# Achieving Synergistic Effects by Combining Different Phytochemicals for the Prevention and Treatment of Cancer

**Article** · January 2014

CITATIONS
0

READS
22

**2 authors:**

Mazen Medhat Alzaharna
Islamic University of Gaza
**15** PUBLICATIONS **43** CITATIONS
SEE PROFILE

Hon-Yeung Cheung
City University of Hong Kong
**130** PUBLICATIONS **1,758** CITATIONS
SEE PROFILE

# Achieving Synergistic Effects by Combining Different Phytochemicals for the Prevention and Treatment of Cancer

**Al ZAHARNA, Mazen[a]; CHEUNG, Hon-Yeung[a,b]***

[a] *Bioactive Research Group, Department of Biomedical Sciences, City University of Hong Kong, Tat Chee Ave., Hong Kong, China*

[b] *Key Laboratory of Biochip Technology, Shenzhen Biotech and Health Centre, City University of Hong Kong, Shenzhen 51807, China*

**Correspondence author; Tel: +852 3442 7746; Fax: +852 3442 0522; Email: bhhonyun@cityu.edu.hk*

## ABSTRACT

**Cancer is a fatal disease which emerges due to the uncontrolled proliferation of cells. It is considered as a complicated health problem with complex processes and redundant signal transduction pathways. The conventional approach of chemotherapy based on the use of mono-target therapeutic agents has demonstrated that it is an inadequate medical treatment for cancer because it may lead cancer cells to develop acquired drug resistance. Botanical extracts of herbal medicines, on the other hand, have been shown to give effective results in prevention or curing of cancer. Many epidemiological studies and human clinical trials revealed that natural compounds, such as flavonoids, polyphenolic compounds and many other phytochemicals play important roles in cancer chemoprevention and chemotherapy. These phytochemicals have been proved to interfere at different stages of cancer including initiation, promotion and progression by acting on multiple signal transduction pathways of cellular proliferation, differentiation, apoptosis and DNA replication. Many recent studies on combination use of different botanical extracts suggested that some additive or synergistic effects may have taken place. In order to achieve a satisfactory, effective and safe medical treatment, combination of different mechanistic based agents is probably a solution to control multiple aberrant pathways in cancer. This report provides a review on the efficacy by combining various phytochemicals, including flavonoids, diterpene lactones and some other chemotherapeutic drugs, for the treatment of cancer.**

***Keywords:*** *Cancer, phytochemicals, combined effects, chemotherapy, efficacy, multiple aberrant pathways; drug resistance*

## INTRODUCTION

Cancer, which is not just one but more than hundred distinct types, is considered as a complicated disease with complex processes and is a major health problem due to the uncontrollable proliferation of tumor cells and the potential of invading other tissue through the blood and lymphatic system.[1-2] After more than half a century of research, people now realize that tumorigenesis is a multiple manner and that these process signify alterations in genetic materials that drive the successive transformation of normal cells into highly malignant copies. Nowadays many types of cancers have been diagnosed in human beings with an age-dependent incidence involving five to six rate-limiting, stochastic events. **Figure 1** illustrates how the huge catalog of cancer cell genotypes is a representation of six essential alterations in cell physiology that collectively rule malignant growth; namely, (1) antigrowth (insensitivity to growth-inhibitory signals), (2) self-sufficiency in growth signals, (3) apoptosis (evasion of programmed cell death), (4) susbtained angiogenesis, (5) limitless replicative potential and (6) tissue invasion and metastatsis. Each of these physiological changes, which are acquired during tumor development, represents the successful falling-out of an anticancer defense mechanism hardwired into cells and tissues.

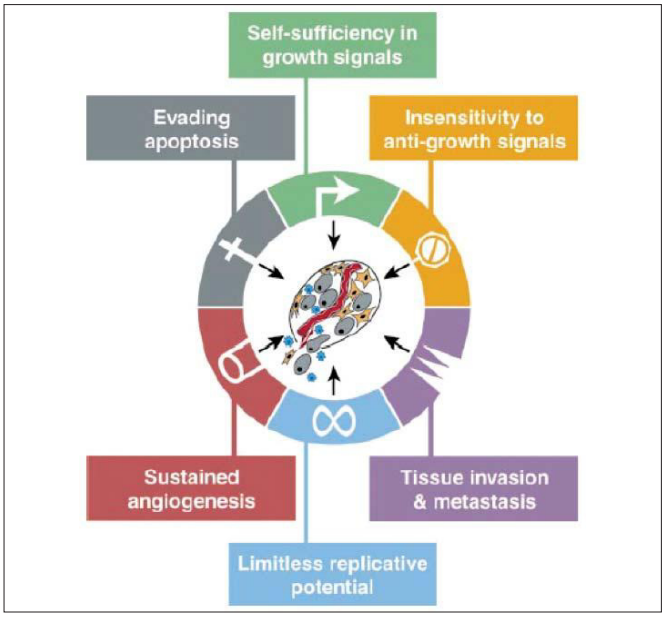


***Figure 1. Acquired characteristics of cancer cell.*** *Most if not all cancers have acquired the same set of functional capabilities during their development, albeit through various mechanistic strategies.*[50]

### Conventional Treatment of Cancers

Up to now surgery, radiation and chemotherapy treatments are the main curative therapies for different types of cancer.[3] Surgery is the most common treatment for most types of cancer. Different surgical procedures can be followed to remove the tumor including Mohs surgery or microsurgery, laser surgery, cryosurgery and amputation. Recently, FDA has announced that for any cancer surgery to be successful it should be done at an early stage of cancer and when cancer is still localized. The surgery should be followed by radiation therapy or chemotherapy in order to kill any cancer cells that were left.[4-6]

The second type of cancer treatment is the radiation therapy which uses high-energy x-rays or other types of radiation to kill cancer cells or stop their growth.[7] Two types of radiation therapy are used depending on the stage of the cancer. The external radiation therapy where an outside machine is used to send radiation toward the cancer and the internal radiation therapy where a radioactive substance sealed in seeds or capsules is placed directly into or near the cancer.[7] However, radiotherapy is not effective in cancer cells that survive in an environment with low oxygen tension due to the increase resistant of cells.[8]

The third main type of cancer treatment is the chemotherapy where drugs are used to induce cell death or cell cycle arrest in cancer cells. Chemotherapy can be systemic where route of entry is through mouth or injection into a vein or muscle, topical when chemotherapy is placed directly onto the skin or regional where the drugs mainly affect cancer cells in one area. The way the chemotherapy is given depends on the type and stage of the cancer being treated.

### PROBLEM OF CONENTIONAL PRACTICES FOR CANCER TREATMENT

Conventional chemotherapy for treatment of cancers, although quite effective, has been associated with toxicities to normal tissue and organs, which is still a major dose limited factor. Furthermore, chemoresistance is another major obstacle for successful treatment of cancer.[9] There is widespread dissatisfaction with surgery, radiotherapy, and especially chemotherapy and hence, treatment of cancer is being re-evaluated around the world.

The traditional model that the malignant phenotype is driven by a dominant signal transduction pathway is becoming increasingly unacceptable. This is due to the appearance of resistance to target- and mechanism-based drugs, and therefore reflects the genetic flexibility of the cancer cell genome as well as the redundancy in the pathways that govern kinase signal transduction networks.[10] Based on this, the traditional mono-target chemotherapy protocol for cancer treatment is becoming increasingly ineffective and may lead cancer cells to develop acquired drug resistance due to the complex signaling pathways involved in cancer.[11] The multi-component therapy in which more than one drug are used at the same time, is the proven cure for cancers.[12]

### ALTERNATIVE APPROACHES TO THE TREATMENT OF CANCER

Different innovative strategies have been adopted to treat cancer in recent years including selective inhibition of anti-apoptotic pathways, antiangiogenic therapy and tissue-selective therapy (including immunotherapy). These overlapping and complementary strategies depend on rational drug combinations aimed at matching targets.[13] For example, reactivation of the apoptotic cascade in apoptosis-reluctant cancer cells where one drug decreases the antiapoptotic block (e.g. inhibitors of apoptosis) allowing the other drug to activate the corresponding apoptotic pathway **(Figure 2)**. Another example is activation of the extrinsic (caspase 8) and intrinsic (caspase 9) apoptotic pathways where both pathways will activate caspase 3 and therefore increase the apoptotic effect. [13-14]

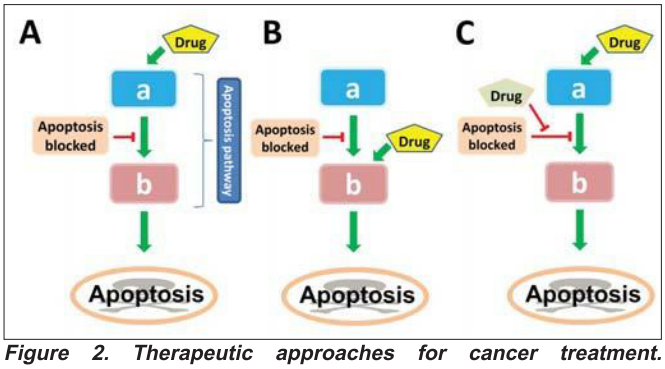


***Figure 2. Therapeutic approaches for cancer treatment.*** *(A) Inhibitors of apoptosis prevent the cell death although the apoptosis pathway is initiated. (B) The use of another drug which bypasses the inhibitors of apoptosis and induces apoptosis downstream of the blockages will lead to cell death. (C) The use of combined drugs where one will initiate the apoptosis pathway while the other one blocks the inhibitors of apoptosis will also lead to cell death.*

The concept of drugs combination, with similar or different modes of action, seeks to result in synergistic or additive therapeutic effects, including increased therapeutic efficacy, decreased host toxicity, and minimal or delayed drug resistance.[14] Drugs that contain several active components have been in use long time ago. Many traditional medicine, including the Chinese medicine, have used mixtures of naturally occurring herbs or herbal extracts.[15] Cancer is a complex disease which involves different signaling pathways and therefore combination therapy in which one or more drugs are used at the same time is the proven cure for cancer.[12]

Polyphenols including flavonoids, diterpene lactones and other phytochemicals have long been known for their antioxidant, anti-inflammatory, antiallergic, antithrombotic, hepatoprotective, antiviral, antibacterial, antiageing and anticarcinogenic activities **(Figure 3)**.[16-21] Epidemiological studies have shown that there is an inverse association between fruits and vegetables consumption, where flavonoids are prominent components, and the risk of various human cancers.[22] The combination effect of flavonoids when combined with other flavonoids, natural compounds or chemotherapeutic drugs was also proved to be a synergistic one by different studies.[22-23]

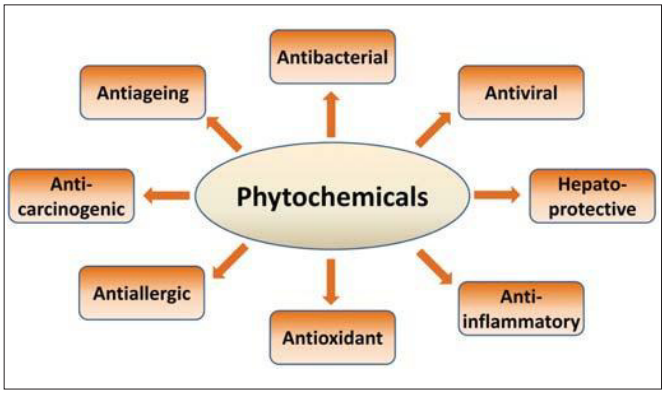


***Figure 3. The different biological activities of phytochemicals.***

The combination therapy can be approached by combination of different mechanism-based agents to control multiple abnormal pathways seen in the tumor.[10] On the other hand, the development of multitarget molecules also seems to be an increasingly reasonable and attractive option.[24] Therefore, based on both of these options, exploration of newer chemical diversity will be an utmost need.[10] The chemical complexity of botanicals makes them important starting materials for the discovery of newer synergistic combinations and single agent multi-target drugs.[25-27]

## EFFECT OF DRUG COMBINATION ON CELL CYCLE

With advancements in our understanding of the basic mechanisms of carcinogenesis, cell cycle physiology, and apoptosis, the effects of chemotherapy on normal and cancerous cells are now better understood. With this knowledge, it is more clear now that a critical role in chemosensitivity is played by the cell cycle in combination chemotherapy.[28] A number of cancers are associated with increase in the activity of Cdks, important molecules for cell cycle regulation, due to gene mutations. Therefore, compounds that can modulate the activity of Cdks are of importance in cancer therapy.[29] Flavonoids are involved in the regulation of many proteins associated with cell growth and differentiation. These include increase in the Cdks inhibitors (p21, p27), or a decrease in cyclins B, D and E and Cdks 2,4 and 6.[30]

Different studies have investigated the effect of drugs combination on the cell cycle arrest. Drugs that block different phases of cell cycle can act in synergy. Shen *et al.* studied the effect of the combination of quercetin (arrest cells at G1 and S phases) and triazofurin (arrest cells at S phase) on the human ovarian carcinoma cells. A synergistic effect on the growth inhibition was observed when the two compounds were used in combination with a CI value of 0.37.[31] Also significant alterations in the cell cycle kinetics induced by the single compounds such as ellagic acid, quercetin and their combinations was observed against human leukemia cells.[23] In one study, the use of Taxifolin in combination with andrographolide increased the percentage of cells arrested at G2/M by increasing the levels of cyclin B and activation of Cdc2.[32] In another study, the combination of silibinin with doxorubicin strongly increased the cell cycle arrest at G2/M compared to the use of single compounds.[33]

## PHYTO-COMPOUNDS USED IN COMBINED THERAPY OF CANCER

There is an increasing evidence indicating the effectiveness of using combined phyto-compounds for the treatment of solid tumors.[14] The combination of anticancer agents which have similar or different modes of action can result in synergistic, additive or antagonistic outcome.[34] Experimental techniques to determine the action of combination drugs and to design effective mixtures have not been completely standardized. It is still being practiced as an art using trial and error methods.[35] The pure natural or synthetic compounds used in western medicine usually aims a single target, while the processed crude multi-component natural products are used in Chinese medicine, in various combinations and formulations, aimed at multiple targets and different symptoms.[36]

Many studies have presented the enhanced effects of drugs combination in treatment of cancer cells using western drugs, isolated pure herbal extracts or a combination of both. A number of good chemotherapeutic agent combinations have been developed to treat cancers and they showed positive cytokinetic and biological interaction with reduced toxicity. Examples include ABV (Adriamycin, bleomycin & vinblastine) or BEP (bleomycin, etoposide & Platinol).[37] Compounds isolated from a single herbal component used in combination, two lignans, asarinin and xanthoxylol inhibited the carcinogenesis in mouse skin and pulmonary tumors.[38]

Other studies indicated that selenium act in synergy with retinoids and vitamin E to inhibit carcinogenesis. It was also found to work in synergy with crambene to kill MCF-7 mammary cells.[39] The combination of EGCG and curcumin showed a synergistic effect on growth inhibition of oral cancer cells. There was an increase dose reduction 4.4-8.5 fold for EGCG and 2.2-2.8 fold for curcumin at ED50 as indicated by the dose reduction index (DRI).[40]

## ROLES OF COMBINED FLAVONOIDS IN TREATMENT OF CANCER

Many research papers have shown a better outcome in cancer treatment when the conventional drug is combined with a herb and others when combination of herbs is used **(Table 1)**. Flavonoids and other antioxidants when used alone could produce beneficial, detrimental, or insignificant effects in cancer patients while if there are combined with other anticancer compounds (i.e., natural compounds or chemotherapy drugs), their effects are more likely to be beneficial or at least not harmful.[42]

Mertens-Talcott *et al.* investigated the combinational effect of quercetin and ellagic acid on cell death in the MOLT-4 human leukemia cell line. The two compounds together reduced more the proliferation and viability and enhanced the induction of apoptosis compared to each alone.[23] In another study, the combination treatment of human gut (HuTu-80 and Caco-2) and breast cancer cells (PMC42) with quercetin and kaempferol was more effective than the additive effects of each flavonol.[43]

**Table 1. Examples of studies on treatment of cancer cells with flavonoids combined with flavonoids, chemotherapy or other phytochemicals**

| Combined compounds | Cancer cells | Synergistic effect | Reference |
|---|---|---|---|
| Quercetin and ellagic acid | Human leukemia (MOLT-4) | Increase induction of apoptosis | 23 |
| Taxifolin and andrographolide | Prostate (DU145) | Increase in cell cycle arrest at G2/M and apoptosis | 32 |
| Silibinin and doxorubicin | Prostate (DU145) | Increase in G2/M arrest and apoptosis | 33 |
| Quercetin and kaempferol | Gut (HuTu-80, Caco-2) and breast (PMC42) | Decreased expression of nuclear proliferation antigen Ki67 and decreased total protein levels | 43 |
| 5-Fluorouracil combined with leuteolin or quercetin | Colorectal (CO115) | Activation of the apoptotic mitochondrial pathway | 44 |
| Curcumin combined with cisplatin or oxaliplatin | Ovarian cancer cells | Increase induction of apoptosis | 46 |

Other studies have investigated the effect of combination of chemotherapeutic drugs with flavonoids. The treatment of colorectal tumor (CO115) with 5-Fluorouracil combined with leuteolin or quercetin increased apoptosis with a significant effect for quercetin which involved the activation of the apoptotic mitochondrial pathway.[44] In another study, the flavonoid silibinin strongly synergized the antiproliferative effect of doxorubicin in prostate carcinoma DU145 cells. This combination was associated with an increase in G2/M arrest and apoptosis compared with treatment of each compound alone.[33] Silibinin also should synergistic cytotoxic effects when combined with chemotherapeutic drugs against breast and lung cancer cells.[45] Curcumin was also shown to be effective in combination treatment. The combination of curcumin with either cisplatin or oxaliplatin increased significantly the cytotoxic effect on ovarian cancer cells by increasing apoptosis.[46] In a recent study done at our laboratory, the flavonoid Taxifolin synergized the effect of Andrographolide by increasing the cell cycle arrest and apoptosis in DU145 cells.[32]

## BENEFITS OF COMBINED USE OF PHYTO-COMPOUNDS FOR PREVENTION AND TREATMENT OF CANCER

Combination or multicomponent therapy, where two or more drugs are used together, usually has one or more of the following objectives: (1) to reduce the frequency of acquired resistance which may arise by combining drugs with minimal cross-resistance; (2) to lower the doses of drugs with getting a similar therapeutic effect so as to achieve efficacy with fewer side effects; (3) to sensitize the cells to the action of one drug through the use of another drug (chemosenstization), this is often achieved by altering the cell-cycle stage or growth properties; and (4) to achieve an enhanced effectiveness through additivity, or better yet, through synergism.[37, 47]

## DETERMINATION OF THE COMBINATION EFFECT

Evaluation of the effect of drug combination is important in all areas of medicine particularly in cancer chemotherapy where combination therapy is commonly used. The *in vitro* studies are usually used to determine the nature and quantitative extent of drugs combination.[48] The combination of two drugs can give synergism, antagonism or additive effect. Synergism means that a combination of two drugs produce a therapeutic effect greater than each of the two drugs alone and more than additive effect (greater than the algebraic sum of the parts), whereas antagonism is an effect which is less than additive.[49]

The two methods which are commonly used in the analysis of drug combination effects are the isobologram and the combination index (CI) where the CI method is the most commonly used.[48] The isobologram method is based on the Loewe additivity model which evaluates the interaction at a chosen effect level and is therefore useful to examine the drug interaction at the corresponding concentration, often the median effect concentration.[50] However the CI method is based on the median-effect principle derived by Chou. The median-effect equation correlates the drug dose and cytotoxicity or cytostatic effect.[51] A software program to calculate combination indices (CI) is available and widely used.[49]

## CONCLUSION

There is an increasing trend nowadays in cancer research to use a combination therapy for several solid tumors where a growing number of *in vitro* and *in vivo* studies show that combinations of natural agents can result in significant activities at concentrations where any single agent is not effective. This urges us to further explore the synergistic effects of dietary phytochemicals in the field of cancer treatment. With holistic clarity of mechanisms, cancer prognosis and treatment will become a rational science, unrecognizable by current practitioners. It will be possible to understand with precision how and why treatment regimens and specific antitumor drugs succeed or fail. As Hanahan and Weinberg anticipated anticancer drugs targeted to each of the hallmark features of cancer; when some, used in appropriate combinations and in concert with sophisticated technologies, such as nano-delivery, will be able to prevent incipient cancers from developing, while others will cure preexisting cancers.[52] Hence, natural products will continue to provide a broad base for the discovery of new drugs and new substances for combinational treatment of cancers.

**Author's background**

***Mr AL ZAHARNA Mazen*** *is a PhD candidate in the Department of Biomedical Science, City University of Hong Kong. He is currently working on a project relevant to the combined effect of bioactive phytocompounds on cancer cells at molecular level. He has a BSc and MSc degree in Medical Technology from University of Malta and The Islamic University of Gaza - Palestine respectively.* ***Dr. CHEUNG Hon Yeung****, who is an associate professor of Pharmaceutical Microbiology & Biotechnology at the City University of Hong Kong since 1989, is a manufacturing pharmacist and biotechnologist. He has more than 40 years of work experiences in industries, academic and consultancy. He was an expert witness in court and a member of the Biotechnology Committee for Hong Kong and Shenzhen Government. Dr. Cheung has published more than 220 papers and articles in many prestigious international journals. His email address: bhhonyun@cityu.edu.hk*

## References


1. Varma A, Padh H, Shrivastava N (2009). Andrographolide: A new plant-derived antineoplastic entity on horizon. *Evidence-Based Complementary and Alternative Medicine*, 2011:1-9.
2. Shayan R, Achen MG, Stacker SA (2006). Lymphatic vessels in cancer metastasis: bridging the gaps. *Carcinogenesis*, 27(9):1729-1738.
3. Shylasree TS, Bryant A, Athavale R (2013). Chemotherapy and/or radiotherapy in combination with surgery for ovarian carcinosarcoma. *The Cochrane database of systematic reviews*, 2:CD006246.
4. Crino L, Weder W, van Meerbeeck J, Felip E (2010). Early stage and locally advanced (non-metastatic) non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology*: *official journal of the European Society for Medical Oncology / ESMO*, 21 Suppl 5:v103-115.
5. Aapro MS (2001). Adjuvant therapy of primary breast cancer: a review of key findings from the 7th international conference, St. Gallen, February 2001. *The oncologist*, 6(4):376-385.
6. Glode LM (2006). The case for adjuvant therapy for prostate cancer. *The Journal of urology*, 176(6 Pt 2):S30-33.
7. Radiation therapy and you: Support for people with cancer. National Cancer Institute. http://www.cancer.gov/cancertopics/radiation-therapy-and-you/allpages. Accessed May 08.
8. Sagar SM, Wong R (2007). in *Annals of traditional Chinese medicine: Alternative treatment for cancer* (Eds.: P.-C. Leung, H. H. S. Fong), World Scientific Hackensack, NJ, p. 363.
9. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG (2013). Cancer drug resistance: an evolving paradigm. *Nature Reviews. Cancer,* 13(10):714-726.
10. Kerr DJ, Thangue NBL (2004). Signal transduction blockade and cancer: combination therapy or multi-targeted inhibitors? *Annals of Oncology*, 15:1727-1729.
11. Faivre S, Djelloul S, Raymond E (2006). New paradigms in anticancer therapy: targeting multiple signaling pathways with kinase inhibitors. *Seminars in Oncology*, 33(4):407-420.
12. Ji HF, Li XJ, Zhang HY (2009). Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? *EMBO Reports*, 10(3):194-200.
13. Blagosklonny MV (2004). Prospective strategies to enforce selectively cell death in cancer cells. *Oncogene*, 23:2967-2975.
14. Yang L, Wu D, Luo K, Wu S, Wu P (2009). Andrographolide enhances 5-fluorouracil-induced apoptosis via caspase-8-dependent mitochondrial pathway involving p53 participation in hepatocellular carcinoma (SMMC-7721) cells. *Cancer Letters*, 276:180-188.
15. Keith CT, Borisy AA, Stockwell BR (2005). Multicomponent therapeutics for networked systems. *Nature Reviews. Drug Discovery*, 4(1):71-78.
16. Middleton E, Jr., Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, 52(4):673-751.
17. Sharma DK (2006). Pharmacological properties of flavonoids including flavonolignans- integration of petrocrops with drug development from plants. *Journal of scientific and industrial research,* 65:474-484.
18. Scalbert A, Johnson IT, Saltmarsh M (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition,* 81(1 Suppl):215S-217S.
19. Singh RP, Dhanalakshmi S, Agarwal R (2002). Phytochemicals as cell cycle modulators--a less toxic approach in halting human cancers. *Cell Cycle*, 1(3):156-161.
20. Tyagi A, Agarwal C, Harrison G, Glode LM, Agarwal R (2004). Silibinin causes cell cycle arrest and apoptosis in human bladder transitional cell carcinoma cells by regulating CDKI--CDK--cyclin cascade, and caspase 3 and PARP cleavages. *Carcinogenesis*, 25(9):17111-11720.
21. Maimone TJ, Baran PS (2007). Modern synthetic efforts toward biologically active terpenes. *Nature Chemical Biology*, 3(7):396-407.
22. Campbell JK, King JL, Harmston M, Lila MA, Erdman JW (2006). Synergistic effects of flavonoids on cell proliferation in Hepa-1c1c7 and LNCaP cancer cell lines. *Journal of Food Science*, 71(4).
23. Mertens-Talcott SU, Talcott ST, Percival SS (2003). Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *The Journal of Nutrition*, 133:2669-2674.
24. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E (2006). Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *Journal of Clinical Oncology,* 24(1):25-35.
25. Liu RH (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. American Journal of Clinical Nutrition, 78(3 Suppl):517S-520S.
26. Bhushan P, Manish G (2007). in *Annals of traditional Chinese medicine: Alternative treatment for cancer* (Eds.: P.-C. Leung, H. H. S. Fong), World Scientific, Hackensack, NJ, p. 363.
27. de Kok TM, van Breda SG, Manson MM (2008). Mechanisms of combined action of different chemopreventive dietary compounds: a review. *European Journal of Nutrition*, 47 Suppl 2:51-59.
28. Shah MA, Schwartz GK (2001). Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. *Clinical Cancer Research,* 7:2168-2181.
29. Senderowicz AM (2003). Cell cycle modulators for the treatment of lung malignancies. *Clin Lung Cancer,* 5(3):158-168.
30. Haddad AQ, Venkateswaran V, Viswanathan L, Teahan SJ, Fleshner NE, Klotz LH (2006). Novel antiproliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. *Prostate Cancer Prostatic Dis*, 9(1):68-76.
31. Shen F, Herenyiova M, Weber G (1999). Synergistic down-regulation of signal transduction and cytotoxicity by tiazofurin and quercetin in human ovarian carcinoma cells. *Life Sciences*, 64(21):1869-1876.
32. Zhang ZR, Al Zaharna M, Wong MM, Chiu SK, Cheung HY (2013). Taxifolin enhances andrographolide-induced mitotic arrest and apoptosis in human prostate cancer cells via spindle assembly checkpoint activation. *PLoS ONE*, 8(1):e54577.
33. Tyagi AK, Singh RP, Agarwal C, Chan DC, Agarwal R (2002). Silibinin strongly synergizes human prostate carcinoma DU145 cells to doxorubicin-induced growth Inhibition, G2-M arrest, and apoptosis. *Clinical Cancer Research*, 8(11):3512-3519.
34. Johnson KR, Young KK, Fan W (1999). Antagonistic interplay between antimitotic and G1-S arresting agents observed in experimental combination therapy. *Clinical Cancer Research,* 5(9):2559-2565.
35. Hemalswarya S, Doble M (2007). in *Annals of traditional Chinese medicine: Alternative treatment* for *cancer* (Eds.: P.-C. Leung, H. H. S. Fong), World Scientific Hackensack, NJ, p. 363.
36. Liao H, Banbury LK, Leach DN (2007). Elucidation of danzhixiaoyao wan and its constituent herbs on antioxidant activity and inhibition of nitric oxide production. *Evidence-Based Complementary and Alternative Medicine,* 4(4):425-430.
37. Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK (2006). Systems biology and combination therapy in the quest for clinical efficacy. *Nature Chemical Biology,* 2(9):458-466.
38. Lee KH (2000). Research and future trends in the pharmaceutical development of medicinal herbs from Chinese medicine. *Public Health Nutrition,* 3(4A):515-522.
39. Wallig MA, Heinz-Taheny KM, Epps DL, Gossman T (2005). Synergy among phytochemicals within crucifers: does it translate into chemoprotection? *Journal of Nutrition*, 135(12 Suppl):2972S-2977S.
40. Khafif A, Schantz SP, Chou TC, Edelstein D, Sacks PG (1998). Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells. *Carcinogenesis*, 19(3):419-424.
41. Adams LS, Seeram NP, Hardy ML, Carpenter C, Heber D (2006). Analysis of the interactions of botanical extract combinations against the viability of prostate cancer cell lines. *eCAM*, 3(1):117-124.
42. Boik J (2001). in *Natural Compounds in Cancer Therapy*, 1st ed. (Ed.: S. Farnell), Oregon Medical Press, pp. 51-61.
43. Ackland ML, Waarsenburg S, Jones R (2005). Synergistic antiproliferative action of the flavonols quercetin and kaempferol in cultured human cancer cell lines. *In Vivo*, 19(1):69-76.
44. Xavier CP, Lima CF, Rohde M, Pereira-Wilson C (2011). Quercetin enhances 5-fluorouracil-induced apoptosis in MSI colorectal cancer cells through p53 modulation. *Cancer Chemotherapy and Pharmacology,* 68(6):1449-1457.
45. Raina K, Agarwal R (2007). Combinatorial strategies for cancer eradication by silibinin and cytotoxic agents: efficacy and mechanisms. *Acta Pharmacologica Sinica*, 28(9):1466-1475.
46. Montopoli M, Ragazzi E, Froldi G, Caparrotta L (2009). Cell-cycle inhibition and apoptosis induced by curcumin and cisplatin or oxaliplatin in human ovarian carcinoma cells. *Cell Proliferation*, 42(2):195-206.
47. Miles D, von Minckwitz G, Seidman AD (2002). Combination versus sequential single-agent therapy in metastatic breast cancer. *Oncologist*, 7 Suppl 6:13-19.
48. Zhao L, Wientjes MG, Au JL (2004). Evaluation of combination chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses. *Clinical Cancer Research,* 10(23):7994-8004.
49. Chou TC (2006). Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacological Reviews*, 58:621–681.
50. Tallarida RJ (2001). Drug synergism: its detection and applications. *Journal of Pharmacology and Experimental Therapeutics*, 298(3):865-872.
51. Chou TC, Talalay P (1984). Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Advances in Enzyme Regulation*, 22:27-55.
52. Hanahan D, Weinberg RA (2000). The hallmarks of cancer. *Cell*, 100:57-70.