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# A Short Review on the Anti-Cancer Activities of Natural Polyphenols

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**ABSTRACT**

Cancer continues worldwide disease taking the life of individuals irrespective of their age. There are various types of cancer detected by the doctors worldwide. Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth. The cause of cancer can be external and internal. It can affect any organs of the human being. Plant derived polyphenols plays a vital role in protecting humans from cancerous diseases. Polyphenols play a major role as a powerful natural anti oxidant which can protect the cells. The review focuses on the role of natural polyphenols as an anti cancer agent.

**Key-words:** Natural Polyphenols, Anti-Cancer Activities, cancer

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**Introduction:**

The reactive oxygen species (ROS) play an important role in many diseases like cancer. Natural polyphenols are the biggest entity of phytochemicals, and have gained more attention as a potential agents for prevention and treatment of oxidative stress-related diseases. Natural polyphenols are secondary metabolites of plants, and many of them have been found in plant based foods. More than 20,000 polyphenols compounds have been identified in various plants. Firstly, they occurred in conjugated forms, with one or more sugar residues linked to hydroxyl groups, but direct linkages of the sugar to an aromatic carbon also exist. Linkage with other compounds, such as amines, carboxylic and organic acids, lipids and association with other phenol were established.[1] Polyphenols could be divided into different groups by the number of phenol rings that they contain and the basis of structural elements that bind these rings, which were classified into several subclasses, such as the phenolic acids, flavonoids, stilbenes and lignans.[2,3]

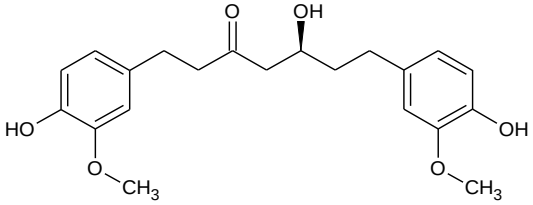
**Anti Cancer Activity:**

The anticancer effects of polyphenols have been established at body parts including mouth, stomach, duodenum, colon, liver, lung, mammary gland or skin. Many polyphenols, such as proanthocyanidins, flavonoid, resveratrol, tannins, epigallocatechin-3-gallate, Gallic acid and anthocyanins, have been tested; all of them showed protective effects in some models although their mechanisms of action were found to be different [4] Dietary polyphenols may exert their anticancer effects via a array of mechanisms such as removal of carcinogenic agents, modulation of cancer cell signaling and antioxidant enzymatic activities, and induction of apoptosis and cell cycle arrest. Some of these effects can be attributed to, at least partly, to their indirect antioxidant activities.

Natural polyphenols might have a better protective effect on metastatic breast cancer. Scientists therefore investigated the effects of proanthocyanidins on a highly metastatic mouse mammary carcinoma cell line. In vitro treatment of breast cancer cells 4T1 resulted in significant inhibition of cellular proliferation and viability, and induction of apoptosis in 4T1 cells in a time- and dose-dependent manner after exposed with proanthocyanidins. Moreover, the metastasis of tumor cells to the lungs was inhibited significantly and the survival of the mice was enhanced. All these data demonstrated that proanthocyanidins possess chemotherapeutic efficacy against breast cancer including inhibition of metastasis [5]

Resveratrol could inhibit each stage of multistage carcinogenesis, scavenge incipient populations of androgen-dependent prostate cancer cells through androgen receptor antagonism, and scavenge incipient populations of androgen-independent prostate cancer cells by short-circuiting the epidermal growth factor-receptor-dependent autocrine loops in the cancer cells. The identification of resveratrol as a cancer preventive agent is largely owed to its high abundance in nature.[6]

Curcumin has been studied in multiple human cancers including melanoma, head and neck, breast, colon, pancreatic, prostate and ovarian cancers .[7] Curcumin, a hydrophobic natural product, comprises two phenolic rings. Each ring is replaced with methoxy ether functionality in the ortho-position and attached to each other by an aliphatic unsaturated heptene linker in the para-position with an α,β-diketonic functionality on carbon-3 and-5.



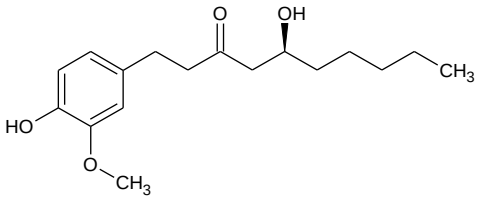

Curcumin

Curcumin's anti-oxidant and free-radical quenching properties play an significant role in the inhibitory effects on the initial stages of carcinogenesis. It has been shown that curcumin has the ability to suppress UV irradiation-

induced DNA mutagenesis and induction of cellular SOS functions.[8] : Curcumin has been found to possess anticancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis. Curcumin has shown anti-proliferative effect in multiple cancers, and is an inhibitor of the transcription factor NF-B and downstream gene products (including c-myc, Bcl-2, COX- 2, NOS, Cyclin D1, TNF-a, interleukins and MMP-9). Curcumin asserts its anti-tumor activity in cancer cells by altering the deregulated cell cycle via (a) cyclin-dependent (b) p53-dependent and (c) p53-independent pathways. Such influences of curcumin upon key signal transduction pathways of cell cycle and effectiveness in animal model systems have qualified it as a multiple edged sword in combating the deadly disease-cancer.[9]

Curcumin has been shown to suppress transformation, proliferation, and metastasis of tumors. These effects are mediated through its regulation of various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes. Curcumin has been shown to have protective and therapeutic effects against cancers of the blood, skin, oral cavity, lung, pancreas, and intestinal tract, and to suppress angiogenesis and metastasis in rodents. Curcumin's ability to affect gene transcription and to induce apoptosis in preclinical models is likely to be of particular relevance to cancer chemoprevention and chemotherapy in patients [10]

Ginger is rich with many active components. Gingerols and shogaols are responsible for the medicinal properties of ginger. The 6-gingerol, a major pungent ingredient of ginger is a potent anti angiogenic activity in vitro and in vivo.



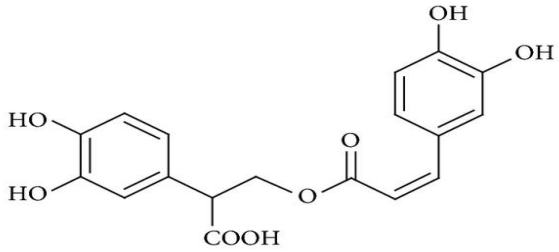

6 gingerol

And 6-gingerol may inhibit tumor growth and metastasis via its anti-angiogenic activity. The proposed mechanisms of action of gingerol involved in anticancer and chemo preventive properties via multiple pathways that includes the inhibition of cyclooxygenase -2 (COX-2) expression by inhibiting p38 MAPK–NF-κB (mitogen activated protein kinase – necrosis factor kappa B) signaling pathway.[11] Ginger is a natural antioxidant and anti carcinogenic dietary component. The treatment with ginger on ovarian cancer cells in vitro revealed that inhibition in growth of cells effectively by 6- shogaol and also inhibition of NF- kB activation and decreases VEGF (growth factor) and IL-8 secretion. Ginger components modulate secretion of angiogenic factors in ovarian cancer cells in vitro and act as potent chemopreventive dietary agent. [12]

Garlic contains several potentially important agents that possess anti carcinogenic and antitumor activities. The data obtained from the epidemiological, clinical, and laboratory investigations suggested that garlic contained many biologically and pharmacologically important compounds, which are beneficial to human health from cardiovascular, neoplastic, and several other diseases including cancer. Naturally occurring sulfur-containing compounds (OSCs), especially garlic compounds (GCs) and isothiocyanates (ITCs) are the two essential, effective and promising potent chemo preventive compounds induce apoptosis in multiple cell lines and experimental animals. [13] The multiple mechanisms involved in anti carcinogenic effects of garlic and its organosulfur compounds (OSCs) that include suppression of cell proliferation by blocking cell cycle progression and/or inducing apoptosis, inhibition of DNA adduct formation, modulation of carcinogen metabolism, up regulation of antioxidant defence and DNA repair systems. The dietary agents such as garlic with its rich array of bioactive OSCs will modulate cancer cascades and acts as potential chemo preventive and chemotherapeutic agents. Organo sulphur compounds of garlic inhibit carcinogen activation, enhance phase 2 detoxifying processes, cause arrest at G2/M phase of cell cycle, induce mitochondrial apoptotic pathway and increase acetylation of histones, like modulation of cellular redox state, influence gap-junctional intercellular communication and participate in the development of

multidrug resistance , involvement in signal transduction and post-translational modification.[14] The mode of action of or the basic mechanisms involved in control of breast cancer include the suppression of DNA adduct formation, the activation of metabolizing enzymes that detoxify carcinogens, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis.[15]The derivatives of garlic contain alk(en)yl sulfides with different numbers of sulfur atom (i.e., mono-, di-, and trisulfide). The cell growth was significantly suppressed by diallyl trisulfide, which is a major constituent of the garlic oil, but not by diallyl monosulfide and diallyl disulfide. The cell cycle was arrested at G2/M phase, the cells with sub-G1 DNA content, and the cells with caspase-3 activity were significantly increased by diallyl trisulfide treatment. The anticancer effect of diallyl trisulfide was examined using human colon cancer cells HCT-15 and DLD-1 and observed that disrupted microtubule network formation of the cells.[16]

Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid commonly found in plants belonging to the Boraginaceae and the subfamily Nepetoideae of the Lamiaceae family.




Rosmarinic acid

The compound has a number of important biological activities, e.g. antiviral, antibacterial, anti inflammatory, anticancer and antioxidant.[17] In colon cancer HT-29 cells, rosmarinic acid (RA) at reduced the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced (cyclooxygenase-2) COX-2 promoter activity and protein levels. RA also reduced TPA induced transcription from a control activator protein-1 (AP-1) promoter-luciferase construct and repressed binding of the AP-1 factors, c-Jun and c-Fos to COX-2 promoter oligonucleotides harboring a cAMP-response element (CRE). RA also antagonized the activation of the extracellular signal-regulated protein kinase-1/2 (ERK1/2). Thus the anticancer effect of RA may be due to its ability to inhibit COX-2 activation by AP-1 inducing agent.[18] In human breast cancer MCF7 cell line, Rosmarinic acid reportedly inhibited DNA methyltransferase activity.[19] In human leukemia U937 cells, RA reportedly sensitized TNF-alpha-induced apoptosis through the suppression of tumor necrosis factor-alpha (TNF-alpha)-induced nuclear transcription factor-kappaB (NF-kappaB) activation and reactive oxygen species (ROS) generation. Activation of caspases was also noted following rosmarinic acid treatment. Rosmarinic acid suppressed NF-kappa B activation through inhibition of phosphorylation and degradation of Ikappa, Balpha, and nuclear translocation of p50 and p65. This was correlated with suppression of NF-kappaB-dependent anti-apoptotic proteins (IAP-1, IAP-2, and XIAP).

Ursolic acid form sage effectively inhibits angiogenesis, invasion of tumor cells and metastasis and suppresses the lung colonization of B16 melanoma cells in vivo. Salvia officinalis water extract also showed a significant decrease in the induced oxidative damaged cells in rats and seems to have the ability to prevent colorectal cancer cell growth .Salvia officinalis also demonstrated a strong cytotoxic activity of the extract on human prostate carcinoma cells.[20,21,22]

The aqueous extract and the fraction of cinnamon (procyanidins) inhibit vascular endothelial growth factor subtype 2 (VEGFR2) kinase activity, thereby inhibiting the angiogenesis involved in cancer.[23]

Fenugreek seed extract significantly inhibited 7,12-dimethylbenz(a)anthracene-induced mammary hyperplasia and reduces its incidence in rats and advised that the anti-breast cancer protective effects of fenugreek could be due to increased apoptosis. Further, alcoholic whole plant extracts of fenugreek showed in vitro cytotoxicity

against different human cancer cell lines such as IMR-32, a neuroblastoma cell line, and HT29, a cancer cell line.[24] Treatment with fenugreek extract showed growth inhibitory effects on breast, pancreatic and prostate cancer cell lines but primary prostate or immortalized prostate cells remained unaffected. Inhibition of cancer cell growth by Trigonella is attributed to its ability to induce death of cell, despite simultaneous up regulation of growth stimulatory pathways in normal cells[25] The ethanol extract of trigonella in Ehrlich Ascites Carcinoma cells induced cancer in Swiss albino mice. The mice inoculated with Ehrlich Ascites Carcinoma and treated with trigonella leaf extract showed increased life span in comparison with the tumor control, owing to the anticancer activity of fenugreek leaf extract in animal models.[26] A diet fed with fenugreek seed powder decreased colon tumor incidence and hepatic lipid peroxidation in 1,2-dimethylhydrazine treated rats and also increased activities of catalase, superoxide dismutase, glutathione S-transferase and glutathione peroxidase in liver showed that diosgenin could modulate the STAT3 signaling pathway in hepatocellular carcinoma by suppressing the activation of c-Src, JAK1 and JAK2. Diosgenin also showed down regulation of the expression of various STAT3-regulated genes, inhibited proliferation and potentiated the apoptotic effects of paclitaxel and doxorubicin, suggesting that diosgenin could be a novel and potential treatment option for hepatocellular carcinoma and other cancers.[27]
The anticancer properties of allspice may attributed to its ability to influence cytochrome P450 (CYP) activity and thereby influence carcinogen bioactivation. [28] Evidence exists that allspice can alter the proliferation of several cultured cancerous cells. While cell viability was reduced about 50% when allspice extract was added to prostate cancer cells (LNCaP cells), it did not influence the viability of cultured human prostate cancer cell lines (DU145) or cervical epithelial carcinoma (HeLa) cells.[29]
Kluth et al. (2007) examined the influence of several spice extracts on phase I and II enzymes in cultured human liver carcinoma and human colon adeno carcinoma cells, and they suggested a shift in the nuclear transcription factor Nrf2 was responsible for the induction. Clove extracts might interfere with β-catenin activity and thereby decrease colon carcinogenesis.[30]

The anticancer properties of basil can be attributed to its ability to influence viral infections. Individuals with hepatitis B are found to be at increased risk for hepatocellular carcinoma studies also evaluated the antiviral activities of basil extract and selected basil constituents in a human skin basal cell carcinoma cell line (BCC-1/ KMC) and a cell line derived from hepatoblastoma HepG2 cells (2.2.15) against several viruses, including hepatitis B[31]

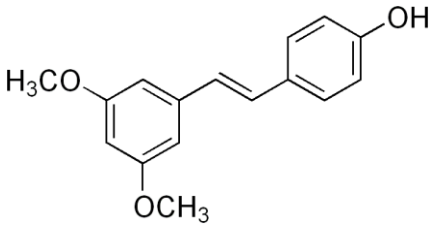
Significant information points to the ability of saffron to inhibit cancer. Aqueous saffron extract have been reported to inhibit chemically induced skin carcinogenesis. Both changes in carcinogen bio-activation and tumor proliferation were established. Saffron infusion given orally either before or after DMBA treatment increased GST, GPx, catalase, and superoxide dismutase in liver[32]

Anthocyanins are the most abundant flavonoid constituents of fruits and vegetables. Anthocyanins have been shown to exhibit anti-carcinogenic activity against multiple cancer cell types *in vitro* and tumor types *in vivo.* Potential cancer chemopreventive activities of anthocyanins revealed from *in vitro* studies include radical scavenging activity, stimulation of phase II detoxifying enzymes, reduced cell proliferation, inflammation, angiogenesis and invasiveness, and induction of apoptosis and differentiation. The anthocyanins modulate the expression and activation of multiple genes associated with these cellular functions including genes involved in the PI3K/Akt, ERK, JNK, and MAPK pathways. *In vivo* studies have proven that anthocyanins can inhibit cancers of the gastrointestinal (G.I.) tract and topically applied anthocyanins inhibit skin cancer.[33]

Pomegranate possesses inhibitory effects on different type of cancers such as prostate, breast, colon, and lung cancers . Different mechanisms have been outlined for pomegranates anti-cancer activities. Pomegranate peel

extract inhibits NF-kB and cell viability of prostate cancer cell lines in a dose-dependent manner .Pomegranate polyphenols, ellagitannins-rich extract and whole juice extract inhibited gene expression of HSD3B2 (3beta-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-ketoreductase family 1 member C3) and SRD5A1 (steroid 5alpha reductase type 1), which are key androgen-synthesizing enzymes in LNCaP, LNCaP-AR, and DU-145 human prostate cancer cells. Since Pg inhibits CYP activity/expression which is necessary for activation of procarcinogens, it may have anti-carcinogenesis effects. Some metabolites of pomegranates chemical components such as 3,8-dihydroxy-6H-dibenzo[b,d]pyran-6-one (urolithin A, UA) which is produced from Ellagitannins (ETs) may also possess anti-cancer effects .[34]

Natural stilbenes are an important group of non flavonoid phytochemicals of poly phenolic structure characterized by the presence of a 1,2-diphenylethylene nucleus. Stilbenes have an strong potential for the prevention and treatment of variety of diseases, which include cancer, due to their antioxidant, cell death activation, and anti-inflammatory properties which associate with low toxicity under in vivo conditions . There are more than 400 more natural stilbenes available. But the area of focus mainly lies in resveratrol and ptero stilbene, both possess high anti oxidant properties. The chemo preventive role of resveratrol have been associated with its antioxidant activity since it was first published that its anticancer activity, affecting all steps in the carcinogenesis process, was linked to the inhibition of cyclooxygenase 2 (COX-2) .[35] Pterostilbene (3,5-dimethoxy-4′-hydroxystilbene) is a natural analog of resveratrol, but with higher bioavailability.




Pterostilbene

Pterostilbene was shown to inhibit growth, adhesion, and metastatic growth and to be an active apoptotic agent. These effects have been shown in different types of cancers such as breast cancer , lung cancer, stomach cancer , prostate cancer , pancreatic cancer , melanoma , or colon carcinoma. Stilbene may also induce cell death, also, by autophagy owing to the anti cancer property.[36]

**References:**

1. Kondratyuk, T.P.; Pezzuto, J.M.J.P. Natural product polyphenols of relevance to human health. Pharm. Biol. 2004, 42, 46–63.
2. Spencer, J.P.; Abd, E.M.M.; Minihane, A.M.; Mathers, J.C. Biomarkers of the intake of dietary polyphenols: Strengths, limitations and application in nutrition research. Br. J. Nutr. 2008, 99, 12–22. 5.
3. Kuhnau, J. The flavonoids. A class of semi-essential food components: Their role in human nutrition. World Rev. Nutr. Diet. 1976, 24, 91–117.
4. Johnson, I.T.; Williamson, G.; Musk, S.R. Anticarcinogenic factors in plant foods: A new class of nutrients. Nutr. Res. Rev. 1994, 7, 175–204
5. Mantena, S.K.; Baliga, M.S.; Katiyar, S.K. Grape seed proanthocyanidins induce apoptosis and inhibit metastasis of highly metastatic breast carcinoma cells. Carcinogenesis 2006, 27, 1682–1691.
6. Stewart, J.R.; Artime, M.C.; O'Brian, C.A. Resveratrol: A candidate nutritional substance for prostate cancer prevention. J. Nutr. 2003, 133, 2440S–2443S.
7. Aggarwal S, Takada Y, Singh S, Myers JN, Aggarwal BB. Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kB signaling. Int J Cancer. 2004;111:679–692. doi: 10.1002/ijc.20333.

8. Oda Y. Inhibitory effect of curcumin on SOS functions induced by UV irradiation. Mutat Res.1995;348:67–73. doi: 10.1016/0165-7992(95)00048-8
9. Sa G, Das T (2008) Anti cancer effects of curcumin: cycle of life and death. Cell Div 3: 14
10. The Potential Role of Curcumin in Cancer Prevention and Treatment. A Pramela Rani1, and V Saikishore
11. Shukla, Y.& Pal, S.K. (2004). Dietary cancer chemoprevention: An overview. Int .J. Hum Genet.4: 265–276
12. Rhode, J., Fogoros, S., Zick, S., Wahl, H., Griffith, K.A., Huang, J. & Liu, J.R. (2007). Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC Compl Altern Shukla, Y., Pal, S.K. (2004). Dietary cancer chemoprevention
13. A. Herman-Antosiewicz, A.A. Powolny & S.V. Singh., Acta Pharmacol Sin., 2007, 28(9),1355.
14. . S. Melino, R. Sabelli & M. Paci., Amino Acids., 2011. 41(1),103
15. A. Tsubura, Y.C. Lai, M. Kuwata, N.Uehara & K. Yoshizawa., Anticancer Agents Med Chem. 2011, 11 (3), 249.
16. T. Seki, T. Hosono, T. Hosono-Fukao, K. Inada, R.Tanaka, J. Ogihara & T.Ariga., Asia Pac J Clin Nutr., 2008, 17(1), 249.
17. Petersen M, Simmonds MS: Rosmarinic acid. Phytochemistry, 2003; 62(2): 121-5.
18. Scheckel KA, Degner SC, Romagnolo DF: Rosmarinic acid antagonizes activator protein- 1-dependent activation of cyclooxygenase-2 expression in human cancer and nonmalignant cell lines. J Nutr, 2008; 138(11): 2098-105.
19. Paluszczak J, Krajka-Kuźniak V, Baer-Dubowska W: The effect of dietary polyphenols on the epigenetic regulation of gene expression in MCF7 breast cancer cells. Toxicol Lett, 2010; 192(2): 119-25
20. Jedinák A, Mucková M, Kost'álová D, Maliar T, Masterova I (2006) Antiprotease and antimetastatic activity of ursolic acid isolated from Salvia officinalis. Z Naturforsch C 61: 777-782.
21. Pedro D, Ramos A, Lima C, Baltazar F, Pereira-Wilson C (2010) Modulation of DNA damage prevention and signaling pathways in diet induced colon cancer prevention. BMC Proceedings 4: 53.
22. Loizzo MR, Tundis R, Menichini F, Saab AM, Statti GA, et al. (2007) Cytotoxic activity of essential oils from labiatae and lauraceae families against in vitro human tumor models. Anticancer Res 27: 3293-3299.
23. J. Lu, K. Zhang, S. Nam, R. A. Anderson, R. Jove, and W. Wen, "Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling," Carcinogenesis, vol. 31, no. 3, pp. 481–488, 2010
24. In vitro cytotoxicity of Calotropis procera and *Trigonella foenum-graecum* against human cancer cell lines J. Chem. Pharm. Res., 2 (2010), pp. 861–865
25. Fenugreek: a naturally occurring edible spice as an anticancer agent Cancer Biol. Ther., 8 (2009), pp. 272–278
26. Anticancer activity of *Trigonella foenum-graecum* on Ehrlich Ascites carcinoma in Mus musculus system J. Pharm. Res., 3 (2010), pp. 1181–1183
27. Phytochemical protection against diethylnitrosoamine induced hepatocarcinogenesis by *Trigonella foenum graecum* in female rats Arab J. Nucl. Sci. Appl., 45 (2) (2012), pp. 523–536
28. Kluth D, Banning A, Paur I, Blomhoff R, Brigelius-Flohe R. Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic Biol Med. 2007;42:315–25
29. Lee Y.H, Hong S.W, Jun W, editors. Anti-histone acetyltransferase activity from allspice extracts inhibits androgen receptor-dependent prostate cancer cell growth. Biosci Biotechnol Biochem. 2007;71:2712–9.
30. Kluth D, Banning A, Paur I, Blomhoff R, Brigelius-Flohe R. Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds. Free Radic Biol Med. 2007;42:315–25
31. Fung J, Lai C.L, Yuen M.F. Hepatitis B and C virus-related carcinogenesis. Clin Microbiol Infect. 2009;15:964–70
32. Das I, Das S, Saha T. Saffron suppresses oxidative stress in DMBA-induced skin carcinoma: A histopathological study. Acta Histochem. 2010;112:317–27.
33. Anthocyanins and their role in cancer prevention -Li-Shu Wang and Gary D. Stoner,
34. Comprehensive Review of Punica granatum (Pomegranate) Properties in Toxicological, Pharmacological, Cellular and Molecular Biology Researches
35. M. Jang, L. Cai, G. O. Udeani et al., "Cancer chemo preventive activity of resveratrol, a natural product derived from grapes," Science, vol. 275, no. 5297, pp. 218–220, 1997
36. Y. Wang, L. Ding, X. Wang et al., "Pterostilbene simultaneously induces apoptosis, cell cycle arrest and cyto-protective autophagy in breast cancer cells," American Journal of Translational Research, vol. 4, no. 1, pp. 44–51, 2012