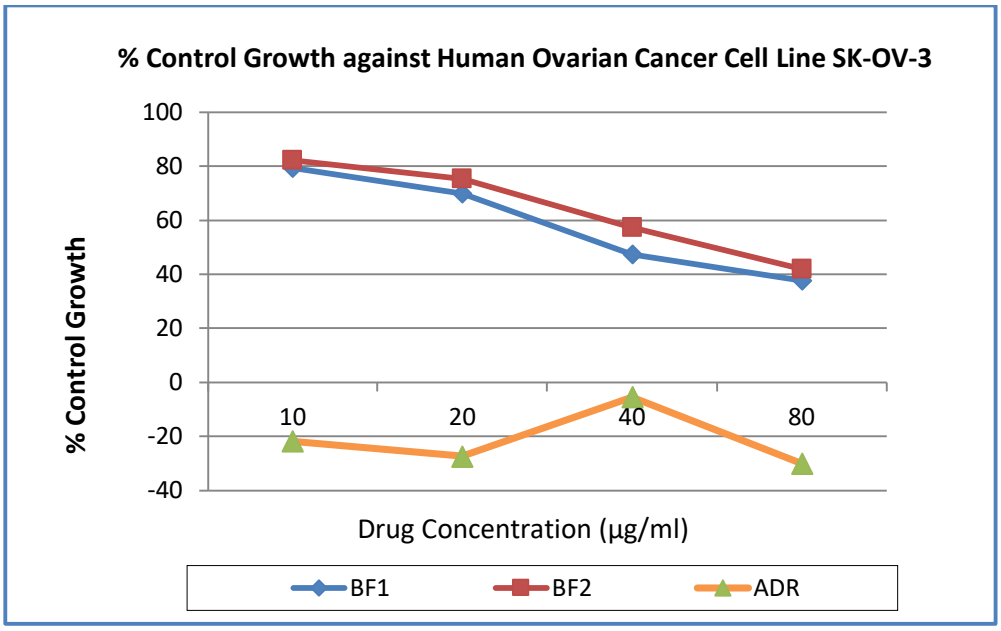


Figure 2 Growth Curve of Buccal film against Human Ovarian Cancer Cell Line SK-OV-3

In vitro anticancer screening against human cancer cell lines that are Human Ovarian Cancer Cell Line SK-OV-3 by SRB assay indicates that buccal film 1 and buccal film 2 did not show any potency at this concentration against these cell lines as compared with standard drug.

CONCLUSION

Higher plants are act as a source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. From the above results, it can be concluded that the buccal film of Kaempferol has exhibited anticancer activities but in lesser extent. This obtained activity may be due to the active principle and variation of other excipients in the optimized formulation.

Hence to put into a nutshell, the active principle Kaempferol and other excipients may be responsible for anticancer activities. Hence it needs to further development of the novel drug delivery formulations to pin point the activity of drug.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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