CHAPTER – II

REVIEW OF LITERATURE

2. AA PATHWAY INHIBITORY NATURAL PRODUCTS AS ANTICANCER AND ANTI-INFLAMMATORY AGENTS

Natural constituents are widely distributed in various natural sources including plants and microorganisms. Research on natural products has been gained importance in drug discovery field due to their safety profile in toxic studies (Kim et al., 2009, Pan et al., 2009, Samadi et al., 2015, Geldenhuys et al., 2012). Hence, researchers are considering natural poducts as alternatives to synthetic drugs. For instance, NSAIDs are synthetic molecules exhibit cancer chemopreventive potentials. However, several side effects associated with usage of NSAIDs hampered their clinical applications (Mau et al., 2013, Bjarnason et al., 1993). In this scenario, several AA pathway inhibitory natural agents have been identified as cancer chemopreventive and therapeutic agents. Apigenin , anthocyans, baicalein , berberine, curcumin, diallyl sulfide, ellagic acid, epigallocatechin gallate, eugenol, fisetin, garcinol, genistein, (6)-gingerol, guggulsterone, indole-3-carbinol, lycopene, nordihydroguaiaretic acid, piperine, quercetin, resveratrol, silibinin, sulforaphane, thymoquinone, triptolide, ursolic acid and wogonin are some of the important natural products that are isolated from various natural sources (most of them are derived from plants), which have been reported to possess cancer chemopreventive and therapeutic potential by targeting AA pathway.

2.1. Apigenin

Apigenin, a flavanoid widely distributed in plants, is known to possess anticancer and cancer preventive properties (Figure 2.1). Various studies have been carried out to establish the mechanism of action of apigenin underlying it`s chemopreventive and anticancer effect. Apigenin showed chemopreventive activity against UV- or chemically-induced skin cancer. Van Dross et al. (2007) carried out several experiments to explore the mechanism of apigenin's chemopreventive activity and they

found that apigenin suppressed the UVB-induced increase in COX-2 protein and mRNA in mouse and human keratinocyte cell lines. Tong et al. (2014) reported that apigenin inhibited UVB-induced cutaneous proliferation and angiogenesis mediated by HuR, thrombospondin-1 and COX-2. Byun et al (2013) found that apigenin exerts potent chemopreventive activity against UVB-induced skin inflammation by targeting Src and COX-2. Tong et al., (2007) reported that apigenin inhibited COX-2 expression in 308 keratinocytes. The studies suggested that COX-2 inhibition activity of apigenin might be one of the mechanisms that were involved in its preventive effect of UVB-induced carcinogenesis.

Apigenin down-regulated PMA--induced COX-2 expression in breast cells (Yi Lau and Leung, 2010). Apigenin treatment inhibited phosphorylation of ERK-1/2. Apigenin blocked ERKs triggered transactivation of AP-1 or CRE, which can be located at COX-2 promoter region (-72/-53). Reporter gene assay as well as electrophoretic mobility shift assays (EMSA) illustrated that apigenin inhibited transcription factor binding at COX-2 promoter region in a dose-dependent manner that led to suppression of COX-2 expression. The study proved that COX down-regulation is key mechanism involved in antiproliferative effect of apigenin in pro-tumorigenic breast cell lines (Yi Lau and Leung, 2010).

Wang and Huang (2013) reported that apigenin inhibited *Helicobacter pylori*-induced inflammation and carcinogenesis in MKN45 gastric cancer cell line (Table 1). Apigenin treatment caused increase in the IκBα expression, and inhibition of NF-κB activation as well as COX-2, ICAM-1 and other pro-inflammatory cytokines expressions in MKN45 cells. The study concluded that apigenin had anticacinogenic effect on the *H. pylori*-infected gastric adenocarcinoma cells and can be potential chemopreventive agent for gastric cancer (Wang and Huang, 2013).

In other studies, apigenin inhibited the growth pancreatic cancer cells *in vitro* and *in vivo* by IKK-β-mediated NF-κB activation (Wu et al., 2014). Apigenin inhibited NF-κB activation and suppressed expression of NF-κB-responsive genes in human prostate carcinoma PC-3 cells (Shukla and Gupta, 2004). Apigenin suppressed inducible COX-2 expression in human colon epithelial cells and inhibited PGE_2 levels in colon cancer cells HCA-7 cells and human colon epithelial cells (Al-Fayez et al.

2006). Epidemiologic studies suggested that apigenin is related to a decreased risk of certain cancers particularly cancers of the breast, digestive tract, pancreatic, skin, prostate, colon (Shukla and Gupta, 2010). Apigenin prevented DMBA-induced hamster buccal pouch carcinogenesis by modulating the expression pattern of inflammatory (NF-κB and COX-2), cell proliferative (Cyclin D1), apoptotic (p53, Bcl-2, Bax, Caspase-3 and 9) and angiogenic (VEGF) markers. Recently, Shukla et al. (2015) found that apigenin Inhibited prostate tumorigenesis, tumor growth and metastasis in TRAMP mice, which correlates with inhibition of NF-κB activity. Further studies demonstrated that apigenin downregulated the expressions of various cell survival mediators including COX-2. Several studies proved that targeting NF-κB mediated COX expression may be one of the underling mechanisms for cancer preventive effect of apigenin.

Apigenin is non-toxic and antigenotoxic in nature (Liu et al., 2015, Hashemi et al., 2010, Siddique et al., 2010). Apigenin exhibited protective effect against chemically-induced hepatotoxicity in rats (Ali et al. 2014). However, oral bioavailability of apigenin is relatively low because of its poor lipid and water solubility, which has restricted its clinical development (Ding et al. 2014). Interestingly, solid dispersions of apigenin with carbon nanopowder were enhaced bioavailablity of apigenin in rats (Ding et al. 2014). The results obtained from previous studies warranted clinical trials for examining the effect of apigenin on cancer prevention.

2.2. Anthocyans

Anthocyans i.e. anthocyanins (cyanidin-3-glucoside, cyanidin-3-galactoside, delphinidin-3-galactoside and pelargonidin-3-galactoside) and anthocyanidins (cyanidin, delphinidin, pelargonidin, peonidin and malvidin) are most abundant flavonoid constituents of many fruits and vegetables (Seeram et al., 2003) (Figure 2.2). Anthocyanidins, the aglycones of anthocyanins, are responsible for various colors of the many types of fruits and vegetables (Zafra-Stone et al., 2007). The epidemiological evidences and results of *in vitro* and *in vivo* studies demonstrated the chemopreventive potentials of anthocyans or anthocyan-containing fruit or vegetable extracts against various cancers (Seeram et al., 2003; Cooke et al. 2005; Bishayee et al. 2015). Molecular studies have been carried out by several research groups to establish the molecular mechanism of anthocyans for anticancer and chemopreventive activities.

Cyanidin-3-glucoside (C3G), an anthocyanidin, is distributed in various vegetables and fruits especially in edible berries, and showed anticancer and chemopreventive properties (Table 2.1) (Pratheeshkumar et al., 2014). C3G significantly decreased the production of UVB-induced pro-inflammatory cytokines, inhibited activation of MAPKs, ERK 1/2, JNK1/2, p38, and MKK4. C3G also suppressed UVB-induced COX-2, PGE_2 and inducible nitric oxide synthase **(**iNOS) levels in mice with UVB-induced skin cancer. The study concluded that C3G inhibited UVB-induced skin carcinogenesis by inhibiting various inflammatory mediators including COX-2 (Pratheeshkumar et al., 2014). In another study, C3G suppressed carcinogen-induced COX-2 expression in mouse epidermal cells (Lim et al. 2011). Further in detailed studies demonstrated that C3G showed chemopreventive effect by targeting carcinogen-induced Fyn kinase/MAPKs/AP-1/NF-κB/ COX-2 pathway in mouse epidermal cells (Lim et al. 2011). Kim et al. (2010) reported that cyanidin inhibited UVB-induced carcinogenesis in epidermal skin cells by targeting MKK4/MEK1/Raf-1/NF-κB/AP-1/COX-2/PGE_2 pathway. Cyanidin is known to possess anticancer activities against prostate cancer. In a study, cyanidin inhibited PGE_2, COX-2 and NF-κB in LNCaP prostate cancer cells (Muñoz and Watkins, 2006). Cyanidin might prevent the binding of NF-κB on COX-2 gene promoter in LNCaP prostate cancer cells. This may be the underlying molecular mechanism behind chemopreventive activity of cyanidin against prostate cancer (Muñoz and Watkins, 2006).

Delphinidin, a dietary anthocyanidin, inhibited UVB-induced carcinogenesis in JB6 P+ mouse epidermal cells by inhibition of MAPKK4/AP-1/NF-κB/COX-2/PGE_2 pathway (Kwon et al. 2009). Kang et al. (2008) reported that delphinidin inhibited tumor promoter TPA-indced neoplastic transformation in mouse epidermal cells due to its inhibition activities of Raf1, MEK and COX-2. Peonidin is another dietary anthocyanidin, which has shown chemopreventive activity against various cancers. Peonidin inhibited induced COX-2 expression, and also inhibited TPA-induced neoplastic transformation mouse epidermal cells (Kwon et al., 2007).

Zikri et al (2009) reported that black raspberries extract and its anthacyanins inhibited the tumorigenesis of carcinogen-induced cancer in the rat esophagus by inhibiting COX-2. The studies denoted that anthocyanins of BRB exerted anticancer and chemopreventive effect though inhibition of COX-2 and other inflammatory mediators. Protocatechuic acid (PCA) and is one of the constituents of black

raspberries inhibited carcinogenesis of N-nitrosomethylbenzylamine (NMBA)-induced esophageal cancer in rats (Peiffer et al. 2014). Further mechanistic studies demonstrated that PCA and other anthocyanins constituents of black raspberries suppressed expression of COX-2 and iNOS genes, and inhibited NF-κB activation in chemically-induced esophageal cancer in rats (**195**) (Peiffer et al. 2014). PCA can be an ideal chemopreventive molecule because it is stable and less toxic (Bishayee et al., 2015).

Anthocyanins of soybean inhibited inflammation in *H. pylori* infected human gastric epithelial cells (Kim et al., 2013). Further studies demonstrated that anthocyanins excerted antiinflammation activity through inhibition of MAPK/IκBα/NF-κB/COX-2 pathway. The study demonstrated that anthocyanins of soybean can be chemopreventive agents against *H. pylori* associated infections including gastric cancer (Kim et al. 2013). Blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry extracts have shown anti-proliferative effect against human colon (HT-29, HCT116), oral (KB, CAL-27), breast (MCF-7), and prostate (LNCaP) cancer cells (Seeram et al., 2006). Further studies demonstrated that berries extract contained various polyphenols including anthocyanins. Moreover, the study demonstrated that COX-2 inhibitory activities of polyphenols of berry extract may be responsible for their cancer chemopreventive activity (Seeram et al., 2006). Anthocyanin containing bilberry and chokeberry extracts substantially inhibited aberrant crypt foci (ACF) formation induced by AOM in rats (Lala et al., 2006). Anthocyanins containing bilberry extract downregulated *COX-2* gene expression in rats with colon cancer (Lala et al., 2006). Previously, we have reported that black currant skin extract (BCSE) containing high amounts of anthocyanins inhibited diethylnitrosamine (DENA)-initiated hepatocarcinogenesis in rats by targeting NF-κB/COX-2/iNOS pathway (Thoppil et al., 2012; Bishayee et al. 2013). Further toxicity studies demonstrated the safety profile of black currant skin extract against cardiotoxicity in rats. Chemopreventive effect of BCSE with a good safety profile encouraged the development of black currant anthocyanins as the chemopreventive agents for liver cancer (Bishayee et al. 2013).

The results of *in vitro* and *in vivo* studies warranted clinical studies of anthocyans to develop them as chemopreventive agents for many cancers. Several studies proved that anthocyans are less toxic with antioxidant potentials (Bishayee et al. 2015). In a

study, anti-genotoxic effects of anthocyanidins are reported (Rocco et al., 2015). As of today, the available data is not sufficient to conclude that anthocyans are less/non-toxic. Further, a detailed and long term toxicity studies on anthocyans are required. Poor bioavailability of anthocyans is a major challenge in development of anthocyans as cancer chemopreventive agents. Anthocyanins are less effective chemopreventive than anthocyanidins due to presence of glycone moieties (Sogo et al., 2015, Galvano et al., 2004). Anthocyanins are absorbed from the stomach as well as intestines and transferred to the kidney or liver. Further anthocyanins are metabolized into various metabolites and enter into blood. Anthocyanin metabolites are present in high concentration in the blood than their parent anthocyanins (Fang, 2014). Gut microflora degrades anthocyacnins and hampers the absorption process. This is one of the reasons for poor bioavailability of anthocyacnins (Fang, 2014). Some reports contradicted these findings and demonstrated that anthocyanin glycosides remained intact during absorption by intestine (Fang, 2014). Although anthocyans are offered substantial chemopreventive and therapeutic potential against various cancers, there is paucity of research because of contradicted reports on their bioavailability.

2.3. Baicalein

Baicalein (5,6,7-trihydroxyflavone), found in the roots of *Scutellaria baicalensis* and *Scutellaria lateriflora*, inhibits both 12-LOX and 15-LOX, but not 5-LOX (Table 2.1&Figure 2.3) (Kim et al., 2014). Baicalein has been reported to exhibit anticancer activities against pancreatic, prostate, lung, breast, and gastric cancers (Table 1.1). Baicalein arrested cell cycle and induced apoptosis in PC-3 and DU-145 prostate cancer cell lines (Pidgeon et al. 2002). Nie et al. (2006) reported that baicalein inhibited 12-LOX activity, which in turn blocks PI3K/Akt/PKCζ/Sp1/AP2 mediated VEGF expression in prostate cancer cells. There by, baicalein prevented prostate tumor angiogenesis and progression (Nie et al. 2006). Timár et al. (2000) found the preventive role of 12-LOX inhibitory activity of baicalein in metastatic process of prostate cancer. Baicalein inhibited spreading of 12-LOX transfected human prostate carcinoma PC-3 cells and prevented metastasis (Nie et al. 2003). Baicalein induced apoptosis in PANC-1, Capan2, MiaPaca2, and HPAF pancreatic cancer cells (Ding et al. 1999). Baicalein showed antiproliferative effect and induced apoptosis in pancreatic cancer cells *in vitro* and *in vivo* (Tong et al., 2002(a)). Baicalein downregulated Bcl-2, increased Bax, increased cytosolic cytochrome *c*, and activated

of caspase-9 but not caspase-8 in pancreatic cancer cells. These findings demonstrated that 12-LOX inhibitory baicalein induced apoptosis through mitochondria-mediated pathway (Tong et al., 2002(a)) (**211**). Baicalein suppressed 12-LOX gene expression in human lung nonsmall carcinoma H460 cells (Leung et al., 2007). Baicalein inhibited H460 cells proliferation, arrested cell cycle at S-phase and induced apoptosis. Taken together, the results of the studies demonstrated that baicalein exhibited anticancer activity against human lung nonsmall carcinoma by downregulating 12-LOX (Leung et al. 2007). Baicalein inhibited cell proliferation and induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cells through intrinsic pathway (Tong et al., 2002(b)). Mitogenic effect of a LOX product, 12-hydroxyeicosatetraenoic acid (12-HETE) in breast cancer cells was inhibited by baicalein. Overall results demonstrated that anticancer and pro-apoptotic potentials of baicalein against breast cancer were might be due to its 12-LOX inhibition activity (Tong et al. 2002(b)). Baicalein inhibited 12-HETE and 12-HETE-induced cell proliferation in human gastric cancer AGS cells (Chen et al. 2008). Baicalein inhibited 12-HETE-induced proliferation of and triggered induced apoptotic death in AGS cells by inhibiting the ERK1/2-PKC pathway (Chen et al. 2008). Blocking of the 12-LOX pathway by baicalein was associated with inhibition of gastric cancer cell proliferation and induction of apoptosis (Wong et al. 2001). *In vitro* and *in vivo* studies demonstrated that baicalin showed anticancer and apoptosis-inducing potentials in colon cancer (Kim et al. 2014, Kim et al. 2012). Baicalin modulated cell cycle progression and induced cell death in sodium butyrate-stimulated human colon adenocarcinoma HT-29 cells (Kovaríková et al., 2004). Baicalein inhibited cell proliferation and induced apoptosis in hepatocellular carcinoma *in vitro*, *in vivo*, which are associated with suppression of 12-LOX expression (Xu et al. 2012). Mechanistic studies demonstrated that baicalein reduced Bcl-2 protein expression, increased Bax, activated caspase-3 and ERK-1/2 in hepatocellular carcinoma cells. The effects of baicalein were reversed by 12-LOX activity (Xu et al. 2012). The study concluded that baicalein inhibited cell proliferation and apoptotic induction in hepatocellular carcinoma by inhibition of 12-LOX activity (Xu et al. 2012).

Bioavailability of baicalein is poor due to internal metabolization (Jie et al. 2006, Xing et al., 2005). Several strategies have been developed to enhance the bioavailability of baicalein (Yu et al. 2012, Liang et al., 2013). Encapsulation of baicalein with long-

circulating nanoliposomes enhanced oral bioavailability of baicalein in mice (**222**) (Liang et al., 2013). Oral bioavailability and solubility of baicalein-nicotinamide cocrystal is greater than free baicalein (**223**) (Huang et al. 2014). Baicalein is not mutagenic and genotoxic and showed safety profile in preclinical and clinical toxicity studies (Fox et al., 2012, Ueng et al. 2001, Li et al. 2014). Baicalein showed protective effect against benzo(a)pyrene- and aflatoxin B_1-induced genotoxicities (Ueng et al. 2001). In a Phase I, randomized, double-blind, single-dose trial, baicalein is safe with no signs of toxicity at oral doses of 100-2800 mg in healthy humans (Li et al. 2014). Baicalein showed good safety profile and better pharmacokinetic properties in humans. Several studies demonstrated that baicalein did not show any serious adverse effects. Over all studies demonstrated that baicalein exterts cancer preventive and therapeutic potentials by targeting 12-LOX. Preclinical studies exhibited encouraging results that warranted further studies for clinical practice of baicalein.

2.4. Berberine

Berberine (an isoquinoline alkaloid), found in *Coptis chinensis* and many other plants, has been reported to possess various bioactivities including anticancer activity and is under clinical trials for several diseases (Liu et al. 2015). Berberine exhibited anticancer and chemopreventive effects on intestine, oral, breast and skin cancers by targeting multiple pathways including AA pathway (Table 1). Berberine inhibited growth of hepatocellular carcinoma *in vitro* and *in vivo*, suppressed $cPLA_2$ and COX-2 gene expressions, and elevated the ratio of AA to PGE_2. The study clearly demonstrated that berberine showed anticancer and chemopreventive effects by inhibiting AA pathway (Li et al. 2015). Berberine prevented *in vitro* and *in vivo* growth as well as migration and invasion of colorectal cancer cells by targeting COX-2/PGE_2-JAK2 and STAT3-MMP-2/-9 signaling pathways (Liu et al. 2015). Li et al., (2015(**b**)) reported that berberine prevented colon carcinogenesis by inhibiting AMP-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) and NF-κB/COX pathways. Chidambara et al. (2012) found that berberine has the ability to cause cell cycle arrest, induce apoptosis and inhibit inflammation in colon cancer cells by targeting multiple pathways including NF-κB/COX-2 pathway. Berberine inhibited proliferation of colon cancer cells *in vitro* and COX-2 expression at mRNA and protein levels in HT-29 human colon cell line (Tai and Luo, 2003). Down-regulation of COX-2 expression by berberine might be one of the mechanisms for

its antiproliferative effect on HT-29 human colon cell line (Tai and Luo, 2003). Berberine inhibited COX-2 transcriptional activity in colon cancer cells *in vitro* (Fukuda et al., 1999). Bererine prevented chemically-induced tumorigenesis of colon cancer in rats by down-regulating COX-2 expression (Wu et al. 2010). Berberine inhibited development of intestinal tumorigenesis in Apcmin/+ mice (Cao et al., 2013). Ancillary molecular studies demonstrated that suppression of Wnt and EGFR signaling pathways and COX-2 expression by berberine may be underlying mechanism involved in its cancer preventive activity (Cao et al., 2013). Manoharan et al. (2011) found that berberine prevented DMBA-induced oral carcinogenesis *in vivo* by targeting multiple pathways including NF-κB/COX-2. Kuo et al (2005) demonstrated that berberine-induced apoptosis in oral cancer cells mediated through inhibition of COX-2/Akt pathway. Berberine down-regulated COX-2 expression decreased PGE_2 levels and hampered binding activity of transcriptional factor AP-1 in oral cancer OC2 and KB cells. Taken together, berberine inhibited COX-2 expression by preventing the binding of AP-1 on COX-2 gene followed by reduction of PGE_2 levels (Kuo et al. 2004). Berberine inhibited melanoma cell migration by inhibiting expression of COX-2, PGE_2 and PGE_2 receptors. There by, berberine prevented invasion and metastasis of melanoma (Singh et al. 2011). Berberine blocked metastasis of melanoma cells by activating AMPK and inhibition of ERK signaling pathway and COX-2 expression (Kim et al. 2012). Berberine exhibited antiangiogenic activity that was mainly mediated through the inhibition of various proinflammatory and pro-angiogenic mediators including VEGF, COX-2, NO, NF-κB and HIF (Hamsa and Kuttan, 2012). Berberine exhibited anticancer and preventive effect on breast cancer *in vitro* and *in vivo* by its antiproliferative, pro-apoptotic, and antiangiogenic potentials (Barzegar et al., 2015, Pazhang et al 2012). Pazhang et al (2012) reported that the apoptotic effect of berberine may be mediated by reduction of the COX-2 and survivin human ductal breast epithelial tumor cells.

Oral bioavailability and solubility of berberine is low. Hence, various nanoparticulate delivery systems have been developed to enhance the bioavailability of berberine and its anticancer potential (Tan et al., 2011). Recently Guamán et al., (2015) synthesized new berberine derivatives with improved anticancer potency and bioavailability. Aforementioned studies suggest that berberine can be novel chemopreventive agent for several cancers based on future studies.

2.5. Curcumin

Curcumin is a constituent of turmeric, a powdered rhizome of *Curcuma longa*, which has been used for many centuries as part of diet or a flavoring or a coloring agent as well as home remedy for various diseases. Turmeric is widely used in Ayurveda, Unani, and Siddha medicines (Anand et al. 2008). From last few decades, curcumin has been reported to possess chemopreventive and therapeutic potentials against several cancers, including cances of such as skin, lung , pancreas, , liver, thyroid, oral cavilty, breast, intestine, cervix and blood, by targeting multiple pathways including AA pathway (Lev-Ari et al., 2006, Dhillon et al., 2008, Li et al. 2004, Lev-Ari et al., 2014, Shishodia et al., 2003, Labbozzetta et al., 2009, Lee et al., 2009, Sharma et al., 2004, Su et al., 2006, Goel et al., 2001, Binion et al., 2008, Xu et al., 2014, Yoysungnoen-Chintana et al., 2014, Divya and Pillai, 2006, Prakobwong et al., 2011, Yoysungnoen et al., 2006, Anto et al., 20002, Saroj et al., 2007, Sharma et al., 2006, Marín et al., 2007, Darvesh et al., 2012). Several clinical trials on curcumin are in progress for several types of cancers (Sharma et al., 2004, Saroj et al., 2007).

Several studies demonstrated that curcumin inhibits AA-mediated $cPLA_2$, COX-2 and 5-LOX pathways. Curcumin selectively inhibits COX-2 expression and/or catalytic activity than COX-1 and reduced production of PGs such as PGE_2, PGD_2, $PGF_{2\alpha}$, and $TXAB_2$ as well as suppresses EP4 receptor (Rao et al., 1993, Hann et al., 2013). Hong et al. (2004) found that curcumin inhibited AA metabolism by blocking the phosphorylation of $cPLA_2$, downregulating COX-2 expression and inhibiting the 5-LOX catalytic activity. Curcumin inhibits production of LTs and 5(S)-, 8(S)-, 12(S)-, and 15(S)-HETE (Huang et al., 1991) In another study, curcumin was found that soybean LOX L1 catalyzed the oxygenation of curcumin and that curcumin can act as a LOX substrate (Schneider et al., 1998). In X-ray crystallographic, mass spectroscopic and molecular docking studies performed by Skrzypczak-Jankun et al., (2000), it was demonstrated that 4- hydroxyperoxy-2-metoxyphenol, a degradation product of curcumin was located near the soybean LOX catalytic site (Skrzypczak-Jankun et al., 2000). These studies elucidated the mechanism of inhibition of LOX catalytic activity by the curcumin.

Curcumin prevented or inhibited pro-inflammatory cytokines, LPS, carcinogens and UV-radiation mediated inflammation and carcinogenensis by targeting AA pathway.

Curcumin inhibited TNF-α-induced $cPLA_2$ expression by targeting MAPK/NF-κB/p300 mediated pathway (Lee et al., 2011). Curcumin inhibited IFN-α-induced activations of NF-κB and COX-2 in A549 lung cancer cells (Lee et al. 2005). Curcumin downregulated expressions of LPS-induced COX-2 and other proinflammatory mediators by inhibiting MAPK/NF-κB/AP-1 pathway (Guimarães et al., 2013, Kang et al., 2004, Shah et al., 2010). Curcumin inhibited cigarette smoke extract-induced $cPLA_2$ expression by targeting MAPK/NF-κB/AP-1 mediated pathway (Cheng et al., 2009). Curcumin downregulated smokeless tobacco and it's constituent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced COX-2 expressions and its transcriptional factor NF-κB activity (Sharma et al., 2006). Curcumin inhibited TPA/PMA-induced COX-2 expression by blocking MAPK/NF-κB mediated pathways (Chun et al., 2003, Lee et al., 2005) (Fig. 2). Curcumin also inhibited chemical carcinogen B(a)P-induced stomach carcinogenesis, N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)-induced duodenal carcinogenesis, and AOM-induced colon carcinogenesis and moderately inhibited DMBA-induced breast carcinogenesis in mouse (**278**) (Huang et al., 1997). Curcumin inhibited UVB-induced COX-2 expression by targeting MAPK/AP-1 mediated pathway (Cho et al., 2005). In another study, Tsai et al. (2012) suggested that curcumin suppressed UVB-induced photocarcinogenesis by blocking NF-κB/COX-2/PGE_2.

Curcumin is recognized as Generally Recognized As Safe (GRAS) by the United States Food Drug Administration and the Joint FAO/WHO Expert Committee on Food Additives approved daily intake of curcumin level of 0.1–3 mg/kg-body weight. A study conducted by the US National Cancer Institute (NCI) demonstrated that oral dosage of curcumin up to 3.5 g/kg body weight in rats, dogs, or monkeys for up to three months, did not cause any adverse effects. In a Phase I clinical trial, curcumin oral administration up to 3.6 g/day for four months was tolerated by patients with colorectal cancer (Sharma et al., 2004). Moreover, curcumin protects against toxicity induced by various toxins (Poapolathep et al., 2015, Yu et al., 2015). Curcumin is non-toxic and antigenotoxic in nature (Sharma et al., 2004, Poapolathep et al., 2015, Yu et al., 2015).

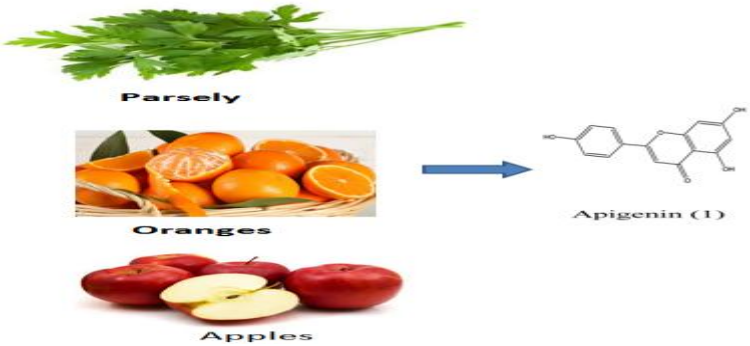


Figure 2.1. Apigenin from various fruits and vegatables

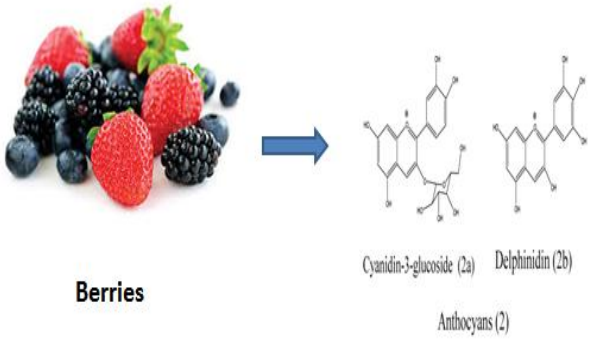


Figure 2.2. Anthocyans from berries and other fruits

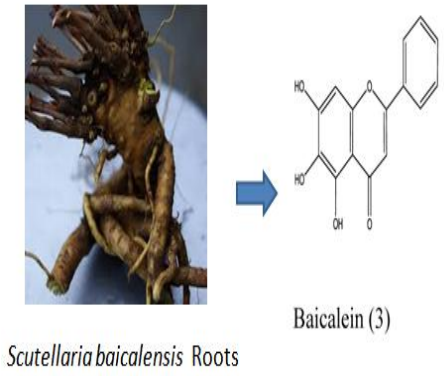


Figure 2.3. Baicalein from *Scutellaria baicalensis* roots

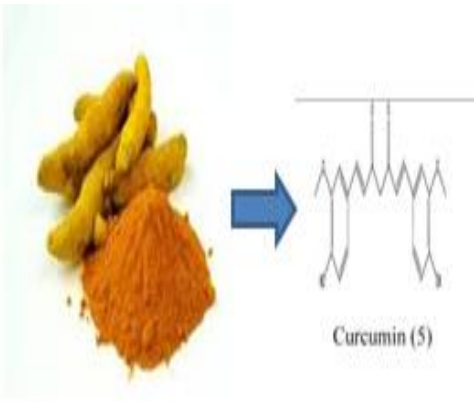


Figure 2.4. Curcumin from *Curcuma longa*

Systemic bioavailability of curcumin is low due to internal rapid metabolism and poor aqueous solubility. Curcumin was excreted in feces in native form or transformed form when administered orally (Ravindranath and Chandrasekhara, 1980). Curcumin biotransformed to monoglucoronide conjugates in rats after intraperitoneal and intravenous administration (Pan et al., 1999). Internal metabolization of curcumin caused loss of its chemopreventive potential. Curcumin metabolites produced by reduction or conjugation of curcumin reduced its ability to inhibit chemically-induced COX-2 expression. Tetrahydrocurcumin, hexahydrocurcumin, and curcumin sulfate, metabolites of curcumin, exhibited moderate chemically-induced PGE_2 inhibitory activity, and hexahydrocurcuminol, another metabolite, was inactive. Various alternative strategies have been developed to improve its bioavailability (Ireson et al., 2001). Several curcumin analogs have been synthesized with improved bioavailability as well as better chemopreventive potentials (Anand et al. 2008). Several nano material based-drug delivery methods are being developed to overcome bioavailability problem of curcumin towards its better chemopreventive actions (Aras et al., 2014, Catania et al., 2013). Piperine, a natural anticancer agent, enhances bioavailability of curcumin by inhibiting curcumin glucuronidation (Grill et al. 2014). Piperine (20 mg/kg body weight) in combination with curcumin (2 g/kg body weight) caused 154% increase in systemic bioavailability of curcumin in rats (Shoba et al., 1998). On the other hand, co-administration of piperine (20 mg) with curcumin (2 g) caused 2000% increase in bioavailability in humans. Gülseren et al. (2014) reported that bioavailability of curcumin was substantially improved by piperine in oil-in-water emulsions. These results suggest that curcumin and combination with a bioactive natural product like piperine can be an alternative strategy to overcome the bioavailability problem of curcumin as well as better chemopreventive and therapeutic approach for several cancers.

2.6. Diallyl sulfides

Diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) are dietary phytochemicals of garlic (*Allium sativum*), have been shown to possess a preventive effect against various cancers including skin and colon (Table 2.1&Figure 2.6) (Cherng et al., 2011, Lai et al., 2013). Cherng et al., (2011) found protective effect

of DAS in UVB light-induced skin lesion in SKH-1 hairless mice with simultaneous inhibition of transcription factors NF-κB activity, COX-2 and PGE_2 levels. DAS, DADS, and DATS downregulated PI3K, Ras, MEKK3, MKK7, ERK1/2, JNK1/2, and p38, inhibited the activities of MMPs, activation of NF-κB and expression of COX-2 that led to the inhibition of cell proliferation of human colon cancer colo 205 cells (Lai et al., 2013). DAS, DADS and DATS exhibited antiproliferative effects in HEK 293T cells and inhibited COX-2 expresion in the same cells (Elango et al., 2004). COX-2 inhibition and antiproliferative effects of DAS were correlated. Results of previous studies warranted detailed pre-clinical studies to develop DAS, DADS, and DATS as cancer chemopreventive agents.

2.7. Ellagic acid

Ellagic acid, a hydrolyzed metabolite of ellagitannin found in certain fruits, nuts and vegetables, has been reported to possess anti-cancer, chemopreventive, anti-oxidant and anti-inflammatory activities (Zhao et al. 2013, Umesalma and sudhandiran, 2010). It exhibits anticancer and chemopreventive potentials against various cancers including skin and pancreas by targeting various pathways, including AA pathway. Zhao et al (2013) found that ellagic acid inhibited pancreatic cancer growth in PANC-1 xenografted mice by suppressing various pro-tumorigenic mediators. Umesalma and sudhandiran (2010) found that ellagic acid prevented chemical carcinogen 1,2-dimethylhydrazine-induced rat colon carcinogenesis by targeting NF-κB/COX-2 pathway. Ellagic acid alone or in combination with resveratrol prevented DMBA-induced skin carcinogenesis as well as suppressed various pro-tumorigenic mediators including COX-2 (Kowalczyk et al. 2010). Karlsson et al., (2010 found that ellagic acid suppressed expression of COX-2 and cPLA2α as well as inhibited production of PGE_2, while it had no effect on the constitutively expressed COX-1 protein in LPS-induced human monocytes. Urolithins, ellagic acid-derived metabolites produced by gut microbiota, inhibited MAPK/NF-κB/COX-2/PGE_2 pathway in human colonic fibroblasts thereby exhibiting anti-inflammatory properties (González et al., 2010). Ellagic acid showed anti-inflammatory effect against carrageenan-induced inflammation by suppressing various pro-inflammatory mediators including COX-2

(El-Shitany et al., 2014). The major disadvantage of ellgagic represents poor bioavailability due to its rapid elimination from the body after administration (Seeram et al., 2004, Murugan et al., 2009). Various strategies have been developed to enhace the bioavailability of ellagic acid. For instance, ellagic acid in combination with phospholipid improved its bioavailability (Murugan et al., 2009). Ellagic acid is non-toxic and non-mutagenic in nature. Moreover, it exhibits protective effect against chemicals-induced toxicity (Kaur et al. 1997, Saba et al., 2013, Ceribaşi et al., 2010, Tasaki et al., 2008). Aforementioned studies warranted further studies on ellagic acid to develop it as cancer chemopreventive and therapeutic agent.

2.8. Epigallocatechin-3-gallate (EGCG)

Epigallocatechin-3-gallate (EGCG), a polyphenol found in white, green and black tea, has been reported to possess chemopreventive effect against several cancers by targeting multiple pathways including COX-2 (Singh and Katiyar, 2013, Harper et al. 2007, Singh and Katiyar, 2011). EGCG inhibited proliferation of skin cancer cells *in vitro* (Singh and Katiyar, 2013). Further studies demonstrated that EGCG inhibited COX-2 by inactivation of PGE_2/EP_2 receptor/cAMP mediated activation of PI3K/AKT pathway. β-catenin and its gene products c-Myc, VEGEF, MMP are inhibited due inactivation of upstream PI3K/AKT pathway by EGCG. The study concluded that EGCG inhibited skin carcinogenesis by targeting COX-2/PGE_2/EP_2R/cAMP/PI3K/AKT/β-catenin/c-Myc/VEGEF/MMP mediated pathway (Singh and Katiyar, 2013). EGCG inhibited early stage of prostate cancer development in Transgenic Adenocarcinoma Mouse Prostate (TRAMP) mice model (Harper et al. 2007). Further mechanistic studies demonstrated that EGCG inhibited COX-2 and other cell survival proteins in prostrate tumor tissue. EGCG inhibited cell migration or invasion in melanoma cells, which was associated with a reduction in the levels of COX-2, PGE_2 and PGE_2 receptors (EP2 and EP4) (Singh and Katiyar, 2011). EGCG exhibited pro-apoptotic potential in HCC cells through down-regulation of COX-2 and modulation of pro-apoptotic mediators (Chen et al., 2008).

Metabolic degradation and low availability of EGCG are major challenges in preclinical and clinical evaluation of EGCG as cancer chemopreventive agent. EGCG is metabolized in liver and by intestinal microbes (Feng, 2006). Efflux transproters are involved in accumulation of EGCG in cells (Vaidyanathan and Walle, 2003). The accumulation of (-)-epicatechin (EC), a non-gallate catechin, was significantly lower than that of EGCG. In toxic studies, EGCG was less toxic at low doses (≤ 10 μmol/L (*in vitro*); ≤2000 mg/kg/body weight/day to mice (*in vivo*) (Kucera et al., 2015, Isbrucker et al., 2006) . Oral doses of EGCG of up to 800 mg per day up to ten days regular administration were found to be safe in humans (Ullmann et al. 2004). Hence, further detailed studies including clinical trials are needed to develop EGCG as a cancer preventive and therapeutic agent.

2.9. Eugenol

Eugenol (4-allyl-2-methoxyphenol), a chemical constituent of *Syzigium aromaticum* (cloves), presents in some aromatic plants, such as cinnamon, nutmeg, basil and bay leaves (Table 2.1 & Figure 2.9). Eugenol possesses antioxidant, anti-inflammatory and anticancer properties. The molecular mechanism is by targeting multitargeting pathways including AA pathway.

Eugenol inhibited the proliferation of HT-29 human colon cancer cells and downregulated COX-2 gene expression (Kim et al. 2003). Eugenol suppressed COX-2 gene expression in LPS-stimulated mouse macrophage cells (Kim et al. 2003). The study suggested that eugenol showed anticancer and anti-inflammatory activities through COX-2 inhibition and it can be chemopreventive agent against colon cancer (Kim et al. 2003).

Kaur et al. (2010) carried out extensive study to demonstrate the chemoprotective effect of eugenol against skin cancer and mechanistic aspects involved. Eugenol treatment delayed or prevented skin tumorigenesis *in vivo*. Eugenol inhibited cell proliferation and induced apoptosis in skin cancer cells. Further mechanistic studies demonstrated that eugenol suppressed expression of COX-2 and other proinflammatory cytokines, inhibited PGE_2 levels as well as NF-κB activity. This indicated that eugenol blocked transcription factor NF-κB activity thereby suppressed

gene expressions of COX-2 and other inflammatory mediators in skin cancer cells. This study concluded that eugenol can be good chemopreventive agent against skin cancer (Kaur et al. 2010).

Hussain et al. (2012) reported that eugenol alone and in combination with sulforaphane (a natural compound present in cruciferous vegetables such as broccoli, brussels sprouts or cabbages) showed significant anticancer activity against HeLa human cervical cancer cells through suppression of COX-2 and other inflammatory mediators. In another study, eugenol alone and in combination with gemcitabineinhibited growth and induced apoptosis in HeLa cells (Hussain et al. 2011).

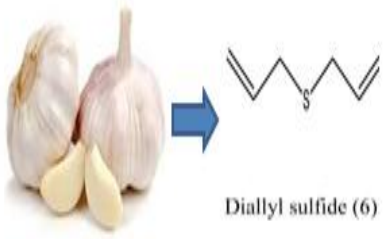


FIgure 2.5. Diallyl sulphide from garlic (*Allium sativum*)

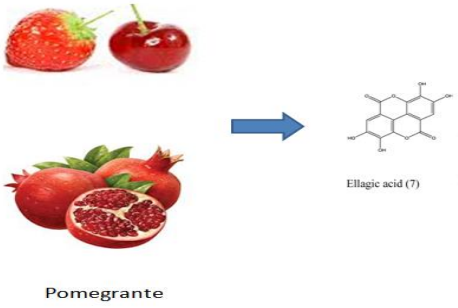


FIgure 2.6. Ellagic acid found in certain fruits, nuts and vegetables

Figure 2.7. Epigallocatechin-3-gallate (EGCG), a polyphenol found in white, green and black tea

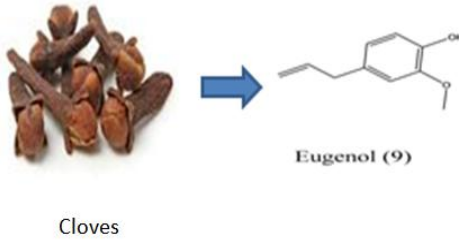


Figure 2.8. Eugenol (4-allyl-2-methoxyphenol), a chemical constituent of *Syzigium aromaticum* (cloves)

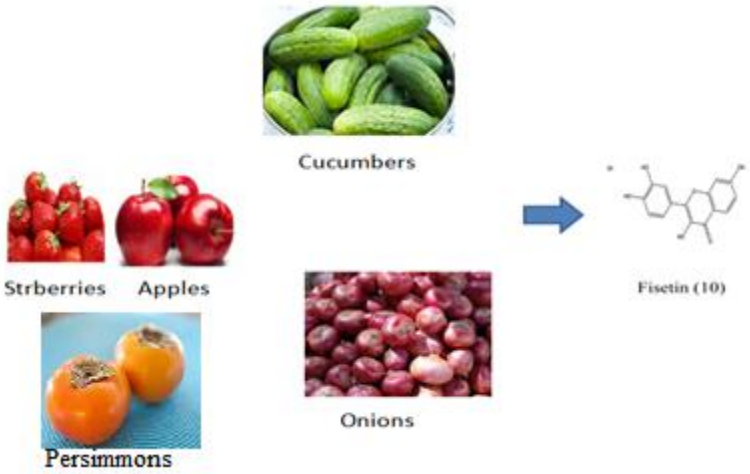


Figure 2.9. Fisetin (3, 7, 3′, 4′-tetrahydroxyflavone), a flavonol, is found in many vegetables and fruits, such as strawberries, apples, persimmons, onions and cucumbers.

Further gene expression studies demonstrated combined treatment enhanced COX-2 gene suppressive effect compared to eugenol alone (Hussain et al. 2011). These studies proved that eugenol alone or in combination with sulforaphane or gemcitabine can be useful for cancer chemoprevention of cervical cancer.

Eugenol has been reported to possess genotoxic and carcinogenuc properties (Martins et al., 2011, Jin et al., 2013). Aspirin eugenol ester, a synthetic hybrid molecule, exhibits comparable anti-inflammatory activity with aspirin and eugenol, but it is not or less toxic (Li et al., 2013, Jian-yong et al., 2012). The results of the study warranted further studies to develop aspirin eugenol ester as a chemopreventive agent.

2.10. Fisetin

Fisetin (3, 7, 3′, 4′-tetrahydroxyflavone), a flavonol, is found in many vegetables and fruits, such as strawberries, apples, persimmons, onions and cucumbers (Figure 2.10). Fisetin has been reported to possess anti-inflammatory and anticancer activities. Fisetin reduced inflammatory mediators such as COX-2, PGE_2 as well as its receptors (EP1-EP4) and other pro-inflammatory cytokines and inhibited activation of NF-κB in UVB-exposed SKH-1 hairless mouse skin (Pal et al. 2015) (Fig. 2). Melatonin potentiated the anti-tumor effect of fisetin by inhibiting COX-2/iNOS and NF-κB/p300 signaling pathways in melanoma cells (Yi et al. 2014). Fisetin suppressed the growth of colon cancer cells and induced apoptosis by targeting Wnt/EGFR/NF-κB/COX-2 signaling pathways (Suh et al. 2009). Fistein downregulated COX-2 expression but not effected expression of COX-1 and suppressed production of PGE2 in colon cancer cells (Suh et al. 2009). Fisetin inhibited TNF-induced NF-κB activation and its gene products including COX-2 (Sung et al. 2007). Fisetin mediates antitumor and anti-inflammatory effects targeting TNFR1, TRADD, TRAF2, NIK, and IKK/NF-κB/COX pathways (Sung et al. 2007). Results of ongoing studies are encouraging and warranted further studies to develop the fisetin as chemopreventive and therapeutic agent for several cancers.

2.11. Garcinol

Garcinol, a polyisoprenylated benzophenone from the fruit rind of *Garcinia indica*, possess anti-inflammatory and anticancer activities). Garcinol inhibited

PGE_2 production in cell-free system as well as in IL-1β-stimulated A549 human lung carcinoma cells and LPS-stimulated human whole blood (Koeberle et al. 2009). Garcinol inhibited carcinogen 4-nitroquinoline 1-oxide (4-NQO)-induced oral carcinogenesis *in vivo* and suppressed COX-2 expression (Yoshida et al. 2005). Garcinol suppressed formation of AOM-induced colonic ACF in rats and suppressed formation of $O(2)^{(-)}$ and COX-2 expression in LPS- and IFN-γ-treated mouse macrophage RAW 264.7 cells (Tanaka et al. 2000). Garcinol and its derivatives cambogin, garcim-1 and garcim-2 inhibited AA release and its metabolites in LPS-stimulated macrophages and intestinal HT-29, HCT-116 and IEC-6 cells (Hong et al. 2006). Garcinol inhibited phosphorylation of ERK1/2 and $cPLA_2$, and activation of NF-κB and COX-2 expression. Garcinol showed anti-inflammatory and cancer chemopreventive effects (Hong et al. 2006).

Garcinol has also been reported to possess 5-LOX inhibitory activity and suppressive effect on LTB4 production in cancer cells (Han et al. 2015, Koeberle et al., 2009, Chen et al., 2012). Structure activity relationship studies demonstrated that 13,14-dihydroxy groups are key for its 5-LOX inhibitory potency as well as its anticancer activity (Han et al. 2015). In DMBA-treated hamster cheek pouch carcinogenesis model, garcinol suppressed LTB4 biosynthesis and inhibited tumor-associated inflammation, cell proliferation, tumor growth and number of cancer lesions (Chen et al., 2012). This study demonstrated that garcinol exerted anticarcinogenesis through targeting 5-LOX pathway (Chen et al., 2012).

2.12. Genistein

Soy isoflavones have been identified as dietary components having an important role in reducing the incidence of various cancers. Genistein, the predominant isoflavone found in soy products, has been known as cancer chemopreventive agent for various cancers. Recently, clinical studies have confirmed the chemopreventive effect of genistein (Messing et al., 2012).

Genistein and other soy isoflavones have been found to be effective not only in reducing COX-2 expression, but also for antagonizing AA for controlling PGE_2 production and invasiveness of MDA-MB231 breast cancer cells through downregulation of EGFR and HER-2/neu activity and by modulating the level of NF-

κB expression (Sanjeev et al. 2008). Genistein prevented inflammatory responses by inhibiting $sPLA_2$ activity (Kattepura et al. 2010).

Soy isoflavones especially genistein reduced COX-2 expression in MCF-7 breast cancer cells, which could be the mechanism underlying in prevention of breast carcinogenesis (Lau and Leung, 2006). Chung et al. (2014) demonstrated that genistein inhibited TPA-induced COX-2 expression and transcriptional activity of NF-κB in MCF10A human breast epithelial cells by blocking ERK-mediated phosphorylation of p65. The study supported the chemopreventive effect of genstein against breast cancer.

Dai et al. (2015) investigated the effects and mechanism of genistein on hepatocellular carcinoma. They found that genistein inhibited hepatocellular carcinoma cell migration by reversing the epithelial-mesenchymal transition and suppressing COX-2 expression. Hwang et al., (2005) demonstrated that the combination of 5-fluorouracil and genistein exert a novel chemotherapeutic effect in colon cancers through inhibition of AMPK/COX-2 expression.

A phase 2 randomized, placebo-controlled trial investigated whether daily, oral genistein (300 or 600 mg/day as the purified soy extract (G-2535) for 14 to 21 days before surgery alters molecular pathways in bladder epithelial tissue in 59 subjects diagnosed with urothelial bladder cancer (median age, 71 years). The study demonstrated that oral administration of genistein had moderate effect and no significant change was observed in COX-2 and phosphorylate-EGFR compared to placebo (Messing et al., 2012).

7,3',4'-trihydroxyisoflavone (THIF) a metabolite of soy isoflavone daidzein, exhibits chemopreventive potential in UVB-induced skin cancer. Lee et al. (2011) demonstrated that THIF, effectively inhibits UVB-induced COX-2 expression through inhibition of MMK4 kinase and NF-κB transcription activities in mouse skin epidermal JB6 P+ cells. These results suggested that THIF can be chemopreventive agent against UVB-induced skin cancers by targeting COX-2 pathway.

Ongoing studies demonstrated that soy isoflavones especially genistein has potential chemopreventive effect against various cancers. Further clinical trials warranted to

confirm the chemopreventive effect of genistein for clinical applicability to patients with cancers.

2.13. [6]-Gingerol

[6]-Gingerol, a naturally occurring phenol, is one of the constituents of *Zingiber officinale* Roscoe, (ginger) and exhibits various bioactivities including anticancer and antiinflammatory activities. [6)]-gingerol has been reported to possess anticancer and chemopreventive potentials against several cancers, including skin, colorectal, gastrointestinal and pancreatic (Prasad and Tyagi, 2015, Kim et al. 2007). [6]-gingerol suppressed UVB-induced translocation of NF-κB from cytosol to nucleus via prevention of IκBα phosphorylation and COX-2 expression and transactivation in HaCaT cells *in vitro* (Kim et al. 2007). [6]-gingerol suppressed COX-2 expression at mRNA and protein levels, and translocation of NF-κB in UVB irradiated skin of hairless mice (Kim et al. 2007). (6)-gingerol suppressed TPA-induced COX-2 gene expression in skin of mouse by inhibiting p38 MAP kinase-NF-κB signaling pathway (Kim et al. 2005, Kim et al. 2004). These findings suggest the chemopreventive effect of (6)-gingerol on UVB-induced skin disorders, including cancers (Kim et al. 2007). Jeong et al. (2009) found that]6]-gingerol inhibited growth of colon cancer by inhibiting LTA_4 hydrolase.]6]-Gingerol suppressed PGE_2 levels in IL-1β stimulated human oral keratinocytes (Kono et al, 2014). Overall, studies demonstrated that [6]-gingerol exerts anticancer and cancer preventive activities by targeting AA pathway. Recent reports on genotoxic effects of [6]-gingerol are controversial, however detailed investigations are required to develop [6]-gingerol as cancer chemopreventive and therapeutic agent to capitalize on promising results in preclinical models (Yang et al. 2010, Yang et al. 2011).

2.14. Guggulsterone

Guggulsterone, a resin of the *Commiphora mukul* tree, has been reported to possess chemopreventive and therapeutic potentials against several cancers (Sarfaraz et al. 2008, Macha et al. 2011). Sarfaraz et al. (2008) found that guggulsterone prevented TPA-induced skin tumor promotion by inhibiting various signaling pathways including MAPK/NF-κB/COX-2. Macha et al., (2011) reported that guggulsterone prevented smokeless tobacco and nicotine-induced head and neck cancer by targeting NF-κB/STAT/COX-2 mediated pathways. Guggulsterone inhibited TNF, phorbol

ester, cigarette smoke condensate, and IL-1 induced-NF-κB activity and expression of pro-inflammatory mediators including COX-2 (**352**) (Shishodia and Aggarwal, 2004). Yamada et al. (2004) found that guggulsterone inhibited chemically-induced esophageal adenocarcinoma by inhibiting NF-κB/COX-2 pathway. Guggulsterone inhibited LPS-induced activation of NF-κB and expression of various proinflammatory mediators including COX-2 in mouse inner medullary collecting duct-3 and human middle ear epithelial cells (Kim et al., 2015; Song et al. 2010). Xu et al. (2014) reported that guggulsterone reverses imatinib resistance and induces apoptosis in imatinib-resistant leukemic cells by inhibiting COX-2 and P-glycoprotein (P-gp). This study suggested that guggulsterone can be useful to sensitize the chemotherapeutic drugs in cancers. Aforementioned studies warranted detailed pre-clinical and clinical studies to develop guggulsterone as a cancer chemopreventive and therapeutic agent.

2.15. Indole-3-carbinol

Indole-3-carbinol, found in cruciferous vegetables such as cabbage, broccoli, Brussels sprouts, and kale, has been shown to have anti-inflammatory and cancer preventive potentials (Royston et al., 2015). Indole-3-carbinol suppressed TNF-α, IL-1β, PMA, LPS, and cigarette smoke-induced NF-κB activation and NF-κB regulated gene products including COX-2 in myeloid, leukemia, and epithelial cells (Takada et al. 2005). Indole-3-carbinol caused TNF-α-induced IκB-α kinase (IKK) suppression, consequently blocked IκBα phosphorylation, ubiquitination and degradation that prevented p65 phosphorylation, nuclear translocation and p65 acetylation. This led to downregulation of NF-κB-regulated gene products such as COX-2, cyclin D1, MMP-9, survivin and other apoptosis inhibiting proteins. There by indole-3-carbinol inhibited cell proliferation and induced apoptosis. 3,3′-Diindolylmethane (DIM), a digested product of indole-3-carbinol, inhibited inflammation by targeting NF-κB/COX pathway (Kim et al. 2010). DIM inhibited 2,3,7,8 tetrachlorodibenzo(p)dioxin (an environmental xenobiotic)-stimulated recruitment of aryl hydrocarbon receptor (AhR) and acetylated histone H4 to the COX-2 promoter and prevented COX-2 gene expression in MCF-7 breast cancer cells (Degner et al. 2009). There by it prevented carcinogenesis. Combinations of indole-3-carbinol and silibinin effectively inhibited a tobacco constituent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and LPS-induced lung tumorigenesis in mouse by

suppressing proinflammatory and procarcinogenic mediators including COX-2 (Song et al., 2015). Results of *in vitro* and *in vivo* anticancer studies warranted clinical trials on indole-3-carbinol. However, poor bioavailability of indole-3-carbinol is a major problem for clinical practice (Song et al, 2014). To overcome this problem, song *et al* prepared liposomal indole-3-carbinol and administered it to animals with lung tumor through nasal route. Intranasal administration of liposomal indole-3-carbinol significantly enhanced the delivery and antitumor potential of indole-3-carbinol than the oral route of administration (Song et al. 2014).

2.16. Lycopene

Lycopene and other natural colour pigments, namely betanin, bixin, chlorophyll and beta-carotene, were isolated from *Beta vulgaris, Prunus cerasus, Lycopersicum esculentum, Spinacia oleracea, Daucus carrota*, and *Bixa orellana,* respectively. These natural compounds, alone and in combination showed COX inhibitory activity and inhibition of growth of breast, colon, stomach, and lung cancer cells (Reddy et al., 2005).

Lycopene showed potential anticancer effect on colon cancer *in vitro* and *in vivo* (Tang et al. 2011). Anticancer effect of lycopene against colon cancer is associated with down regulation of COX-2 gene expression and inhibition of PGE_2 levels (Tang et al. 2011). A study by Tang et al (2012) demonstrated that lycopene and fish oil acted synergistically as chemopreventive agents against colon cancer using a mouse xenograft model of colon cancer. Molecular studies demonstrated that lycopene and fish oil inhibited colon cancer progression through inhibition of COX-2 and PGE_2 (Tang et al 2012).

Lycopene-rich tomato-based foods believed to reduce the risk of prostate cancer (segev and native, 2006). Bonvissuto et al., (2011) reported that lycopene downregulated gene expression of COX-2, 5-LOX, and iNOS in LPS stimulated-peritoneal macrophages and in the prostate of rats. Moreover, lycopene inhibited IκB-α and NF-κB binding activity. COX-2 and other proinflammatory mediators inhibitory activities of lycopene are associated with reduced prostate inflammation. Ivanov et al. (2007) reported that lycopene suppressed proliferative and survival of androgen-responsive LNCaP and androgen-independent PC3 prostate cancer cells. In a clinical study, lycopene treatment did not change COX-2 gene expression in the prostate

cancer microenvironment in patients with prostate cancer (Chan et al. 2011). Lin et al., (2014) reported that lycopene inhibited neuroinflammatory responses in microglia. Further studies demonstrated that lycopene inhibited expression of COX-2 and other inflammation mediators in mouse and rat microglia. Lycopene inhibited LPS-induced NF-κB and AP-1-DNA binding activityas well as COX-2 expression. In another study, lycopene activated adenosine monophosphate-activated protein kinase (AMPK) and heme oxygenase-1 (HO-1) which led to downregulation of LPS-induced COX-2 expression in microglia (Lin et al., 2014). This study demonstrated that lycopene inhibited neuroinflammation by targeting COX-2. Hence, it can be useful as chemopreventive agent for neuroinflammation-associated disorders including cancer.

Lycopene exhibits anti-mutagenic and anti-oxidant activities (Polívková et al. 2010). Morevoer, lycopene shows protective effect against chemically-induced toxicity (Sheik and Thiruvengadam, 2013). The oral bioavailability of lycopene is low due to poor intestinal absorption (Faisal et al. 2013, Böhm and Bitsch, 1999). A novel lipid based solid dispersion formulation was developed by Faisal et al. (2013) to enhance the oral bioavailability of lycopene. Chen et al. (2014) developed lycopene micelles and lycopene chylomicrons to enhace the oral bioavailability of lycopene.

2.17. Nordihydroguaiaretic acid

Nordihydroguaiaretic acid (NDGA) is a constituent of the creosote bush *Larrea divaricata* and is known to possess anti-inflammatory and anticancer activities by targeting multiple signaling pathways including LOX pathway (Avis et al., 1996, Blecha et al., 2007). NDGA blocked growth factor–mediated 5-LOX pathway in proliferating lung cancer cells (Avis et al., 1996). 5-LOX inhibitory activity of NDGA is associated with reduction of growth of lung cancer. The study summarized that anticancer activity of NDGA against lung cancer was due to its LOX inhibitory activity (Avis et al., 1996). Nie et al (2006) carried out a detailed investigation on chemopreventive role of 12-LOX inhibitory NDGA in prostate cancer using an animal model (Nie et al. 2006). The overall study summarized that NDGA inhibited 12-LOX activity, which in turn blocked PI3K/Akt/PKCζ/Sp1/AP2 pathway mediated VEGF expression in prostate cancer cells and consequently prevented prostate cancer angiogenesis and progression (Nie et al. 2006). NDGA inhibited cell proliferation and invasion in head and neck as well as colon cancer cells (Koontongkaew et al.,

2010). NDGA substantially inhibited the growth of human breast carcinoma in both animal and cell-based models (Cui et al., 2008). NDGA reactivated methylation-silenced E-cadherin gene in breast cancer cells, which suggest NDGA may act as natural effective epigenetic modifiers in the prevention and treatment of cancer (Cui et al., 2008(a)). NDGA inhibited cell proliferation of LoVo human colonic cancer cells (Melstrom et al. 2008). Cui et al. (2008**(b)**) reported that NDGA reactivated methylated silenced-tumor suppressor gene p16INK4a and arrested cell cycle at G1 phase in T47D and RKO human cancer cells. Youngren et al. (2008) reported that NDGA inhibited the growth of MCNeuA breast cells *in vivo* and blocked IGF-1 and c-erbB2/HER2/neu receptor-associated signaling pathways. NDGA inhibited dihydrotestosterone-induced growth of LAPC-4 prostate cancer cells (Ryan et al., 2008). Antitumor activity of NDGA against human esophageal adenocarcinoma in pre-clinical model was reported by Chen et al. 2002. McDonald et al., (2001) synthesized several NDGA analogs and evaluated their anticancer activities against H-69 small cell lung cancer cell line. They simplified the structure of NDGA by removal of the methyl groups has produced a new lead compound 4, which was 10 times more the potent than parent molecule NDGA as a anticancer agent against H-69 small cell lung cancer cells McDonald et al., (2001). Tetra-*O*-methyl nordihydroguaiaretic acid (M4N), a NDGA derivative, suppressed growth of Hep 3B hepatocellular carcinoma, LNCaP prostate carcinoma, HT-29 colorectal carcinoma, MCF7 breast carcinoma, and K-562 erythroleukemia xenograft tumors in mice (Park et al. 2005). Pharmacokinetic analysis following oral and intra venous administration of M4N to mice indicated an absolute bioavailability (approximately 88%) for oral M4N. Minimal drug-related toxicity was observed in M4N treated mice (Park et al. 2005).

NDGA was once classified as “generally recognized as safe” by US FDA, and used as an antioxidant food additive. Later this classification was withdrawn based on toxicity studies in rats demonstrated that NDGA caused severe nephrotoxicity and other adverse effects (Grice et al. 1968). Carcinogenic effect of NDGA was reported by several researchers. NDGA increased hepatic tumor number, and increased the prevalence of thymus and lung tumors. Taken together, the study demonstrated that NDGA reduced tumor growth rates and increased tumor formation (Spindler et al. 2014). Several studies proved that it expand the life span of drosophila and mice

(Spindler et al. 2014). LOX inhibitiory activity of NDGA may be one of the underlying mechanisms for its life expansion potential.

Lambert et al. (2001) found the lethal dose 50% (LD_{50}) of NDGA was found at 75 mg/kg (intraperitoneal route). NDGA was not overtly toxic at low doses. However, high doses of NDGA have been associated with several adverse effects including, nephrotoxicity, and hepatotoxicity includes liver failure, and renal cell carcinoma in humans (Jian et al., 2010). Further detailed studies are necessary to get clarity on toxic effect of NDGA which is a major problem in clinical application of NDGA. LD_{50} of a NDGA analog M4N was found at 1000 mg/kg (intraperitoneal injection) whereas LD_{50} of parent molecule NDGA was found at 75 mg/kg (Chang et al. 2004). In another study, tetra-*O*-substituted NDGA analogs exhibited less toxicity than NDGA (Meyers et al. 2009). These studies warranted development of less toxic derivatives of NDGA which should be explored as anti-inflammatory and anticancer agents.

2.18. Piperine

Piperine, an alkaloid, present in *Piper nigrum* (black pepper) and *Piper longum* (long pepper), and has been reported possess anti-inflammatory, antioxidant and anticancer activities against colon, lung, breast, gastric and prostate cancers (Liu et al. 2010, Yaffe et al., 2015, Lin et al., 2014, Do et al., 2013, Xia et al., 2015, Samykutty et al., 2013). Ying et al. (2013) reported that piperine inhibited COX-2 and iNOS gene expression and PGE2 and NO production in LPS-induced RAW264.7 cells. Piperine inhibited LPS-stimulated activation of NF-κB by suppressing the degradation of IκB and the translocations of p65 subunit of NF-κB from the cytosol to the nucleus (Ying et al. 2013) . Piperine significantly inhibited PMA-induced nuclear translocation of NF-κB, C/EBP and c-Jun, activation of the Akt and expression of ERK and COX-2 in RAW 264.7 macrophages (Fig. 2) (Kim et al., 20102). Liu et al. (2010) reported that piperine significantly inhibited the cancer cell proliferation and inflammatory mediators including NF-κB and COX-2. Piperine inhibited PLA_2, COX and LOX *in vitro* (Stöhr et al., 2001, Son et al., 2014, Prasad et al., 2004). Piperine, an anticancer agent, enhanced bioavailability of curcumin and resveratrol by inhibiting their glucuronidation (Grill et al. 2014, Jeremy et al. 2011). Piperine has good absorption efficacy and can`t under go any metabolic degradation during the

absorption process (Suresh and Srinivasan et al., 2007). Moreover, it exhibited safety profile in toxicity studies (Piyachaturawat et al., 1983). The studies warranted that piperine can be potent cancer chemopreventive agent based on further studies.

2.19. Quercetin

Quercetin, a flavonoid and polyphenol found in many fruits and vegetables, has been found to have a role in inhibiting various cancers including colon, liver and lung (Warren et al., 2009; Turner et al., 2009). Quercetin inhibited PGE_2 levels in HCA-7 human colon cancer cells cells (Al-Fayez et al., 2006). Quercetin potentially suppressed the formation of preneoplastic lesions, inhibited proliferation and increased apoptosis during colon carcinogenesis. It is possible that the effects on proliferation and apoptosis resulted from suppressive effect of quercetin on expression of proinflammatory mediators including COX-2 (Warren et al., 2009; Turner et al., 2009). A reporter gene assay has revealed that quercetin suppressed transcriptional activity of the COX-2 gene in DLD-1 human colon cancer cells (Mutoh et al., 2000(a)). A structure-activity study demonstrated that the hydroxyl groups on the B ring and an oxo group at the 4-position of the C ring of quercetin are important in the suppression of COX-2 transcriptional activity (Mutoh et al., 2000(a)). Quercetin contains a resorcin-type structure that may play a crucial role in the inhibition of COX-2 gene expression in colon cancer cells (Mutoh et al., 2000(b)).

Quercetin suppressed COX-2 gene expression and NF-κB activity in cooking oil fumes (COF) induced CL-3 human lung adenocarcinoma cells. This study demonstrated chemopreventive effect of quercetin on lung cancer cells (Lin et al., 2002). Granado-Serrano et al. (2012) demonstrated that quercetin inhibited inducible COX-2 expression and NF-κB activity in a human hepatoma cell line (HepG2). Quercetin downregulated NF-κB and COX-2 expressions and upregulated Nrf2 expression in Ochratoxin A (OTA)-teated HepG2 cells (Ramyaa et al., 2014). As a consequence, quercetin suppressed OTA-induced oxidative stress in HepG2 cells. These observations suggest that quercetin may contribute as a chemopreventive agent for liver cancer. Quercetin selectively inhibits GII $sPLA_2$, which may be one of the reasons for its anti-inflammatory activity (Guardia et al, 2001, Lindahl and Tagesson, 1993). Double bond and double-bonded oxygen in the oxane ring, and hydroxyl group in 5-position of flavonoids including quercetin are responsible for their PLA_2

inhibitory activities. Further, they demonstrated that the hydroxyl groups in 3'- and 4'-positions of flavonoids are important for their selective GII $sPLA_2$ inhibitory activities (Lindal and Tagesson, 1997).

Quercetin enhances drug sensitivity in cancers (Cipák et al., 2003, Chan et al., 2003, Wang et al, 2015). Recently, Atashpour et al (2015) found that quercetin enhanced anticancer effects of doxorubicin in human colorectal HT29 cancer cells. Quercetin can also reverse the multidrug-resistance in cancer cells, thereby enhances the anticancer effect of various drugs (Jakubowicz-Gil et al. 2005, Asaum et al., 2000, Atashpour et al. 2015). Quercetin is non-toxic and exhibits protective effect against drug-induced toxicity (Yagmurca et al., 2015, Saleem et al., 2015, Shokoohinia et al., 2015). Quercetin exhibited substantial chemopreventive and therapeutic potential against various cancers with out any toxic effects. However, clinical use of quercetin is limited due to its poor bioavailability (Ratnam et al., 2006). Various strategies have been developed by several research groups to improve the bioavailability of quercetin (Bagad and Khan 2015, Gao et al. 2014). Gao et (2014) encapsulated quercetin into biodegradable monomethoxy poly(ethylene glycol)-poly(ε-caprolactone) micelles and found enhanced anticancer activity by increasing its bioavailability.

2.20. Resveratrol

Resveratrol, a stilbene, is found in grapes, berries, peanuts, red wine and many other plants (Table 2.1&Figure 2.16) (Bishayee, 2009). During the last decade, several preclinical and clinical studies have shown that resveratrol can prevent or inhibit the progression of a wide variety of age-related diseases, including cancer (Aggarwal et al., 2004; Bishayee, 2009, Carter et al., 2014). Many studies suggest that resveratrol exhibits chemopreventive potential against several cancers at all stages i.e., initiation, promotion, and progression by targeting multiple pathways including, AA pathway (Bishayee, 2009).

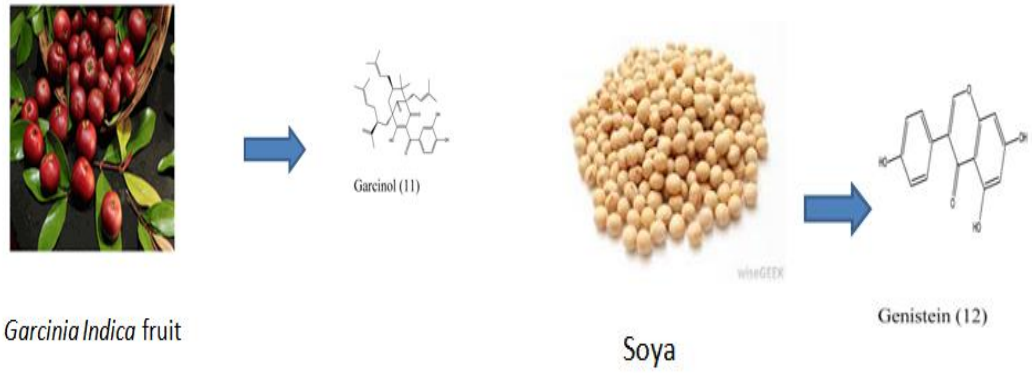
Bishayee and colleagues (2010) previously reported that resveratrol prevented -DENA-induced hepatocarcinogenesis in rats by suppressing the levels of full form HSP70, COX-2 and NF-κB. Subsequent report from the same laboratory documented that resveratrol reversed DENA-induced alterations of the level and expression of several proinflammatory cytokine, such as TNF-α, IL-1β and IL-6, during rat liver carcinogenesis (Mbimba et al., 2012). Ferruelo et al. (2014) found that resveratrol

exerts anticancer activity on androgen hormone-resistant prostate cancer PC-3 cell line by blocking NF-κB/COX-2/PGE_2 pathway. Resveratrol induced intranuclear accumulation of COX-2 and promoted colocalization of COX-2 with Ser15-phosphorylated p53 and p300, which caused induction of apoptosis in breast cancer cells (Tang et al. 2006). MacCarrone et al., (1999) reported that resveratrol was found to act as a competitive inhibitor of 5-LOX and 15-LOX and PGH synthase *in vitro*. Sulfate-conjugated resveratrol metabolites exhibit COX-1 and COX-2 inhibitory activities *in vitro* (Juma et al. 2010)). Resveratrol exhibits human CYP inhibitory activities and exhibit selective inhibition on CYP 1A1 (Chun el al. 1999).

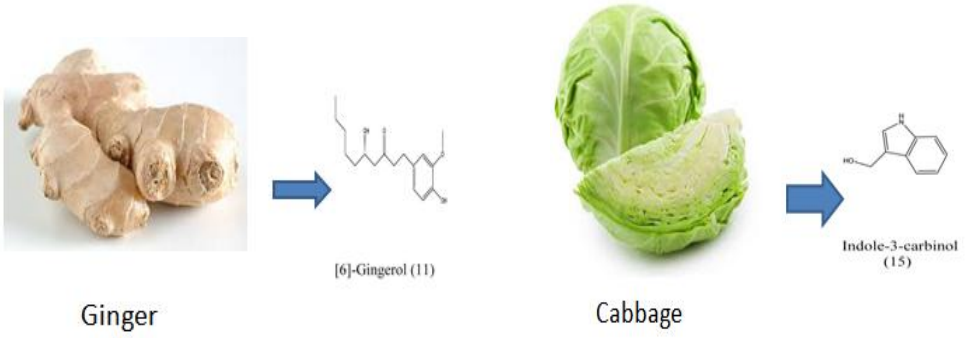
Resveratrol prevents or inhibits chemical carcinogen and pro-inflammatory cytokine mediated inflammatory responses and carcinogenesis by targeting AA pathway. Resveratrol was reported to inhibit PMA-induced COX-2 expression by blocking activation of MAPK/NF-κB/AP-1 (Martín et al., 2004, Kundu et al., 2006, Subbaramaiah et al., 1998). Tsai et al., (2012) reported that pterostilbene, a natural analogue of resveratrol, prevented DMBA/TPA-induced skin tumor formation in mice by blocking MAPK/NF-κB/AP-1/COX-2 pathway. Recently, Cho et al. (2015) reported that resveratrol in combined with ursolic acid effectively inhibited TPA-induced skin tumor promotion by inhibiting MAPK/NF-κB/COX-2 pathway. In another study by Chatterjee et al. (2011) demonstrated that resveratrol prevented DMBA-induced mammary carcinogenesis by blocking 5-LOX. Resveratrol suppressed DMBA-induced mammary carcinogenesis by blocking NF-κB/COX-2 pathway (Banerjee et al., 2002). Aziz et al., (2005) found that resveratrol inhibited UVB-induced carcinogenesis by downregulating COX-2 and cell survival mediators. Another study by Chan et al. (2015) demonstrated that resveratrol suppressed UVA-induced COX-2 expression in retinal pigment epithelial cells. Several reports suggest that resveratrol inhibited LPS-induced expression of COX-2 and other proinflammatory mediators by blocking TLR4/myd88/MAPK/NF-κB mediated pathway (Zhong Y et al. 2012, Zhang et al., 2014, Cianciulli et al., 2012, Zong LM et al., 2012). Resveratrol suppressed pro-inflammatory cytokines (IL-1α, TNF-α, and IFN-γ)-induced expression of COX-2 and production of PGE_2 in HT-29 colon epithelial cells (Serra et al., 2014). Amanda and Robert, (2008) reported that resveratrol inhibited TNF-α-induced expression of COX-2 and pro-inflammatory cytokines in adipocytes by inhibiting NF-κB activity. Resveratrol suppresses an

oncogenic catechol estrogen, 4-hydroxyestradiol-induced progression and development of breast cancer by inhibiting IκB kinaseβ/NF-κB/COX signaling pathway (Park et al. 2012).

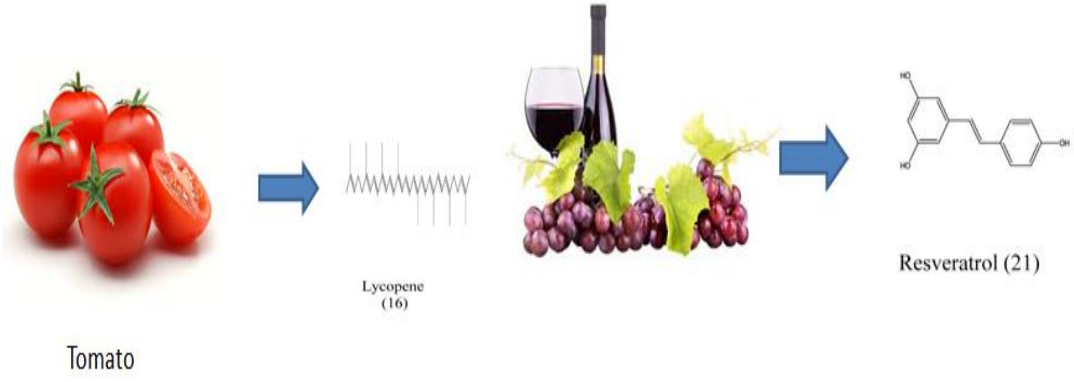
The rapid internal metabolism and poor pharmacokinetic parameters of resveratrol has found to be main obstacle in translating its observed preventive and therapeutic effect in pre-clinical studies to the humans. Resveratrol has been shown to be extensively metabolized through glucuronidation and sulfation (Jeremy et al. 2011). Several studies demonstrated that resveratrol is rapidly absorbed but quickly metabolized-



Garcinol from *Garcinia indica* Genistein from soya



Gingerol from Ginger Indole-3-carbinol from cabbage



Lycopene from Tomato Resveratrol from grapes

Fig. 2.10. Some of the important dietary natural products witn anticancer and antinflammatory activities

within an hour after oral or intravenous administration in humans (Boocock et al. 2007, Walle et al., 2004). Various strategies have been developed by several investigators to enhance bioavailability of resveratrol (Jeremy et al. 2011, Ana et al. 2013; Siddiqui et al., 2015). Ana et al. (2013) developed lipid nanoparticles-based two novel resveratrol nanodelivery systems to improve the oral bioavailability of resveratrol. Piperine, a natural chemopreventive agent, significantly enhances the bioavailability of resveratrol (Jeremy et al. 2011). Piperine enhances the bioavailability of resveratrol by inhibiting glucuronidation of resveratrol (Jeremy et al. 2011). Pterostilbene, a naturally occurring dimethylether analog of resveratrol with chemopreventive potential, exhibited better pharmacokinetic profile than resveratrol in rats (Izet et al., 2011). Several series of synthetic structural analogues of resveratrol with improved bioavailability have been developed with enhanced cancer chemopreventive and therapeutic activities (Aggarwal et al. 2004). Resveratrol exhibits safety profile in toxicity studies (Sangeetha et al., 2013, Tatefuji et al., 2014). Moreover, resveratrol protects against drug/chemical/toxin-induced toxicity (Arslan et al., 2015, Gu et al., 2015, Albuquerque et al., 2015, Raghubeer et al., 2015). Aforementioned studies warranted detailed clinical studies to develop resveratrol as a cancer chemopreventive and therapeutic agent.

2.21. Silibinin

Silibinin is present in *Silybum marianum* or milk thistle which has been reported to possess anti-inflammatory and antitumorigenic activities (Khan et al. 2014, Raina et al., 2013, Kim et al 2009, Hagelgans et al., 2014). Silibinin exhibited anticancer and cancer preventive effects against several cancers including skin, colorectal, breast, liver and prostate (Khan et al. 2014, Raina et al., 2013, Kim et al 2009, Hagelgans et al., 2014). However, the precise underlying mechanism remains to be established.

Khan et al. (2014) reported that silibinin showed anticarcinogenic activity against DMBA/TPA-induced skin tumorigenesis in mice. The anticarcinogenic effect of silibinin is associated with supression of various inflammatory mediators including COX-2 (Khan et al. 2014). Silibinin showed photoprotective in SKH1 hairless mice and prevented photocarcinogenesis via suppression of inflammatory and angiogenic mediators such as transcriptional factors NF-κB, HIF-1α and STAT3, COX2 and

iNOS (Gu et al. 2007). Silibinin showed antiproliferative, pro-apoptotic, and antiangiogenic activities against colorectal carcinoma. These effects of silibinin were due to suppression of COX, NOS, HIF-1α, and VEGF genes expressions, and inhibition of ERK1/2 and Akt signaling pathways (Singh et al. 2008). Raina et al. (2013) found that silibinin exhibited anticarcinogenic and chemopreventive against colorectal cancer by targeting NF-κB/COX pathway. Kim et al (2009) reported that silibinin down-regulated COX-2 and MMP-9 expression in TPA-treated human breast cancer MCF-7 and MDA-MB231 cells. Silibinin suppressed sPLA2s expression and NF-κB in basal and cytokine-induced hepatoma HepG2and prostate PC-3 cancer cells. These findings suggested that silibinin exerted anticancer activity against liver and prostate cancers by targeting NF-κB/sPLA$_2$ pathway (Hagelgans et al., 2014). These studies clearly demonstrated that silibinin exhibited anticancer and caner preventive activities by targeting various inflammation mediators including NF-κB, COX and PLA$_2$.

Silibinin showed cytoprotective effect against toxins and chemical-induced liver toxicity (Beydilli et al., 2015, Kostek et al., 2012). Moreover, silibinin is non-carcinogenic and non-mutagenic in nature (Angeli et al., 2010, Dunnick et al, 2011). Cytoprotective and chemopreventive effects of silibinin warranted clinical trials for its development as anticancer and cancer preventive agent. Poor aqueous solubility and poor bioavailability of silibinin limited its chemopreventive potential at the tumor sites (Gohulkumar et al. 2014). Gohulkumar et al. (2014) suggested that silibinin-loaded nanoparticles (Eudragit® E) can be used as an effective drug delivery system to produce a better chemopreventive response for the treatment of oral cancer.

2.22. Sulforaphane

Sulforaphane, an isothiocyanate present in cruciferous vegetables, has been known to possess anti-inflammatory and cancer chemopreventive properties. Several investigators determined the molecular mechanisms by which sulforaphane prevented inflammation and cancer. Sulforaphane showed oncosuppressive activity against oral cancer *in vivo* and *in vitro* by inhibiting COX-2 expression (Cho et al. 2009). Kim et al. (2014) found that sulforaphane suppressed TPA-induced NF-κB activity and COX-2 gene expression in human mammary epithelial MCF-10A cells by inhibiting ERK1/2-IKKα and NAK-IKKβ-mediated signaling pathways. Shan et al. (2009, 2010)

reported that sulforaphane inhibited COX-2 expression by inhibiting NF-κB-DNA-binding activity and activating p38 MAPK in human bladder T24 cells. Sulforaphane reduced breast cancer cell proliferation by inhibiting COX-2 expression and activation p38 MAPK (Jo et al. 2007). Sulforaphane inhibited epithelial-mesenchymal transition (EMT) via COX-2/MMP2 & 9/miR-200c/ZEB1 and Snail/ZEB1 pathways in human bladder cancer T24 cells (Shan et al. 2013). Sulforaphane inhibited TNF-α-induced NF-κB activity and its regulated gene products and induced apoptosis through activation of reactive oxygen species (ROS)-dependent caspase-3. Sulforaphane inhibited MAPK-NF-κB, C/EBP, CREB/AP-1/COX-2 pathway in LPS-stimulated Raw 264.7 cells (Woo and Kwon, 2007).

Sulforaphane caused detoxification of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons. As a consequence, it prevented mutagenesis or carcinogenesis (Weisburger, 1999, Barcelo et al. 1996, Villa-Cruz et al. 2009, Yoxall et al. 2005). Isothiocyanates prevented lung carcinogenesis induced by a tobacco constituent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Hecht, 1999). Licznerska et al. (2015) found that L-sulforaphane affect the expression of CYP involved in estrogen metabolism of breast cancer tissue. This effect may contribute to its anticancer activity against breast carcinoma. Sulforaphane modulates the xenobiotic-metabolising enzyme systems, shifting the balance of carcinogen metabolism toward deactivation, and this is a mechanism of its chemopreventive activity (Yoxall et al. 2005, Licznerska et al. 2015).

2.23. Thymoquinone

Thymoquinone, a promising natural product derived from black cumin (*Nigella sativa*), has been reported to possess anti-inflammatory and antitumorigenic activities through inhibiting various inflammatory mediators, including COX-2. Xu et al (2014) reported that thymoquinone showed antitumor activity against human cholangiocarcinomas *in vitro* and *in vivo*. Further, mechanistic studies demonstrated that anticancer activity of thymoquinone against cholangio cancer may be possible due to inhibition of PI3K/Akt/NF-κB/COX-2 pathway. Mansour and Tornhamre (2004) reported 5-LOX and LTC4 synthase inhibitory activities of thymoquinone in human blood cells. Thymoquinone inhibited COX-2, NF-κB and proinflammatory cytokines in pancreatic ductal adenocarcinoma implicated in its anti-inflammatory activity

(Chehl et al. 2009) (**500**). Banerjee et al. (2010) synthesized novel thymoquinone analogs which are showed better anticancer activities than natural product thymoquinone against pancreatic cancer. Anticancer activities of thymoquinone analogs are associated with downregulation of NF-κB, COX-2 and other cell survival proteins (Banerjee et al., 2010). Yusufi et al. (2013) synthesized thymoquinone derivatives appended with gallate and found their antitumor activities against pancreatic cancer. These thymoquinone analogues docked on COX-2 protein cavity with better binding affinity than thymoquinone in molecular docking studies (Yusufi et al., 2013). Previously, Sethi et al. (2008) carried out mechanistic studies which demonstrated that thymoquinone inhibited NF-κB activation and NF-κB–regulated gene expression including COX-2, which makes it as a potential inhibitor of cancer cell survival, proliferation, invasion, and metastasis.

Thymoquinone enhanced anticancer activities of gemcitabine and oxaliplatin in pancreatic cancer cells (Banerjee et al. 2009). The study demonstrated the chemosensitization potential of thymoquinone in cancer cells. Moreover, thymoquinone exhibited safety profile in toxicity studies and inhibited drug-induced toxicity (Abdel-Wahab, 2015, Aycan et al. 2015). Based on available published reports, further detailed studies are needed including evaluation of its bioavailability, toxicity and clinical trials in humans to develop thymoquinone as a chemopreventive and therapeutic agent.

2.24. Triptolide

Triptolide, a diterpenoid isolated from *Tripterygium wilfordii*, has been reported to possess anti-inflammatory and immunomodulatory, and anticancer activities against broad range of cancers (Chen, 2001, Kapoor, 2009, Ma et al. 2013, Zhou et al. 2007). Triptolide suppressed cell proliferation, induced apoptosis, inhibited invasion, angiogenesis and metastasis by targeting multiple pathways, including AA pathway (Ma et al. 2013, Zhou et al. 2007, Johnson et al. 2011). Triptolide exhibited substantial anticancer activities against pancreatic cancer *in vitro*, *in vivo* and suppressed COX-2 and VEGF expression (Ma et al. 2013). These finding suggested that down-regulation of COX-2 and VEGF expression might be one of the molecular mechanisms by which triptolide inhibited the growth, induced apoptosis and suppressed angiogenesis of pancreatic tumor (Ma et al. 2013). Triptolide inhibited cell proliferation and induced

apoptosis in human pancreatic cancer cells and suppressed 5-LOX expression and production of LTB4. These findings demonstrated that triptolide exerted anticancer activity against pancreatic cancer by inhibiting of the 5-LOX pathway (Zhou et al. 2007). Sun et al. (2011) found that triptolide suppressed TNF-α-induced COX-2 expression in lung cancer A549 cells. Triptolide inhibited COX-2 expression, NF-κB activation, and phosphorylations of p38, ERK1/2 (p42/p44) and AKT proteins in neuronal cells (Geng et al. 2012). Park et al (2011) found that triptolide sensitized leukemic cells to apoptosis, and inhibited tumor by targeting TNF-TNFR1-TRADD-TRAF2-NIK-TAK1-IKK/NF-κB/COX pathway. Triptolide showed cytotoxic activity against human colon cancer cells (SW114) and myelocytic leukemia (K562) and inhibited productions of PGE2 and NO in cancer cells but not expression of COX-2 and iNOS (Tong et al. 2007). These finding clearly demonstrated that triptolide exerted anticancer property by inhibiting COX-2 and iNOS activities (Tong et al. 2007). Johnson et al. (2011) reported that triptolide inhibited proliferation and migration of colon cancer cells by suppressing cell cycle regulators, cytokine receptors and COX-2 expression.

Severe toxic effects and water-insolubility of triptolide are limited its clinical use (Liu, 2011). New water-soluble triptolide analogs have been synthesized and investigated for their utility as cancer chemopreventive agents (Liu, 2011). PG490-88, a triptolide analog, has been considered to be a safe and potent anticancer agent (Liu, 2011, Fidler et al 2003). PG490-88 has entered into Phase I clinical trial for treatment of prostate cancer in the United States (Liu, 2011). F60008, a semi-synthetic analog of triptolide, is converted to triptolide *in vivo* and induced apoptosis in human tumor cells. In Phase I dose-escalation study of F60008 in patients with advanced solid tumors, F60008 showed promising results that warranted further studies to develop F60008 as an anticancer and chemopreventive agent (Kitzen et al. 2009).

2.25. Ursolic acid

Ursolic acid, a natural pentacyclic triterpenoid distributed in plants, is emerging as a promising compound for cancer prevention and therapy (Shanmugam et al., 2013). Ursolic acid exerts anticancer effects through targeting multiple signaling pathways including AA pathway. Wang et al (2013) reported that ursolic acid inhibited cell

proliferation and induces apoptosis in colon cancer cells through simultaneous modulation of the multiple signaling pathways such as COX-2/PGE_2, Akt/ERK, and p300/NF-κB/CREB2 pathways. Shanmugam et al. (2012) previously reported that ursolic acid showed significant anticancer activity against prostate cancer using TRAMP mice model. Prostate tumor growth inhibitory effect of ursolic acid correlated with expression of COX-2 and other pro-inflammatory mediators. This study demonstrated that COX-2 inhibitory activity may be one of the mechanisms for anticancer activity of ursolic acid against prostate cancer. Tian et al. (2006) reported that ursolic acid effectively inhibited the proliferation of hepatocellular carcinoma using both *in vitro* and *in vivo* models. Further studies demonstrated that ursolic acid induced apoptosis and G0/G1 arrest in HepG2 hepatocellular cancer cells through cleavage of poly-(ADP-ribose)-polymerase (PARP) and downregulation of COX-2 (Tian et al., 2006). This study suggested that ursolic acid as a chemopreventive agent for liver cancer. Subbaramaiah et al., (2000) carried out few experiments using human mammary and oral epithelial cells to establish the mechanism involved in anticancer and chemopreventive activities of ursolic acid. The study demonstrated that ursolic acid acid inhibited PMA-mediated activation of PKC, ERK 1/2, JNK, and p38 MAPK. Additionally, ursolic acid blocked AP-1 activity and the binding of c-Jun to the cAMP response element of the COX-2 promoter that led to suppression of COX-2 expression (Subbaramaiah et al. (2000). This study confirmed anticancer and chemopreventive effects of ursolic acid through targeting COX-2 pathway. Recently, Cho et al. (2015) evaluated the effect of ursolic acid in combination with resveratrol in TPA-promoted skin cancer and found that this natural agent inhibited TPA-induced skin tumor promotion by targeting inflammatory mediators including COX-2.

Ursolic acid inhibited proliferation and induced apoptosis of gastric cancer SGC7901 cells through arresting cell cycle, inhibiting COX-2 expression to reduce PGE_2 production and down-regulating Bcl-2 expression (Zhang et al., 2006). Nataraju et al., (2007) found that ursolic acid inhibited GIIA $sPLA_2$ activity, and acts as anti-inflammatory agent. Several research findings demonstrate the potential chemopreventive effect of ursolic acid against various cancers. However, the hydrophobicity of ursolic acid increases the difficulty in its potential clinical application. To overcome this problem, Zhang et al. (2013) prepared nanoparticle-based delivery system to target ursolic acid effectively into cancer cells. Ursolic acid-

loaded nanoparticles were prepared by a nano-precipitation method using amphilic methoxy poly(ethylene glycol)-polycaprolactone block copolymers as drug carriers. Ursolic acid was easily transported into SGC7901 cells by nanoparticles and distributed around the nuclei of cancer cells (Zhang et al. 2013). Anticancer activities of ursolic acid and ursolic acid-loaded nanoparticles were correlated with COX-2 expression levels in SGC7901 cells. This study demonstrated that nanoparticle coating on ursolic acid improved cell permeability to target in side of the cancer cells there by targeted COX and other signaling molecules led to cytotoxicity (Zhang et al. 2013). Therefore, the study offered a new strategy to improve the antitumor potency of ursolic acid-loaded nanoparticles through nano-drug delivery system. Ursolic acid exhibits antigenotixic and antimutagenic properties (Slamenová et al., 2006, Miyazawa et al., 2005).

2.26. Wogonin

Wogonin, a flavonoid, is present in *Scutellaria baicalensis* and *Scutellaria barbata* and has been reported to exhibit anticancer and anti-inflammatory properties (Table 1.1) (Chen et al. 2008). Kimura and Sumiyoshi, (2013) reported that wogonin inhibited COX-2 expression in LPS-stimulated THP-1 macrophages. Wogonin inhibited COX-2 in tumor associated macrophages. Thereby, it exhibited antitumor and antimetastatic actions (Kimura and Sumiyoshi, 2013). Wogonin suppressed PMA-induced COX-2 mRNA and protein levels in human lung epithelial cancer cells (Chen et al. 2008). Wogonin inhibited COX-2 expression by blocking MEK1/2/AP-1 (Chen et al. 2008). Combination of 5-fluorouracil and wogonin inhibited growth of SMMC-7721 hepatocellular carcinoma by downregulating COX-2 expression (Zhao et al., 2013). Wogonin supressed COX-2 expression in UVB-treated HaCaT cells (Kimura and Sumiyoshi, 2011). The results of the previous studies demonstrated that wogonin exerted chemopreventive and anticancer potentials by blocking COX-2 pathway and warranted pre-clinical and clinical evaluation to develop it as a cancer preventive/therapeutic agent.

2.27. Other AA pathway inhibitory natural products

6-Shogaol (ginger), caffeic acid (coffee), methyl jasmonate (many plants), withanolides (*Withania somnifera*), rosmarinic acid (*Cordia verbenacea*) and chebulagic acid (*Terminalia chebula*) are some of the other important natural agents, which have been identified and characterized as chemopreventive and anticancer potentials against several cancers by targeting AA pathway. 6-Shogaol, a constituent of ginger, has been found to possess chemopreventive potentials against several cancers by inhibiting COX-2 pathway (Prasad and Tyagi, 2015, Wu et al., 2010, Gan et al., 2013). 6-shogaol potentiated antitumor activity of gemcitabine against human pancreatic tumors to by blocking TLR4/NF-κB/COX-2 mediated pathway (Zhou et al. 2014). Caffeic acid exhibits cancer chemopreventive potentials by inhibiting COX-2 expression (Kang et al. 2009, Kuo et al., 2015). Withanolides are found in aswagandha (*Withania somnifera*), which have been found to possess selective COX-2 inhibition and tumor growth supressive activities (Mulabagal et al. 2009, Prabhakaran et al., 2012). Methyl jasmonate, a plant stress hormone, induced apoptosis in human prostate cancer cells by 5-LOX mediated pathway (Ezekwudo et al., 2007). Scheckel et al., (2008) found that rosmarinic acid suppressed AP-1-mediated COX-2 expression in human cancer and non-malignant cells. Anticancer and anti-inflammatory activities of rosmarinic acid were also reported previously.

Chebulagic acid is isolated from the fruits of *Terminalia chebula* as a COX-2 and 5-LOX dual inhibitor. Further *in vitro* studies demonstrated that chebulagic acid exhibits antiproliferative activities against several cancer cell lines and induces apoptosis in COLO-205 cells (Reddy et al. 2009 (a)). Chebulagic acid downregulates COX-2 and 5-LOX in LPS- stimulated RAW 264.7 by blocking NF-κB/MAPK mediated signaling pathways (Reddy et al. 2009 (b)). Capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide), a principal pungent ingredient of hot red and chili peppers, has been reported to possess cancer chemopreventive potentials by targeting COX-2 pathway (Oyagbemi et al., 2010). Some reports demonstrated that capsaicin exhibits carcinogenic property by activating several pro-carcinogenic mediators including COX-2 (Liu et al., 2015). However, the role of capsaicin in carcinogenesis is controversial and warrants further investigation (Liu et al., 2012).

Overall, research on development of AA pathway inhibitory natural products in cancer prevention and therapy is in good progress. However, some of the previous reports suggest that several AA pathway inhibitory natural products exhibit toxic effects. Curcumin is characterized as non-toxic and "Generally Recognized As Safe" (GRAS) by the US FDA. However, recently a committee of the FDA voted to prohibit its use intravenously due to inadequate evidence of safety or efficacy. This decision is not yet law in the US, and is currently being challenged. Recent reports on genotoxic effects of (6)-gingerol are controversial and need further investigations. NDGA was once classified as "Generally Recognized As Safe" by US FDA but later this classification was withdrawn due to its toxicity concerns. Toxic effect of NDGA is a major problem in clinical use of NDGA. Eugenol, a cancer chemopreventive agent, exhibits genotoxic and carcinogenic properties. Poor bioavailability of the AA pathway inhibitory natural products is a major problem for their clinical development as cancer chemopreventive and therapeutic agents. In this context, discovery of new inhibitors of AA pathway from natural sources are needed for their development as effective and safe drugs for cancer prevention and therapy.

The present investigation has been undertaken with following objectives

Objectives of the research work

- To screen selected medicinal plants for arachidonic acid metabolizing enzymes (PLA2, COX and 5-LOX) inhibition and selection of plant extract (*Borassus flabellifer* seed coat) with potent activity for isolation of active principle.

- To isolate COX and 5-LOX dual inhibitory dammarane triterpenoid 1 from *Borassus flabellifer* seed coat extract according 5-LOX assay guided fractionation using silica gel column chromatography.

- To elucidate the chemical structure of COX and 5-LOX dual inhibitory dammarane triterpenoid 1 using various advanced spectroscopic techniques including, ^{1}H & ^{13}C NMR, mass spectroscopic and elemental analyses.

- To evaluate anti-inflammatory activity of COX and 5-LOX inhibitory dammarane triterpenoid 1 using *in vitro* and *in vivo* methods.

- To evaluate antiproliferative and pro-apoptotic activities of COX and 5-LOX inhibitory dammarane triterpenoid 1 on different cancer cell lines using *in vitro* methods.