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Original Article

Identification of Suitable Amalgamation with Resveratrol, Epigallocatechin-3-gallate, and Diallyl Trisulfide against Skin Cancer Cell Lines (A431)

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ABSTRACT

Introduction: Resveratrol, Epigallocatechin -3-gallate (EGCG) and Diallyl Trisulfide (DATS) are bioactive compounds in which two polyphenols and an organosulfur are naturally occurring bioactive chemicals that can be efficacious in skin cancer chemoprevention either independently or in combination. **Objectives:** Conglomerate analysis of RES, EGCG, and DATS was performed as a single and in binary combinations to investigate cancer cell viability, *in vitro* in the A431 (human epidermoid carcinoma) skin cancer cell line. **Materials and Methods:** Screening of cytotoxicity potential of bioactive combinations by MTT assay, nuclear fragmentation: Acridine orange / ethidium bromide AO/EtBr assay on skin cancer cell lines (A431). **Results:** Our result showed that the half-maximal growth inhibitory concentration for RES-(IC_{50})-42 µM/mL, EGCG (IC_{50})-44 µM/mL, and DATS (IC_{50})-36 µM/mL whereas the binary combination RES + EGCG (IC_{50})-20 µM/mL; EGCG + DATS (IC_{50})-13 µM/mL; DATS + RES (IC_{50})-24 µM/mL which is evident from the uptake of AO and EtBr dyes by live/dead cells. **Conclusion:** Amalgam of RES, EGCG, and DATS can succor to develop skin cancer treatment better than as a single. Compared to all the combinations EGCG + DATS possess higher anticancer potentials against A431 skin cancer cell line.

Keywords: Skin cancer, Synergistic effect of Resveratrol, Epigallocatechin-3-gallate, Diallyl trisulfide, Dietary bioactive compound.

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INTRODUCTION

Though skin provide the first line of defence against oxidative damage induced by environmental factors, photo oxidative damage plays a vital role of mechanism in skin cancers. The circumstance of skin cancer is linked with dietary factor, longtime exposure to sunlight (UV), noxious agents, microorganisms, and ozone depletion. Epidemiological evidence in Asia over the past decades reveals that a poor-quality diet, chemotherapeutic side effects is also a strong risk factor for multiple malignancies by which rate of skin cancer morbidity is elevated.[1,2] Chemotherapeutic agents, radiotherapy used to treat cancer are ineffective, mostly resistant to skin cancer. The overall positive response to skin cancer monotherapy using conventional anticancer drugs are weak ranges from 4% to 26%.[3] There is a strong association between diet and cancer which has persisted in the early history of medicine. In contrast cancer chemoprevention using bio-active food agents present in common fruits and vegetables so called as phytochemicals which function on distinct mechanism such as overlapping, gene expression regulation in apoptosis and cell proliferation, complementary, transition of detoxifying enzymes.[4-6] A secured chemotherapeutic strategy and chemo preventive nature of Phytochemicals are due to its pleiotropic, non-toxic, and cost-effective by which the therapeutic effects for skin cancer are improved by removing negative effects of chemotherapy, other cancer treatment and aiming metastasis.[3,6,7] Several dietary bioactive agents induce apoptosis like luteoline, and an increase in chemotherapy sensitivity by a dietary supplement indole-3-carbinol by *in vivo* method.[8] To subside the risk of developing cancer several dietary guidelines had been developed by the WHO.[9]

Skin cancer is a multi-factorial disease, hence one strategy using a single dietary bioactive compound in common fruit and vegetable agents may be ineffective to determine efficacy mainly compounds differ in molecular size, polarity, solubility which may affects their availability and distribution.[6,10] A multi-pronged approach such as the concept of using a combination of different dietary bio active agents with different mechanism of action may involve either single or multiple targets with several divergent interactions (synergistic, additive, or antagonistic) are possible making certain combination inefficient. So, it is salient

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to achieve a combination of different dietary bioactive agents which is a recent advancement in cancer chemoprevention that will synergistically interact with each other, thereby increasing the effectiveness of treatment thereby combination regimen reduces the risk of emergent drug resistance.[11,12] It has been reported that exploit of anticancer effect presents in fruits, vegetables are due to an additive or synergistic interaction of the complex mixture of bioactive compound present in them.[13,14] The principal in cancer pharmacologic research is managed with inhibition of proliferation *in vitro* cell as a model. For assessment of bioactive compound combination effects, the current latest technology uses a quantify of synergy as terminus because cell kill is always insignificantly increased by adding another drug to a combination with cytotoxic action. The current expensive anticancer therapies are replaced by use of inexpensive dietary bioactive combination.[13,15]

The use of bioactive compounds that are derived from the diet for chemoprevention which provides a strategy to inhibit cancer that should have limited toxicity. Selected dietary bioactive compound such as Resveratrol (RES), Epigallocatechin-3-gallate (EGCG), and Diallyl Trisulfide (DATS) agents, extensively investigated. Dietary polyphenol such as resveratrol (3,4',5-trihydroxy-trans-stilbene) abundant in berries, peanuts, red wines, grapes, and its skin which is a functional inhibitor for cell cycle and inducer of cell apoptosis in multiple carcinomas with its distinctive structural character with diverse phenolic hydroxyl groups competent of scavenging free radicals to form stronger molecules.[16,17] Resveratrol chemo prevention and therapeutic activity against skin cancer are proposed to be due to properties such as its antioxidant nature, alteration in the expression and function of surviving, a member of the Inhibitors of Apoptosis (IAP) gene.[13,18] Resveratrol proved its efficacy against melanoma and non-melanoma skin cancer in both *in vivo* animal models and widespread data on *in vitro* cell lines confirmed that resveratrol can alter many mechanisms incorporated in apoptosis, cell proliferation, and inflammation reported a CD-1 mice chemically induced skin carcinogenesis was treated with RES as a topical application provided significantly protective effects.[13,19] It has been demonstrated combination of resveratrol with ellagic acid, grape seed extract which acts as potent inhibitors of skin cancer (*in vivo*) by preventing mutation in the *Has-ras* gene.[20]

Epigallocatechin-3-gallate (EGCG) is a flavan-3-ols, and a subclass of flavonoids, which are found mainly in green tea, berries, nuts, red wine, chocolate fruits.[21] It has been reported that Epigallocatechin-3-gallate (EGCG) is poly-phenolic flavanols which exhibit cytotoxic effect, antioxidant, photo-protective against different tumours including skin cancer both *in vivo and in vitro* models. EGCG is known to impede photo-carcinogenesis, chemical carcinogenesis and reduce UV-B induced skin cancer.[22] It has been reported as EGCG exerts direct and selective antiproliferative, pro-apoptotic effects on skin cancer cells (A375 and Hs-2947), without affecting normal cells.[23]

Diallyl Trisulfide (DATS), the important organosulfur components of allium vegetables, such as garlic an oil-soluble fraction which has been used for flavouring and DATS is set on the top of a vegetable pyramid by the National Cancer Institute (NCI).[24] DATS efficacy and biotics function such as anticarcinogenic, chemo preventive and anti-inflammatory reaction were determined by the number such as three sulfur atoms and two allyl sulfides moieties exhibit. DATS triggered DNA damage, induces G2/M arrest by both caspase-dependent and independent apoptosis through p53 pathway in skin cancer cells.[25] Wang *et al.,* 2010 reported cell growth inhibition in A375 and Basal Cell Carcinoma (BCC) by DATS in which integral part more than 80% of skin cancers are due to Endoplasmic Reticulum (ER) stress, and by enhanced levels of Reactive Oxygen Species (ROS).[15] The above selected compounds (RES, ECGC, and DATS) were all most consumed dietary bioactive agents worldwide.

The goal of the current work is to assess the antiproliferative proficiency of RES, EGCG and DATS as single bioactive agents as well as in binary bioactive combinations in skin carcinoma cell line A431.To determine synergistic effects of selected compound using binary combination on A431cell line and HEK 293 (normal human cell line) using 5-fluro-uracil as a positive standard. Though various studies have reported the anticancer properties of single dietary bioactive compound, this is the first proceed of research to explore the combination of RES, EGCG and DATS in A431skin cancer cell line and it may also be contemplated as a pilgrim in skin cancer therapeutics.

MATERIALS AND METHODS

Preparation of study compounds

Resveratrol>99% (HPLC), Epigallocatechin-3-gallate (ECGC)>98% (HPLC), and Diallyl trisulphide (DATS)>98% were all purchased from Sigma Aldrich with analytic or reagent grade products used for assessing their cytotoxic potentials against A431cell line. As well the following combinations RES + EGCG; EGCG + DATS; and DATS + RES were prepared by using fixed ratio (1:1) according to Betina *et al*., 2020 for identifying the suitable bioactive combinations for the treatment.[26]

Cell culture maintenance

Skin carcinoma A431 (human epidermoid carcinoma cells) and normal cell HEK293 were procured from Pune at National Centre for Cell Sciences (NCCS), India. Dulbecco Modified Eagle Medium (DMEM) are used for culturing the cells, which consist of 1.5 g/L glucose, essential nutrients, bovine serum and, 1.5 g/L Na_2CO_3 was supplemented with 2 mM L-glutamine and stabilized salt solution. The cells were grown in a 5% CO_2 at 37°C condition. The concentration of penicillin and streptomycin (100IU/100 g) were calibrated to 1 mL/L. The cells were exposed to various

ratios of hesperidin (10, 20, or 50 M) for around 2 hr before being exposed to 6-OHDA (100 M) for 24 hr.

Cell viability assay

To get the measure of the inhibitory concentration (IC_{50}) of cells a colorimetric assay called as MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used which exploit with colourless dye in tetrazolium and alter it into a formazan salt colorless, later absorbance is quantified by measuring at 570 nm. To seed 100 µL medium containing cells at a density of (1×10^4cells/well) cells per well to obtain 80% cell confluence were seeded in 96-well format. Taking gradient concentration of RES, ECGC, and DATS which were dissolved in DMSO were treated in A431 and HEK293 after 24 hr incubation. The culture medium was maintained at 0.2% with the final concentration of DMSO. With drawl of the spent medium was after 24 hr of incubation, and PBS were used twice to wash the wells. Cells were incubating 37°C in dark for 4 hr by add up 100 µL of MTT (5 mg/mL in PBS) and 100 µL of fresh medium. 50µL of 100% DMSO was added by which product formazan gets dissolved after the medium removed from each well. The A431 cells was measured for optical density using a Biotek multi-mode plate reader (USA) at 570 nm.

$$\% \text{ Cell viability} = \text{OD of experimental sample/OD of experimental control} \times 100$$

Analysis of cell morphology

The A431 cells ($1x10^5$) cells per cover slip and HEK293 were treated with various doses of compound (0.05 mg/mL, 0.025 mg/mL and 0.1 mg/mL) for about 24 hr then static in an acetic acid: ethanol (1:3; v/v). Morphological study of the treated cells was done subsequently, the cover slip was gently mounted on glass slides for the morphological study. Three monolayers micro-graphic are present in each experimental group. Nikon (Japan) bright field microscope inverted light at microscopy at 40X magnifications were used to inspect the morphology variations.

Analysis of apoptotic cell death by fluorescence microscope

Combination of a dye of almost 1µL of (AO: EtBr, 1:1 v/v) was prepared using deionized water on clean microscopic cover slips with 900 µL of cell suspension ($1x10^5$ cells/mL). The A431 cells pre-handled with 6-hydroxydopamine were dealt with distinctive concentrations of hesperidin and collected simultaneously, by using PBS to wash. The procedure was pursed by staining of 10µL of AO/EtBr. Then the cells were imagined under fluorescence microscope (Nikon Eclipse, Inc., Japan) after washing.

Experimental prototype of the binary combination of work

The Inhibitory Concentration (IC_{50}) of RES, ECGC, and DATS were obtained as a single and by binary combination such as RES + EGCG; EGCG + DATS; and DATS + RES with gradient concentration as (0,5,25,50,100, and 200µM) on A431 cell and HEK293 which were all calculated using a Graph-pad prism software, version 5.02. Positive control as a standard drug 5-Fluro-uracil (5FU) was used.

Statistical analysis

Absolutely experiments were done *in vitro* in triplicate. Analysis of data was done by SPSS software. Recorded outcomes were as mean ± SD. GraphPad 5.0 software was used for statistical examination. A value of $^*p<0.05$ was considered as statistically notable.

RESULTS

Preparation of RES, EGCGC and DATS

The selected bioactive compounds such as Resveratrol, Epigallocatechin-3-gallate and diallyl trisulfide was dissolved in 0.2% DMSO with gradient concentration (0, 5, 25, 50,100, and 200µM) both as single as well as in binary combination. Figure 1 depicts the chemicals structure of dietary bioactive compounds which is selected for the present study.

Cell viability assay

The cytotoxicity of RES, EGCG, and DATS as individually and in binary combination against A431 and HEK293 cells was determined using the MTT method. The results are shown in Table 1 depicts the IC_{50} concentration of RES, EGCG, and DATS. Each of three compounds tested as a single showed as RES (IC_{50}) 42µM/L; EGCG (IC_{50}) 44 µM/L and DATS (IC_{50}) 36µM/L. Thus, the result depicts from Figure 2 among the three selected dietary compounds RES, EGCG, and DATS manifest a notable inhibition of cell proliferation in the A431 cells at concentration of (5-200 µM) when compared to the control as 5-fluro-uracil. Table 1 depicts the IC_{50} concentration of DATS against A431 cells was found to be 36 µM/Hence, result could be stated that among the three selected dietary bioactive, DATS as a single bioactive compound resulted a uncommon inhibition of cell proliferation in the A431 cells while for HEK293 cells, the selected bioactive compounds RES, EGCG and DATS did not exhibit any remarkable toxicity.

Binary combination of RES, EGCG, and DATS on A431 cells

To investigate the nature of interactions among selected dietary bioactive compounds, here binary combination regimens were designed at fixed ratios of bioactive compounds (Material and methods). In binary combination bioactive compounds were examined after 24 hr of treatment in A431 cells from the result shown in Table 1 in which EGCG + DATS IC_{50} 13µM/L; RES + EGCG IC_{50} 20 µM/L; revealed that almost all binary combination of dietary phytoconstituents causes a decrease in proliferation

of A431 cells when compared with single Compound. From the Figure 3, A431 cells were treated with binary combination of (RES + EGCG; EGCG + DATS; and DATS + RES) respectively with control as medium alone at gradient concentration (0, 5, 25, 50, 100, and 200 µM). The percentage of viable cells through binary combination at all concentration was <50%, ranging from 10%-25% whereas the percentage of viable through single compound was not <50% ranging from 50%-60% which shows that binary combination of bioactive compounds resulted in greater efficacy in inhibiting the growth and viability of A431 (Figure 3). The effect of binary combination of bioactive compounds was also studied on normal cell HEK29 which show no changes in cellular viability because it is believed that an effective combination of bioactive agents for the management of skin cancer should not be cytotoxic to normal cells.

Analysis of cell morphology

The morphological change in A431cells and HEK293 treated with binary combination of bioactive compounds are depicted in Figure 4 (A) RES + EGCG, (B) EGCG + DATS, (C) DATS + RES that the (a) Absence of any notable morphological alteration in control cells. (a) Control, (b) 10µM, (c) 25µM, and (d) 50µM. In binary combination like DATS + RES, EGCG + DATS appear some changes such as shrinkage of cell, membrane blebbing, floating of cells when contrast with single bioactive compound with A431, while in all treatments with selected bioactive compound with HEK293 depicts the absence of notable morphological changes. Thus, binary combination of bioactive compounds appeared to have some significant morphological alteration and changes (EGCG + DATS), (DATS + RES) when compared with all other compounds as a single and binary combination by which the toxicity effects (EGCG + DATS) disclose that the cytotoxic effects which transpire through disturbance of cytoskeleton, membrane instability in the treated cells.

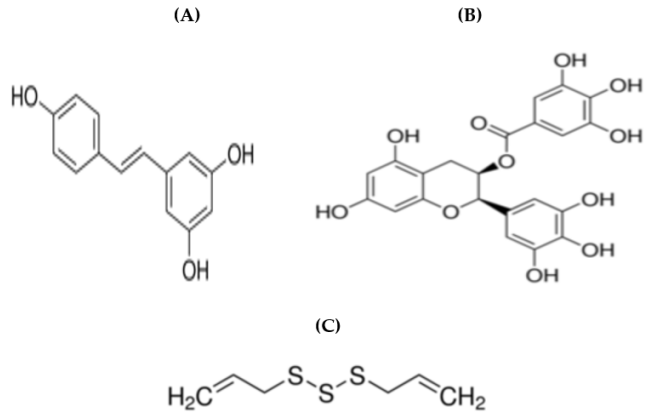

Figure 1: Chemical structure of selected bioactive compounds, (A). Resveratrol, (B). Epigallocatechin-3-gallate, (C). Diallyl trisulfide.

Apoptotic cell death analysis by fluorescence microscope

Apoptotic cell death analysis for binary combination of bioactive compounds was done to examine the apoptogenic activity by fluorescence microscopic analysis. Figure 5 (A, B and C) (AO/EtBr) shows the potency of combination among three binary combinations of bioactive compound in which Figure 5(B) EGCG + DATS; Figure 5(C) DATS + RES; show the significant efficacy against A431 cells. Figure 5(a) depicts the control cells that show only green fluorescence colour indicating no sign of apoptosis while Figure 5(b-d) depicts the fluorescence colour changes of cells from green to orange yellow/red colour in A431, due to effect of the nuclear condensation and initiation of apoptosis attributed to the potency of binary combination EGCG + DATS in a dose-dependent manner.

DISCUSSION

Chemo preventive approach such as intake of fruits and vegetables will reduce skin cancer than by promoting using a habit of sunscreen protection.[12,27] Disruption in critical pathways of the carcinogenesis is postulated by the combination of bioactive over single bioactive compound. In present study, by employing *in vitro* skin cancer model A431, an attempt was made to provoke the chemopreventive combinatorial outcome of bio-active compounds RES, EGCG and DATS. This study clearly indicates that binary combination of bio-active agents exerts more chemopreventive effects rather than single bioactive agent. As shown in Figure 1a, 2c, 3e. None of the single bioactive compound in the present study exhibit greater cytotoxic effects on A431 cell line, while DATS IC_{50} 36µM had some significant effect on the inhibition of cell viability in A431 as a single, when compared with other two bioactive compounds. Because DATS is an organ sulfur compound known to react with sulfhydryl group,

Table: 1: Inhibitory concentration (IC_{50}) (µM/L) of bioactive compound: IC_{50} – Values of respective sample (at 24 hr).

Cell lines	5FU	Fixed ratio and concentration of bioactive compounds for the combination								
		RES	EGCG	DATS	RES + EGCG		EGCG + DATS		DATS + RES	
	IC_{50} (µM/L)				IC_{50} (µM/L)	FR	IC_{50} (µM/L)	FR	IC_{50} (µM/L)	FR
A431	8+0.5	42±0.5	44±0.2	36±0.8	20±1.5	1:1	13±0.5	1:1	24±0.7	1:1
HEK293	8+0.7	NEGLIGIBLE TOXICITY								

Note: 5-FU: 5-Fluorouracil; RES-Resveratrol; EGCG: Epigallocatechin-3-gallate; DATS: Diallyl trisulfide; IC_{50}: Half maximal inhibitory concentration; FR: Fixed ratio.

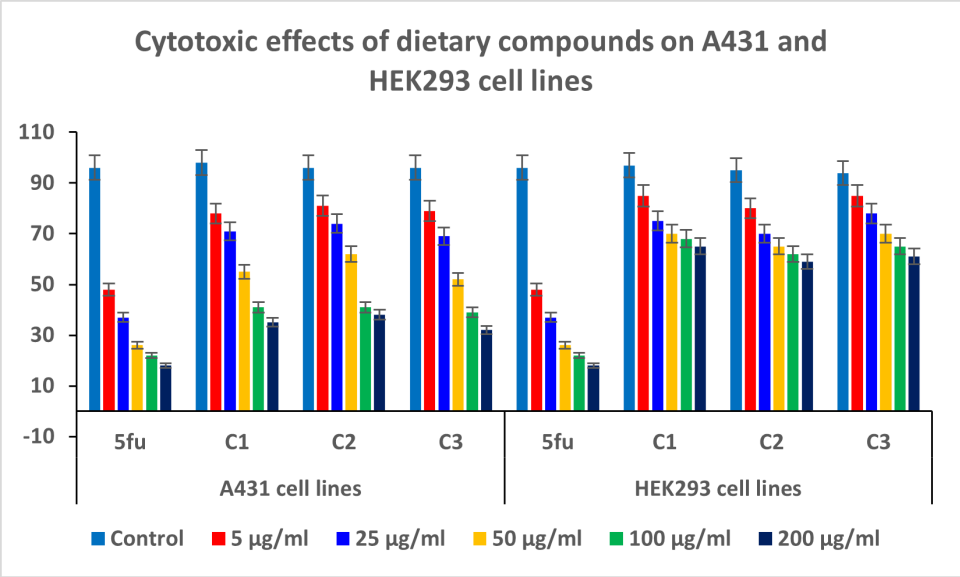

Figure 2: Cytotoxic effect of single dietary compounds RES (C1), EGCG (C2), DATS (C3) in the viability in A431 cell line (a) and HEK293 cell line (b) was confirmed by MTT assay taking 5-fluorouracil as a positive control. Data are indicated as the comparative percentage to control. Bar diagram shows mean ±SD of three dissociated set analysis, *p<0.05.

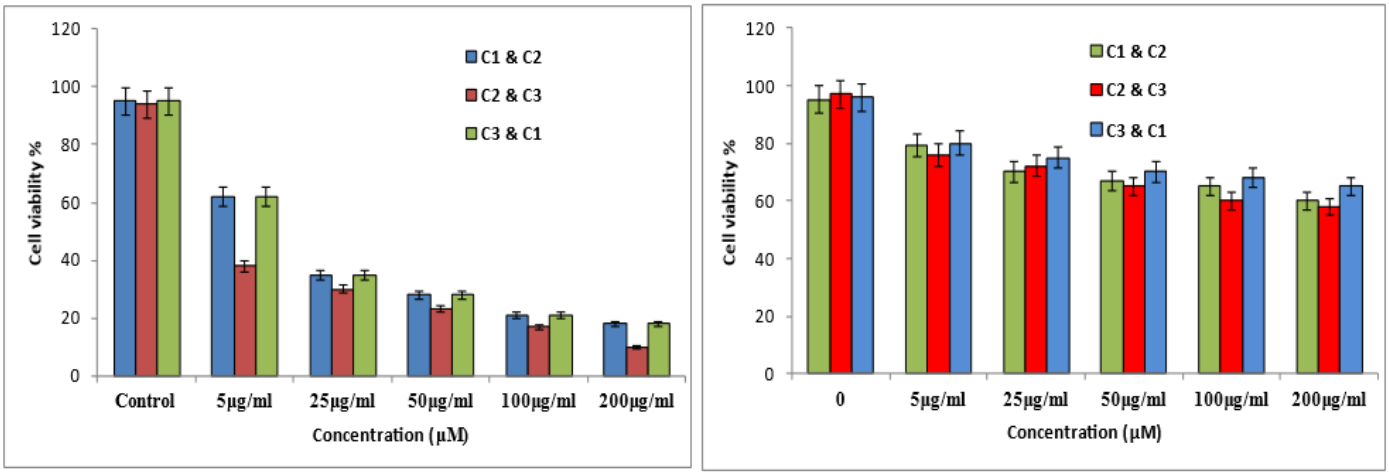

Figure 3: Effect of binary combination (RES + EGCG), (EGCG + DATS), and (DATS + RES) in the viability in A431 cell line, HEK29 cell line was confirmed by MTT assay. Data are indicated as the comparative percentage to control. Bar diagram shows mean ± SD of three distinct set analysis, *p<0.05. (Note: C1-RES, C2-EGCG and C3-DATS).

cysteine modification would be crucial for cell growth inhibition while reported a prominent result by DATS on the inhibition of cell viability IC_{50} 25µM and cause difference in morphology on BCC and A375 cells.[15] It has been reported highest potent anti-proliferative rate in MCF-7 cells of human breast cancer was evinced by DATS.[28] It has been studied anticancer effects of diallyl trisulfide on human colon cancer cells at 100 µg/mL concentration for different interval of time indicated that anticancer potent of DATS due to reaction of its sulfhydryl group with the cysteine group of affected cell and eventually in control for mitotic arrest of cancer cells, microtubule network formation of the cancer cells are distorted by trisulfide, and during at the interphase microtubule fragments could be seen, finally by cell cycle arrest at G2/M phase.[11] Diallyl trisulfide (DATS) can potentially and rapidly release H_2S in the reduced glutathione tumor microenvironment and inflate the anti-tumor effect.[10] In our study A431 cells treated with bioactive combination EGCG + DATS IC_{50} 13µM was found to have the pronounced effective on the cell viability inhibition and caused changes in the morphology at 24 hr treatment in decreasing the rate of tumorgenicity which states that binary combination of bioactive compounds are more efficacious in skin cancer prevention than treatment with each agent alone, despite the fact the these compounds having opposing action on cell differentiation, they still be effective when used as a combination because of the shared property of antiproliferative activity on A431 cells. Naturally EGCG could block most signals

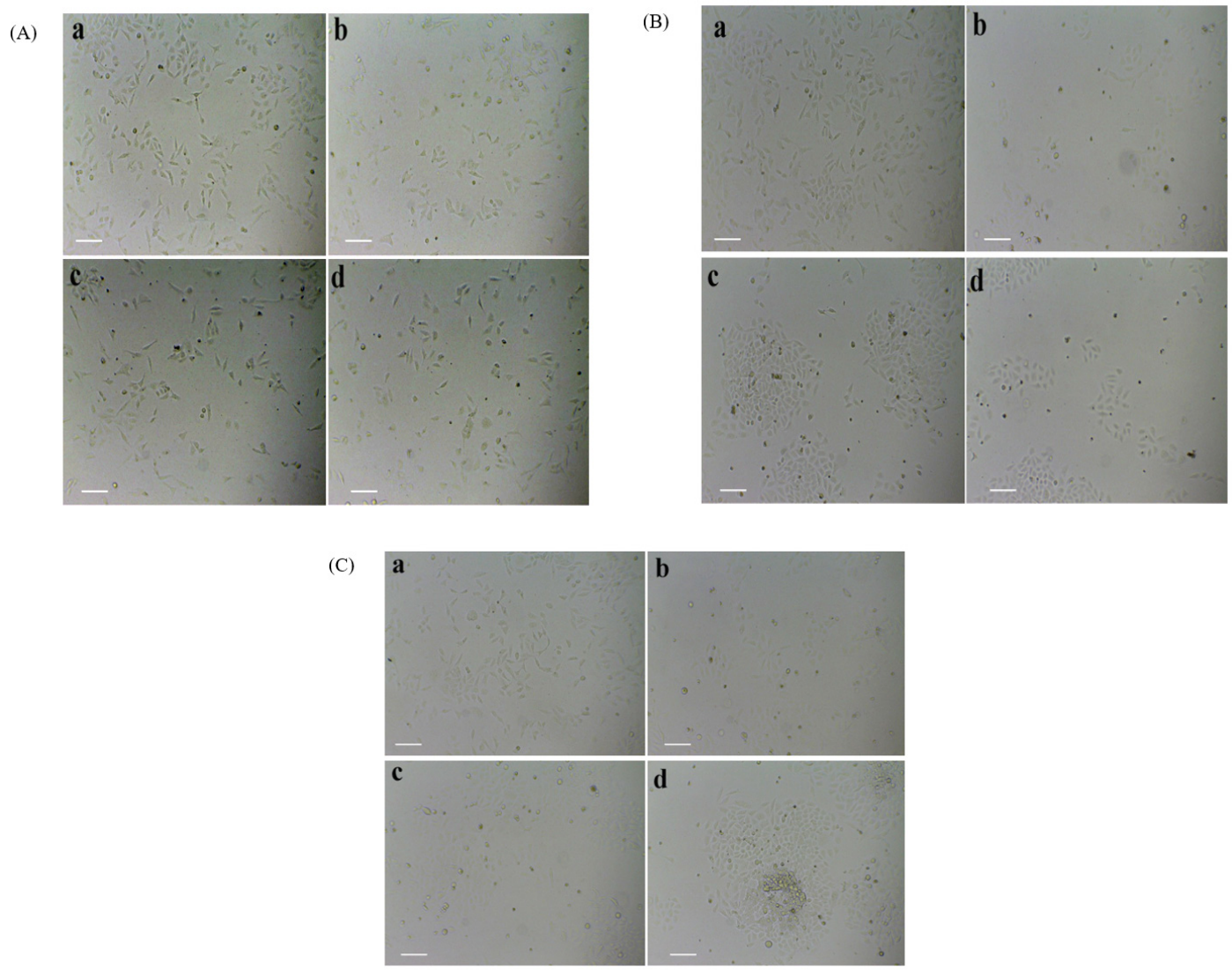

Figure 4: Morphometric analysis of A431 cells after exposure to various concentration of dietary bioactive compounds such as (A) RES + EGCG, (B) EGCG + DATS, and (C) DATS + RES. Inside a. Untreated control; b. 10μM; c. 25μM; d. 50μM; Scale bar = 50μm.

transduction from cell surface to nucleus A431 cells express high level of Epidermal Growth Factors receptors (EGF) which is inhibited by EGCG the tyrosine kinase activity of the EGF-R in intact A431 cell.[22] It has been reported as EGCG + Curcumin resulted potent inhibitory effects against colorectal carcinoma.[4] It has been showed an epigenetic modulator as EGCG which decrease the overall DNA methylation levels in A431 cells and reported that effect of EGCG alone showed absence of notable effect on cell viability and no apparent changes in A431cell morphology.[22] It has been reported combination of EGCG + Curcumin in human keratinocytes.[29] Previous studies also demonstrated combination of resveratrol + curcumin, piperine + resveratrol, curcumin + piperine on breast cancer cell line.[26] It has been reported in a different study identified combination on garlic + tomato on colon cancer.[30] It has been reported combination of resveratrol, ellagic acid and grape seed extract on 3PC, MT1/2 and Ca3/7 cell lines.[20]

Furthermore, the anticancer potency of EGCG is augmented by combining with different of specific polyphenols synergistically.[31] Apoptosis induction by combination of DATS + EGCG on A431 cells may be due to over expression of anti-apoptotic protein such as Bc1-2, IAPs, or down regulation of proapoptotic proteins such as Bax, Apaf-1, and caspase8. DATS-induced apoptosis in BGC823 human gastric cancer cell line was coupled with down-regulation of Bcl-2 and activation of caspase-3.[32] It has been reported the combinational study on isoflavones genistein and the polyphenol thearubigin, inhibited *in vitro* growth of prostate cancer.[33] Currently, study demonstrated that a binary combination treatment with different polyphenols (RES + EGCG) and with other bioactive compound such as organosulfur compound (EGCG + DATS and DATS + RES) is a rational option in order to augment their effectiveness at lower dose by improving specificity, reducing adverse effects, and chemoresistance in which able to inhibit the growth of the A431 cell lines remarkably

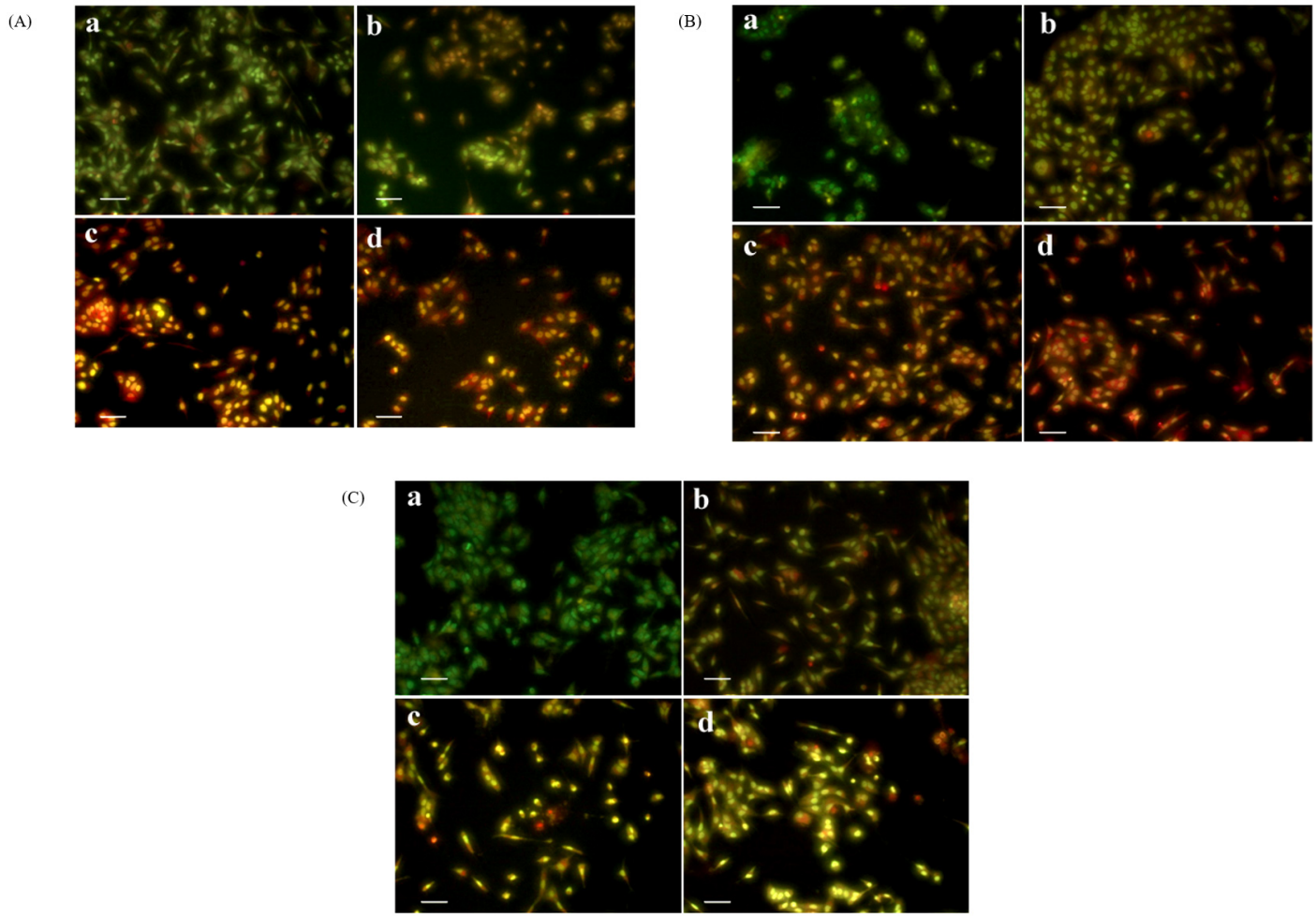

Figure 5: Apoptotic analysis for the binary combination of dietary phytoconstituents such as (A) RES + EGCG, (B) EGCG + DATS, (C) DATS + RES in A431 cells Inside, (a) Untreated, (b) 10 µM, (c) 25 µM, Scale bar = 50 µM.

more than the two compounds administered as a single. It has been also evaluated the combination of bioactive effect of (EGCG + luteolin) on lung cancer cell line, head, and neck, which synergistically increase apoptosis in both cell lines.[1]

To investigate the apoptosis which is a decisive cellular process expressed by every cell to bring about cell death as per to stimulation so skin cancer proliferation can be prevented by inducing apoptosis by AO/Etbr analysis using a binary combination of bioactive compounds. In conclusion, the selected for the present study (RES, EGCG, and DATS) in which the RES and EGCG are the poly-phenolic compounds which had the ability to change different signal transduction pathways involved in carcinogenesis which indicates that binary combination of bioactive treatment with different polyphenols, or other bio active agent is a pragmatic idea in order to ameliorate their potential at different dose by increasing potential targeting, chemo-resistance and reducing hostile effects. It is reasonably estimated at present since different combination of bioactive compounds supplement have been already developed and it is currently recommended for adjuvant therapy as well as for the prevention of several skin disorders.

CONCLUSION

The outcome acquired in present study implicates that selected bioactive compound when combined in which combination could have the potential to exert dominant and synergistic protective effects against carcinogenic insults to skin, to restrain the development of skin cancer and oxidative effects. Thus, the present study reveals findings reported here are appreciable to a bioactive compound's combination RES, EGCG, and DATS, which are already exist on the market as a single or combination with other drugs and prescribed by dermatologists for the adjuvant therapy and the prevention of skin cancer. Compared to all the combinations EGCG + DATS possess good anticancer potentials against A431 skin cancer cell line. Thus, binary combination of selected bioactive compounds synergistically decreased cell viability, initiated apoptosis, exert pleiotropic effect achieving a higher efficacy, potency with reduced toxicity than single bioactive compound effect on A431 cells. The present study of

bioactive combinations is analytic strategy to improve response, tolerability, and decreasing resistance due to non-overlapping mechanism of action and the future *in vitro* studies of selected bioactive compounds may indicates combinations of bioactive agents are able to give the most promising effects than the single bioactive agents *in vivo* experiments also.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

RES: Resveratrol; **EGCG:** Epigallocatechin-3-gallate; **DATS:** Diallyl trisulfide.

SUMMARY

- Dietary bioactive compounds such as Resveratrol (RES), Epigallocatechin -3-gallate (ECGC) and Diallyl Trisulfide (DATS) exert pleiotropic effect when using as a binary combination, also achieving a higher efficacy against skin cancer cell lines.
- In worldwide, most of the phytochemicals producing good activity against numerous diseases with low bioavailability. Here we have attempted to make good combinational drug of dietary phytochemicals with high synergism and good bioavailability.
- Overall, three bioactive combinations of RES + EGCG, EGCG + DATS & DATS + RES were screened on skin cancer cell lines and found EGCG + DATS combinations are exhibit potent anti-cancer activity against skin cancer cell line (A431) with higher synergism.

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