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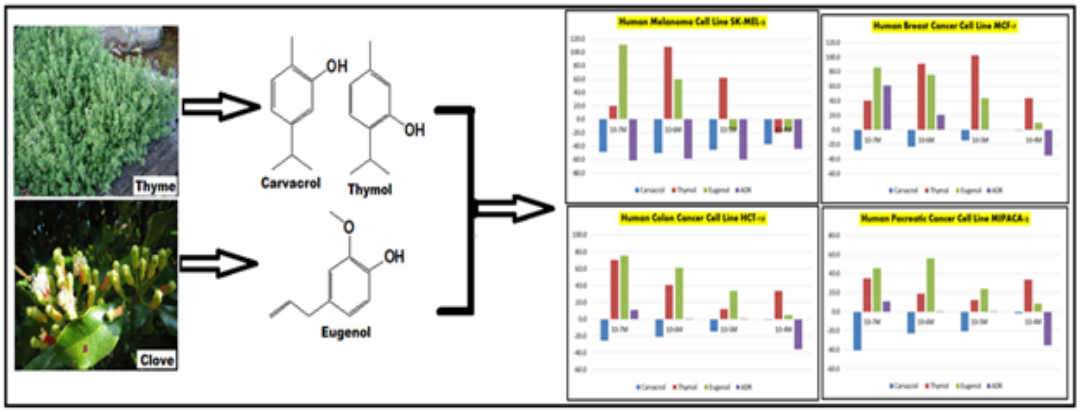
Comparative Anti-Proliferative Studies of Natural Phenolic Monoterpenoids on Human Malignant Tumour Cells

Jamatsing D Rajput, Suresh D Bagul, Samina Tadavi and Ratnamala S Bendre*

School of Chemical Sciences, North Maharashtra University, Jalgaon, Maharashtra, India

Abstract

In present study we report prominent effect of carvacrol (5-isopropyl-2-methyl phenol), thymol (2-isopropyl-5-methyl phenol) and eugenol (4 allyl-2-methoxyphenol) on Human melanoma cells. These three compounds were screened for Anti-proliferative test by using Sulforhodamine B (SRB) assay. For this investigation we used human breast melanoma cell line MCF-7, human skin melanoma cell line SK-MEL-2, human colon melanoma cell line HCT-15 and human pancreatic melanoma cell line MIAPaCa-2. Carvacrol was identified as most potent molecule with low GI_{50} value as 0.1 µL for selected human cancer cell lines; which is comparatively equal to standard drug 14 Adriamycin (ADR). Thymol and Eugenol also exhibited remarkable GI_{50} for human cancer cell 15 lines.



Keywords: SRB; Carvacrol thymol; Eugenol; MCF-7: SK-MEL-2; HCT-15; MIAPaCa-2

Introduction

Cancer is a serious chronic disease that arises due to changes in many physiological processes in body [1,2]. In general, symbols of cancer were found to be sustaining proliferative signalling, avoiding growth suppressors, control cell death, enabling replicative immortality, inducing angiogenesis, and activating incursion and metastasis, along with two emerging symbols including reprogramming energy metabolism and absconding immune damage [3-5]. The search for novel small molecules as drugs is still a priority goal for cancer therapy, due to the rapid development of resistance to chemotherapeutic drugs [6]. In addition, the high toxicity usually associated with some bulky anticancer drugs and their undesirable side-effects increase the demand for novel anti-tumour drugs active towards untreatable tumours [7,8] Natural products have long been a substantial source of treatment for melanoma, which is projected to become the main causes of death in this century [9,10]. The research of last 40 years demonstrated that more than one thousand plants possess significant anticancer properties [11]. while many molecules obtained from these plants have shown wonderful anticancer properties [12,13]. Some of them showed effective delivery in biological system to reduce the action of cancer disease, without toxic and other side effects against healthy cells and tissues [14].

Carvacrol (1), thymol (2) and eugenol (3) are part of a naturally occurring class of compounds known as phenols [15]. They are well-known naturally occurring phenolic monoterpenoids found primarily in oils of oregano, thyme, and marjoram, and are recognized as traditional therapeutic agents [16,17]. All the major groups of angiosperms biosynthesize these biocides [18]. Currently, these biocides are used in food flavouring ingredients and preservatives, as well as a fragrance ingredients in cosmetic formulations [19]. In recent years, significant research has been undertaken as an effort to establish the biological actions of these phenolic monoterpenoids for their potential use in pharmaceutical and agricultural applications [20]. Some results from *in-vitro* and *in-vivo* studies showed that carvacrol,

***Corresponding author:** Ratnamala S Bendre, School of Chemical Sciences, North Maharashtra University, Jalgaon, Maharashtra, India, Tel: +919422211435/+912572257435; E-mail: bendrers@gmail.com

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thymol and eugenol possess a variety of biological and pharmacological properties including anticancer, anti-inflammatory, antioxidant, hepatoprotective, antibacterial, antifungal, spasmolytic and anti-tubercular [21,22]. Perspective view on agricultural applications of these phenolic monoterpenoids, shows that they possess interesting activities against the various insect species (Figure 1) [23].

Due to several remarkable biological activities, these phenolic monoterpenoids are suitable as natural and non-toxic therapeutic agents and are highly promising alternatives to synthetic agents [24]. At the same time these are also suitable starting molecules for synthesis of organic, bio-organic and a natural product analog based fine chemicals [25,26].

In present study we report comparatively noticeable effect of carvacrol, thymol and eugenol on human melanoma cells by using Sulforhodamine B (SRB) protocol [27]. In this investigation we observed that all the three phenolic monoterpenoids possess outstanding anti-proliferative activity against human breast melanoma cell line MCF7, human skin melanoma cell line SK-MEL-2, human colon melanoma cell line HCT-15 and human pancreatic melanoma cell line MIAPaCa-2.

Materials and Methods

Reagent and chemicals

Carvacrol, thymol and eugenol were purchased from Sigma-Aldrich, St. Louis, MO, USA. Stock solutions of carvacrol, thymol and eugenol were prepared in dimethyl sulfoxide (DMSO) and were diluted to final concentration in the culture medium. Final concentration of DMSO employed as vehicle never exceeded 0.03% and had no discernible effects on MCF-7, SK-MEL-2, HCT-15 and MIAPaCa-2 cells in comparison with the untreated control. Tissue culture medium constituents, all chemicals and solvents were analytical grade purchased from HiMedia (Mumbai, India).

Cell culture

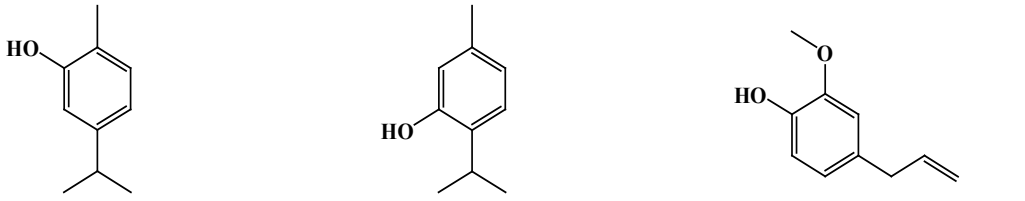
Human breast cancer (MCF-7), human skin cancer (SK-MEL-2) (Figure 2), colon cancer (HCT-15) and human pancreatic cancer (MIAPaCa-2) cell lines were obtained from Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. 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 addition of experimental drugs.

Cytotoxicity assay

Cytotoxicity activity was evaluated by Sulforhodamine B (SRB) assay as described by Vichai et al. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10^{-2} concentration [27]. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µL of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µL of medium, resulting in the required final drug concentrations.

After addition of compounds; the 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 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 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. The dose response parameters were calculated for each test article. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] × 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 LC_{50} (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=-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.



1. Carvacrol, (5-isopropyl-2-methyl phenol)

2.Thymol (2-isopropyl-5-methyl phenol)

3. Eugenol (4-allyl-2-methoxy phenol)

Figure 1: Structure of Carvacrol, thymol and eugenol.

Results

Cytotoxicity of phenolic monoterpenoids against SK-MEL-2 cell line

Preliminary observations suggest that at lower concentration (viz 10^{-7}), carvacrol and thymol shows potent activity but at increasing concentration only carvacrol and eugenol possess better effects on selected cell. A noticeable observation is that from lower to higher concentration only carvacrol shrink the cell during experiment, photograph (Figures 3 and 4) displays effect of phenolic monoterpenoids on cell during testing. The GI_{50} of carvacrol in SK-MEL-2 was determined graphically as 0.1 µL which is equal to the standard drug (ADR). However, GI_{50} of thymol and eugenol are 18.9 µL and 8.6 µL respectively. The overall result showed that carvacrol was identified as most potent molecule against SK-MEL-2.

Cytotoxicity of phenolic monoterpenoids against MCF-7 cell line

Preliminary observation suggests that at lower concentration (viz 10^{-7}), only carvacrol show potent activity against MCF-7 cell, while thymol and eugenol are less effective. Finally overall results suggest that carvacrol is most potent against breast cancer cell. The GI_{50} of carvacrol in MCF-7 cell line is 0.1 µL which is comparatively equal to standard drug (ADR) is 0.1 µL. However, at GI_{50} of thymol and eugenol is 88.4 µL, 33.5 µL respectively.

Cytotoxicity of phenolic monoterpenoids against HCT-15 cell line

Preliminary observation suggests that at lower concentration (viz 10^{-7}), only carvacrol show potent activity against HCT-15 cell, while thymol and eugenol are less effective. Finally overall results suggest that carvacrol is most potent against colon cancer cell. The GI_{50} of carvacrol in HCT-15 cell line is 0.1 µL which is comparatively equal to standard drug (ADR) is 0.1 µL. However, at GI_{50} of thymol and eugenol is also notable 32.9 µL, 28.6 µL respectively.

Cytotoxicity of phenolic monoterpenoids against MIAPaCa-2 cell line

Preliminary observation suggest that at lower concentration (viz 10^{-7}), only carvacrol show potent activity against MIAPaCa-2 cell, while thymol and eugenol are less effective. Finally overall results suggest that carvacrol is most potent against pancreatic cancer cell. The GI_{50} of carvacrol in MIAPaCa-2 (Figure 5) cell line is 0.1 µL which is comparatively equal to standard drug (ADR) is 0.1 µL. However, at GI_{50} of thymol and eugenol is also notable 18.2 µL, 22.5 µL 84 respectively.

Conclusion

This is the comparative study which determined the effect of carvacrol, thymol and eugenol on breast cancer cells, skin cancer cells, colon cancer cells and pancreatic cancer cells. Total results suggested that among the three phenolic monoterpenoids carvacrol possesses interesting cytotoxicity against breast cancer cell line MCF-7, skin cancer cell line SK-ML-2, colon cancer cell line HCT-15 and pancreatic cancer cell line MIAPaCa-2. In summary, the present study demonstrated that carvacrol is a potent anti-cancer natural compound with excellent inducing growth inhibition against various human cancer cells. This finding recommended that carvacrol may be a potential chemotherapeutic agent against cancer war, however further research is essential for the therapeutic entitlements.

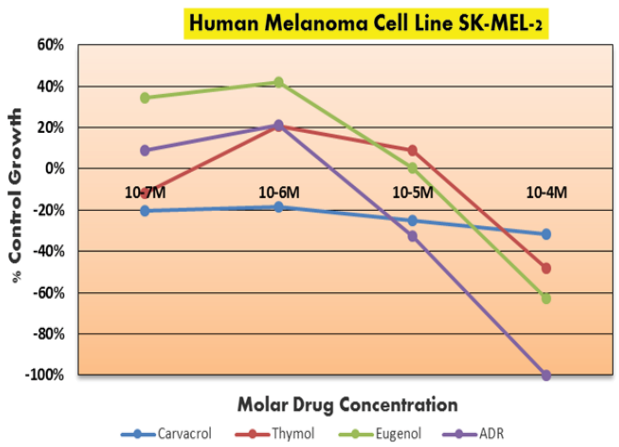


Figure 2: Cytotoxicity of carvacrol, thymol and eugenol on human melanoma cell line SK-ML-2.

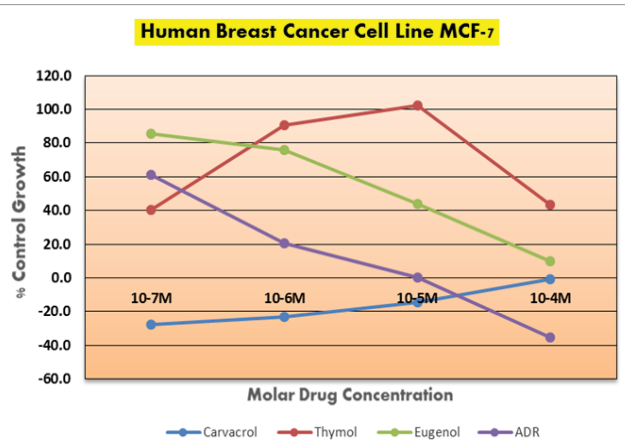


Figure 3: Cytotoxicity of carvacrol, thymol and eugenol on human melanoma cell line MCF7.

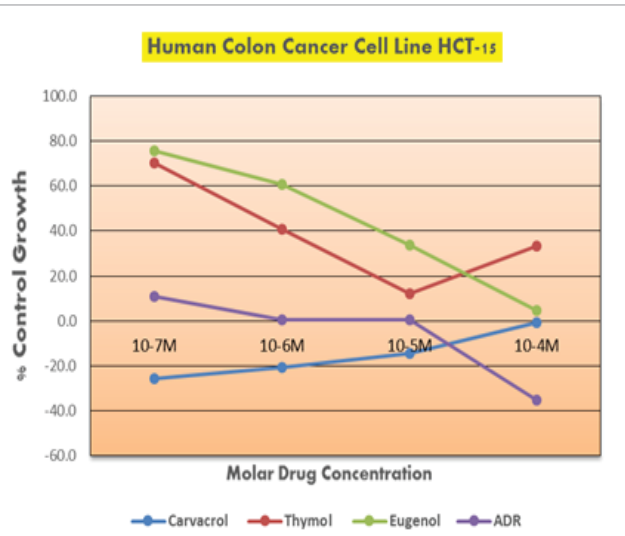


Figure 4: Cytotoxicity of Carvacrol, Thymol and Eugenol on Human Colon Melanoma Cell line HCT-15.

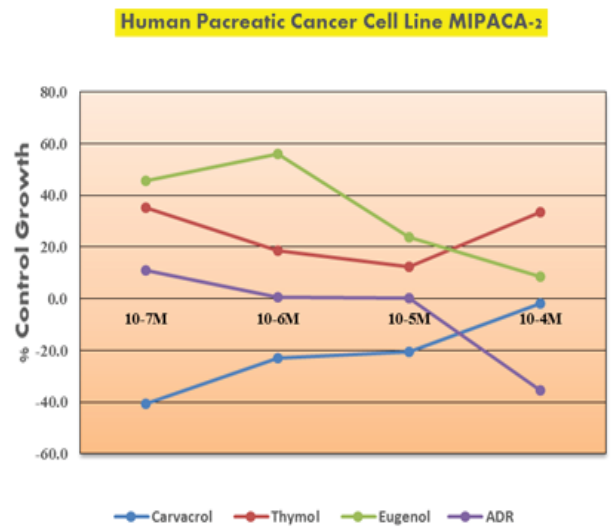


Figure 5: Cytotoxicity of Carvacrol, Thymol and Eugenol on Human Pancreatic Melanoma Cell line MIAPaCa-2.

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