Journal of Animal and Veterinary Advances 9 (7): 1099-1107, 2010
ISSN: 1680-5593


# Biochemical and Physiological Evaluations of Limonoids as Potential Cancer Destroyers

[1]Vahdettin Bayazit and [2]Vahit Konar
[1]Department of Biology, Faculty of Arts and Sciences, Alparslan University, 49100, Mus, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Firat University, Elazig, Turkey

**Abstract:** The aim of this study was evaluated the anticancerogen properties of limonoids and their biochemical structures. The term limonoids was derived from limonin, the first tetranortriterpenoid obtained from citrus bitter principles. Compounds belonging to this group have exhibited a range of biological activities like insecticidal, insect antifeedant and growth regulating activity on insects as well as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities on humans. Limonoids found in citrus fruits have been able to stop the progression of neuroblastomas in the laboratory. It also produces no side effects unlike the currently used toxic cancer drugs. Further research is needed to determine how the substance achieves this effect and how it may be used in human cancers. Both flavenoids and limonoids may be capable of stopping, slowing or killing cancer cells. Ultimately, these compounds could be used as food additives as a preventative measure. The effects of D-limonene and citrus, orange oil and lemon oil on induced neoplasia of the lungs and forestomach of female mice inhibited pulmonary adenoma formation and the occurrence of forestomach tumors, thus demonstrating that non-nutrient constituents of the diet may inhibit carcinogen-induced neoplasia.

**Key words:** Limonoids, cancer, anti material, biochemical structures, biological activities, Turkey

## INTRODUCTION

Limonoids are naturally-occurring compounds found in citrus fruit. This multidisciplinary, multi-state, multi-institute integrated project will focus on the isolation, purification, identification and evaluation of biological activities of limonoids in citrus. The reactivities of limonoids will be tested based on several enzymatic, tumor cell and animal models in order to evaluate the impact of specific limonoids on oral and colon cancer and the ability of limonoids to detoxify certain carcinogens, cancer-causing compounds (Roy and Saraf, 2006). Citrus limonoids have potential for prevention of certain chronic diseases such as cancer and cardiovascular diseases. Exploring biological activities of limonoids is very essential. Educating dieticians and graduate students about phytochemicals is needed to improve human consumption of fruits and vegetables.

Citrus fruits possess a wide variety of bioactive compounds with health-promoting, disease-preventing properties that have been shown to be effective against cancer. Unique among these and less studied are the limonoid glucosides, a class of furan-containing triterpenes found mainly in the Rutacaeae and Meliacease families of fruits and that differ from flavonoids in chemical structure. To date, 53 limonoids have been identified and characterized. Although, limonoid aglycones are water-insoluble compounds that are responsible for a bitter taste in fruits, the limonoid glucosides are water-soluble and tasteless. Two enzymes, UDPG-limonoid glycosyl transferase and limonoid D-ring lactone hydrolase, perform the interconversion of free to carbohydrate-bearing moieties. The transferase gene has been isolated and cloned. Recent studies demonstrated health benefits and chemopreventive action from limonoid ingestion or treatment. For example, limonoid aglycones or glucosides at micromolar concentrations were shown to restrict HIV replication in human mononuclear cells, act in the capacity of antimalarial and anti-inflammatory agents and inhibit proliferation of breast cancer cells in culture (Guthrie *et al*., 2000). In animal models, these compounds have antineoplastic activity against chemically induced cancers of the colon, stomach, buccal pouch and blood.

Limonoid glucosides taken through the diet reportedly lowered serum cholesterol. Some if not all of these properties appear to be associated with the effects on cells and cell growth. This report focuses on the mechanism for blocking cancer cell growth (Kweon *et al*., 2004; Poulose *et al*., 2005). Limonoids are secondary

**Corresponding Author:** Vahdettin Bayazit, Department of Biology, Faculty of Arts and Sciences, Alparslan University, 49100, Mus, Turkey

metabolites produced in plants found in the order Rutales. Within this order, limonoids are most often found in the family Meliaceae and less frequently in the families Rutaceae and Cneoraceae. Limonoids are described as modified triterpenes having a 4,4,8 trimethyl-17 furanyl steroid skeleton. Arrangements of subgroups and ring structures within this basic building block provide a host of characteristics that have generated interest in this plant product.

These characteristics include insecticidal, insect growth regulation, insect antifeedant and medicinal effects to animals and humans such as antibacterial, viral and antifungal properties. Of recent great interest, limonoid's possible anticarcinogenic properties are being explored. Limonoid Anticarcinogenis and Antimutagenic activity. Some of the most exciting applications of limonoids and compounds derived from them are their use in the treatments of specific cancers.

Limonin, nomilin, 12, hydroxyamdorastatin and isofraxinellone are limonoids or their derivatives that have been shown successful in treatments with *in vitro* bioassays on human tumor cell lines. Limonin and nomilin were described earlier as being bitter principals for citrus fruits. Both limonoids have been found to induce increased activity of the detoxifying enzyme glutathione-S-transferase.

The increased enzyme activity was correlated with the ability of these compounds to inhibit chemically induced carcinogenisis in laboratory animals. Administration of nomilin by gavage to a specified strain (ICR/Ha) of mice reduced the incidence and number of forestomach tumors per mouse induced by Benzo(a) Pyrene (BP), a potent epoxide former. Addition of nomilin to the diet at various concentrations inhibited BP induced mice lung tumor formation. This was attributed to the limonoid's inhibition of the formation of BP-DNA adducts in the lung.

Topical application of limonoids was found to inhibit both the initiation and promotion phases of carcinogenisis in the skin of (SENCAR) mice. Nomilin appeared to be more effective during initiation stage induced carcinomas, while limonin was more potent as an inhibitor during the promotion phase of the carcinogenisis (Hasegawa *et al*., 1989). These and other findings suggest citrus limonoids may be useful as cancer chemo-preventative agents.

Recent studies into induced oral cancers in hamsters suggests that limonin can act as a capture chemical, intercepting compounds such as benzo(a)pyrene and other mutagens before the formation of adducts to cell macromolecules. Since oral cancers are on the rise in human populations, the treatment by an easily isolated and obtained compound would be of great interest to the oncology profession. Current literature for the last two years is devoted to the identification of Rutaceae and Meliaceae species containing limonoids.

Extraction and isolation methods make up a bulk of recent publications with eventual study of the limonoids as to biological activity exhibited toward insect species. Lethal concentrations to 50% of test insect speicies (LC50) of most limonoids studied fall in the range of 50 parts per million (ppm) or lower with some exhibiting LC50 s as low as 0.625 ppm depending on the insect studied.

The effect of ring structure and chemical oxidation state parameters is a focus of why limonoids exhibit activity against insect herbivores. The variety of additional biological properties exhibited by limonoids has also contributed to their interest to the scientific community (Jacob *et al*., 2000; Poulose *et al*., 2005). This study reviews the inhibition effects of limonoid components on various cancer or tumour cells.

**Biochemistry and anticancerogenic activities of limonoids:** Nutritional research on the health benefits of substances in plant foods has recently advanced to a new stage. The research frontier has moved from study of classical vitamin deficiency diseases to study of the thousands of phytochemicals that may have important physiological effects.

Recent research suggests that citrus fruit consumers may be getting another health benefit from orange juice and other citrus products called limonoids, which appear to possess substantial anticancer activity. Limonoids are highly oxidized triterpenes present in Rutaceae and Maliaceae families. Several citrus limonoids have recently been subjected to anticancer screening utilizing laboratory animals and human breast cancer cells.

The experimental results described that citrus limonoids may provide substantial anticancer actions. The compounds have been shown to be free of toxic effects in animal models, so potential exists for the use of limonoids against human cancer in either natural fruits in citrus fortified with limonoids or in purified forms of specific limonoids. Although, the initial studies are very promising they have been conducted primarily with *in vitro* cell culture and animal models. Thus, research is needed to determine whether the limonoids may be useful in preventing or treating cancer in humans (Maier *et al*., 1977; Patil *et al*., 2007).

Limonoids are unique highly oxygenated triterpenoid compounds long recognized as significant biologically

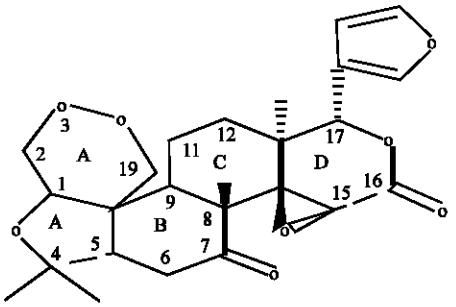

Fig. 1: Limonin ($C_{26}H_{30}O_8$, molar mass 470.52 g $mol^{-1}$, other names limonoate D-ring-lactone, limonoic acid di-delta-lactone)

Table 1: Activity of citrus limonoids against proliferation of breast cancer cells

| Limonoid tested | Estrogen MCG-7 (+) Dependent[a] | Estrogen MDA-MB -435 (-) Independent[a] |
|---|---|---|
| Limonin | 2.00 | 12.50 |
| Limonin glucoside | 35.00 | 75.00 |
| Nomilin | 0.05 | 0.04 |
| Limonoid glucoside mixture | 0.05 | 0.08 |
| Tamoxifen | 0.04 | 90.00 |

[a]IC50 in mg $mL^{-}$ (concentration of test compound that inhibits growth by 50%)

active natural compounds. Citrus limonoids appear in large amounts in citrus juice and citrus tissues as water soluble limonoid glucosides or in seeds as water insoluble limonoid aglycones (Maier *et al*., 1977; Ozaki *et al*., 1995; Tanaka *et al*., 2000a, b, 2001). General formula of limonin was given Fig. 1.

Limonin is a limonoid and a bitter white, crystalline substance found in orange and lemon seeds. It is also known as limonoate D-ring-lactone and limonoic acid di-delta-lactone. Chemically, it is a member of the class of compounds known as furanolactones. Limonoid glucoside concentrations can reach levels of 350-400 ppm in orange juice. The limonoid aglycones are responsible for the development of delayed bitterness in citrus (Maier *et al*., 1977) and are converted to the non-bitter limonoid glucosides during fruit maturation (Hasegawa *et al*., 1989; Maier *et al*., 1977; Poulose *et al*., 2005).

Several citrus limonoids have recently been subjected to anticancer screen procedures utilizing laboratory animals and human breast cancer cells in culture. In mice, it was found that five limonoid aglycones (limonin, nomilin, obacunone, isoobacunoic acid, ichangin) induced significant amounts of Glutathione-S-Transferase (GST) in the liver and intestinal mucosa (Lam and Hasegawa, 1989; Lam *et al*., 1994; Maier *et al*., 1977). GST is a major detoxifying enzyme system which catalyzes the conjugation of glutathione with many potentially carcinogenic compounds which are highly electrophilic in nature.

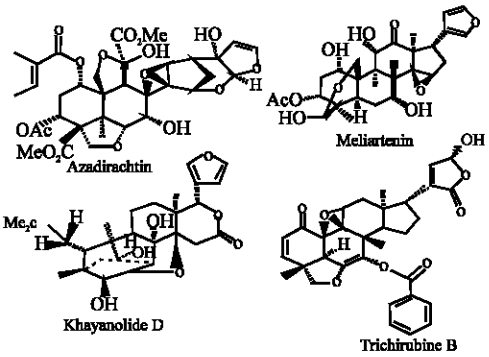


Fig. 2: Examples of structural complexicities in limonoids of Meliaceae

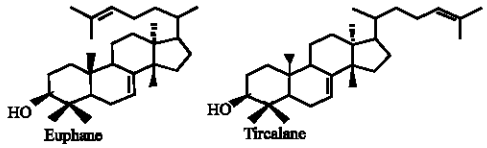


Fig. 3: Precursor of limonoids

A study of the inhibitory effects of two limonoid aglycones (limonin and nomilin) on the formation of benzo(a)pyrene induced neoplasia in the forestomach of ICR/Ha mice showed that incidence of tumors could be reduced by >50% at 10 mg dose (Lam and Hasegawa, 1989).

In hamster, limonin was found to be a potent inhibitor of 7, 12-dimethylbenz(a)anthracene induced oral carcinogenesis (Miller *et al*., 1989). Topical application showed a 60% reduction in tumor burden. Nomilin was less effective. Topical application of the limonin glucoside to the same oral tumors in hamsters showed a 55% reduction in tumor burden (Miller *et al*., 1992).

This observation has added significance considering the predominance of limonin glucoside among the large amount of limonoid glucosides present in fruit tissues and juice. Most recently several limonoid aglycones and a mixture of limonoid glucosides were administered *in vitro* to estrogen dependent and estrogen independent human breast cancer cell lines (Table 1) (Guthrie *et al*., 2000).

The results showed that the limonoids were equally potent as tamoxifen for inhibiting the proliferation of estrogendependent breast cancer cells and more potent

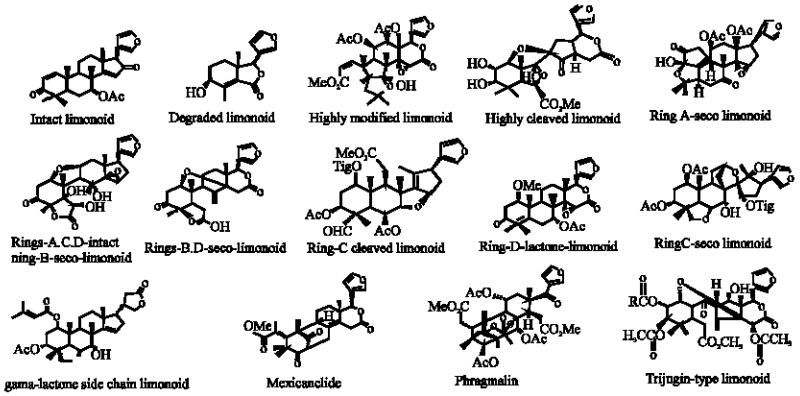


Fig. 4: Types of limonoids

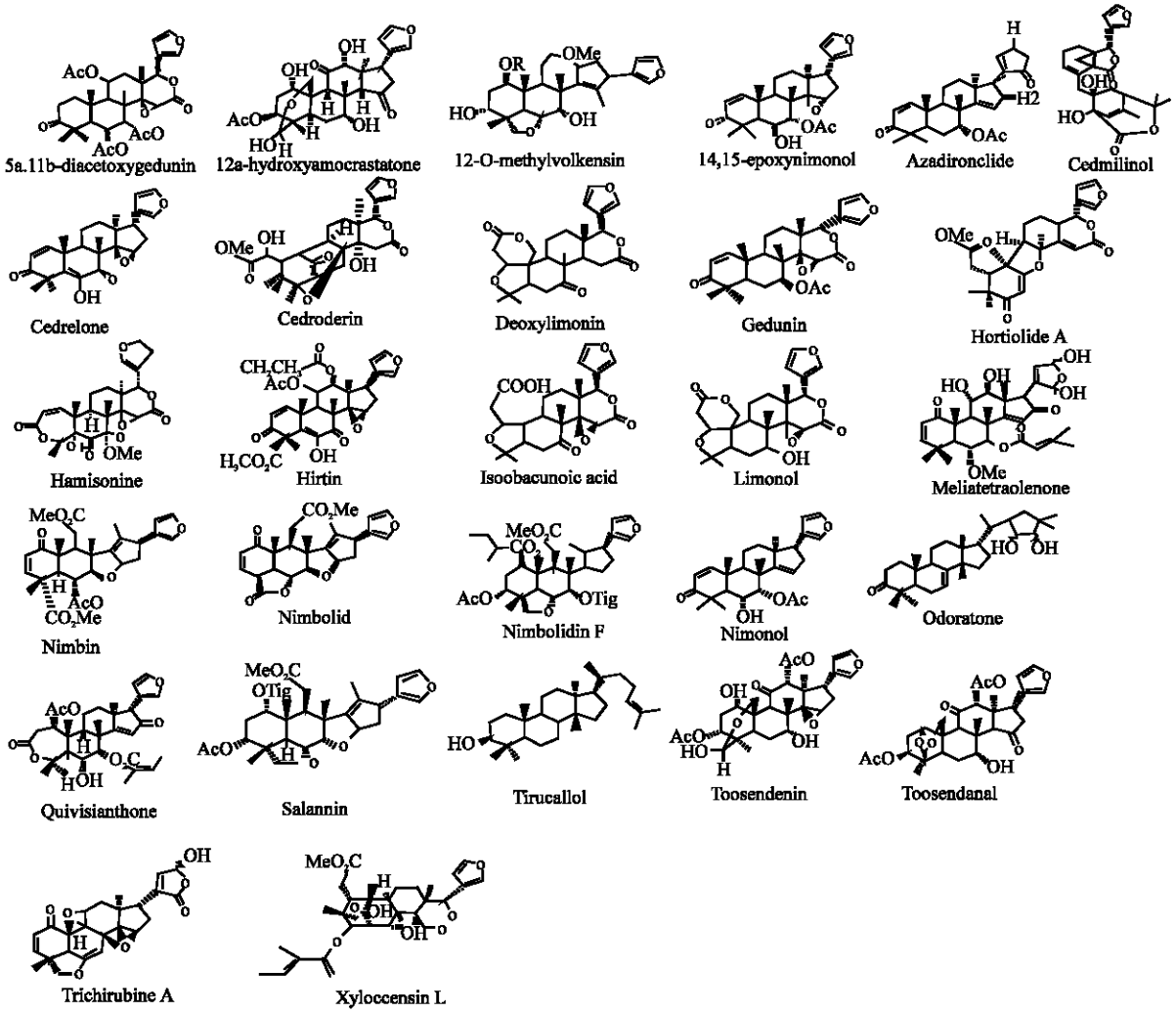


Fig. 5: Biologically important limonoids

than tamoxifen for activity against estrogen-independent cancer cells. Structural complexicities in limonoids of Meliaceae were given in Fig. 2. Limonin and obacunone fed to rats have also been shown to increase GST and quinone reductase activities in liver and colon mucosa ana was correlated with the prevention of colon

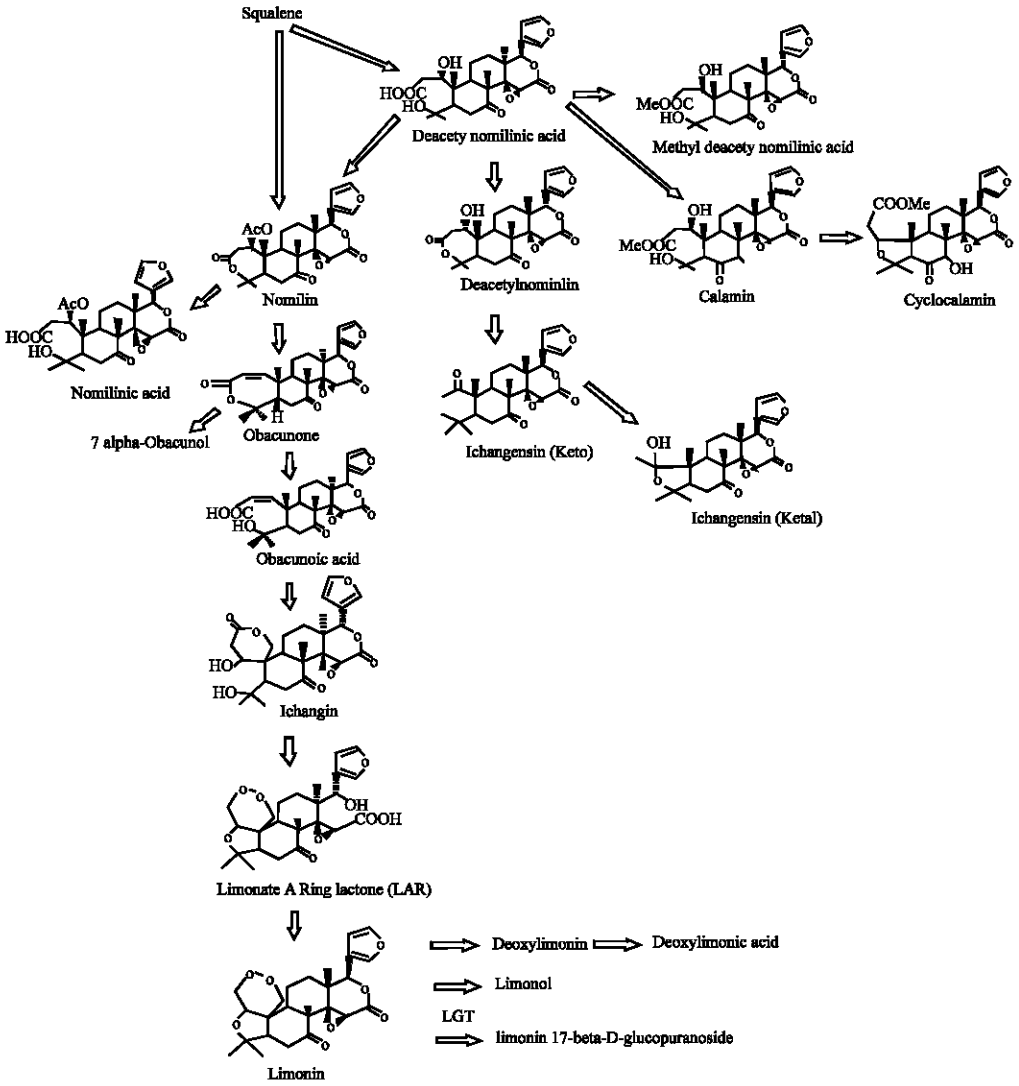


Fig. 6: Biosynthetic pathways of limonoids in Citrus

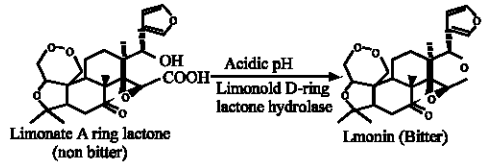


Fig. 7: Machanism of delayed bitterness

carcinogenesi in rats (Jacob *et al*., 2000; Ozaki *et al*., 1995). Some limonoids and the chemical structures of limonin, nomilin and their glucoside derivatives are shown in Fig. 3-7.

## RESULTS AND DISCUSSION

Limonoids are heavily oxygenated, modified triterpenes dominant in the plant family Meliaceae and to a lessor extent in Rutaceae. The latter has received considerable attention due to the bitter taste of most limonoids Rutaceae includes the citrus species of commerce. The major limonoid in this family is limonin, accountable for the bitterness of poor quality juices. Other members of the Meliaceae are far more common in tropical flora and bring important examples of structural diversity and impressive topography in the three dimensional display of chemical functionality an untapped resource.

Limonoids are of moderate polarity being insoluble in water soluble in DMSO, ethanol, toluene; insoluble in hexane. Citrus limonoids are highly oxigenated triterpenoids (Lam and Hasegawa, 1989; Lam *et al*., 1994). Limonoids are abundantly present in Rutaceae (citrus fruits) and Meliaceae (neem) families.

Table 2: Chief sources of limonoids

| Family | Plant species | Plant part |
|---|---|---|
| Meliaceae | *Agalia andamanica* | Leaves |
| | *Azadirachta indica* | Seed oil, leaves, seeds |
| | *Carapa granatum* | Fruits, leaves |
| | *Cipadessa fruticosa* | Fruits |
| | *Khaya anthotheca* | Stem bark, fruits |
| | *Melia azedarach* | Leaves, ripe fruits, kernel |
| | *Munronia henryi* | Whole plant |
| | *Quivisia papinae* | Seeds |
| | *Sandoricum koetjape* | Leaves |
| | *Swietenia mahogany* | Stem bark |
| | *Teucrium tomentosum* | Aerial parts |
| | *Trichilia pallida* | Roots, leaf, seeds |
| | *Trichilia rubescens* | Leaf |
| | *Turraea floribunda* | Seeds |
| | *Turraea wakefieldii* | Root bark |
| Rutaceae | *Bouchardatia neurococca* | Aerial parts |
| | *Citrus reticulata* | Seeds |
| | *Citrus unshiu* | Peels |
| | *Clausena excavate* | Rhizomes and roots |
| | *Hortia colombiana* | Wood |
| | *Raulinoa echinata* | Stems and leaves |
| Simaroubaceae | *Harrisonia abyssinica* | Root bark |
| | *Harrisonia perforate* | Leaves |

Sources of some limonoids were given in Table 2. Thirty six-limonoid aglycones and seventeen limonoid glucosides have been isolated from Citrus and its hybrids. Limonoids impart bitterness to citrus juices and not favored by citrus juice industry for long time. However, bitter limonoid aglycones turn to glucosides with maturity of fruit and limonoid glucosides are tasteless and water soluble. Recent research results shows that limonoids has many biological functions: anticancer activity; antifeedant activity against insects and termites. Limonoids have shown to reduce the risk of following cancers: oral cavity, larynx, esophagus, stomach, pancreas, lung, colon and rectum. More than twenty epidemological studies suggest an inverse relation between consumption of citrus fruits and many types of cancer. Limonoids have ability to induce detoxifying enzyme system, glutathione S-transferase and this may be the possible mode of action of limonoids in cancer chemoprevention (Jacob *et al*., 2000).

There have been enourmously experimental studies concerning DNA, limonoids, tamoxifen, interleukin ana interferon, cancer cells. Limonoids have been shown to inhibit breast cancer cellular proliferation in Estrogen Receptor negative ($ER^-$) ana positive ($ER^+$) cells. Nude mice were given limonoids in both treatment ana adjuvant-based models to observe their effects on the proliferation of established ana resected tumors, respectively. It was found that limonoids, particularly limonin slowed down tumour grwth ana was able to prevent or delay the regrowth of resected tumours in these models. Additionaslly, limonoids were detected in mammary tissue samples of mice from the treatment model experiment indicating that these compounds are bioavailable and were responsible for the observed results.

Limonoids are highly oxygenated, modified terpenoids with a prototypical structure either containing or derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton. All naturally occurring citrus limonoids contain a furan ring attached to the D-ring at C-17 as well as oxygen containing functional groups at C-3, C-4, C-7, C-16 and C-17. The structural variations of limonoids found in Rutaceae are less than in Meliaceae and are generally limited to the modification of A and B rings, the limonoids of Meliaceae are more complex with very high degree of oxidation and rearrangement exhibited in the parent limonoid structure. As a health conscious society with increasing concern and desire for effective cancer treatments, the limonoids are extremely attractive. With over 300 similar compounds, the bioassay of known limonoids for their anticancer and antimutagenic activities will encourage much more investigation. Like many of their secondary plant metabolite cousins, limonoids appear to be a source of countless possible resources that can benefit the human race (Kweon *et al*., 2004).

Extraction methods too should be optimized evaluation and establishment of pharmaco-dynamic and kinetic principles and structure activity relationships should be a key goal associated with limonoids so that they can be safely introduced in the arsenal of pharmaceuticals to safeguard the humanity from the wrath of disease and its discomfort. Recent studies demonstrated health benefits and chemopreventive action from limonoid ingestion or treatment. For example, limonoid aglycones or glucosides at micromolar concentrations were shown to restrict HIV replication in human mononuclear cells act in the capacity of antimalarial and anti-inflammatory agents and inhibit proliferation of breast cancer cells in culture. In animal models, these compounds have antineoplastic activity against chemically induced cancers of the colon, stomach, buccal pouch and blood.

Limonoid glucosides taken through the diet reportedly lowered serum cholesterol. Some if not all of these properties appear to be associated with the effects on cells and cell growth. This study focuses on the mechanism for blocking cancer cell growth (Miller *et al*., 1989, 1992, 2004; Patil *et al*., 2007; So *et al*., 1996). Oranges rich in vitamin C offer another important yet lesser-known

nutritional bonus: citrus limonoids. In laboratory tests with animals and with human cells, citrus limonoids have been shown to help fight cancers of the mouth, skin, lung, breast, stomach and colon. Citrus limonoids are responsible for the bitter taste in citrus fruits. The most prevalent limonoids are limonin and nomilin. They are present in the rutaceous plants that include lemon, lime, orange and grapefruits. An important characteristic of this class of compound is a substituted furan moiety.

It has been determined by animal studies that citrus limonoids and derivatives have certain biological activities that may be used as chemopreventive agents for cancer. Glutathione S-Transferase (GST) is a major detoxifying enzyme system that catalyses the conjugation of glutathione with electrophiles that induce activated carcinogens. The glutathione conjugates are usually less reactive and more water soluble and hence, facilitate excretion. An increase in GST activity caused by a substance is therefore an elevation in the mechanism that protects against the noxious effects of xenobiotics including carcinogens.

Many chemicals that are GST enhancers have been found to inhibit chemically induced carcinogenesis 12, 13. The structures of the naturally occurring furanoids that have been found to induce GST activity range from the simple 2-alkyl substituted compound 2-n-heptyl furan and the sulphur analogue 2-n-butyl thiophen formed during the roasting of meat to more complex molecules such as kahweol and cafestol, isolated from gren coffee beans and salannin identified in the seed of the mythical neem tree of India.

The presence of the furan moiety appears to be essential for enzyme induction. When the furan ring in cafestol is saturated by hydrogenation, its activity as a GST inducer is lost. In the case of limonin and nomilin, the triterpene structure appears to play a part in determining the relative GST inducing activity of these compounds. The structure activity relationships of limonin and nomilin have been studied and discussed.

The chemical structures of limonin, nomilin and their glucoside derivatives are shown in. A great number of epidemiological studies have shown that citrus fruit consumption is protective in a variety of human cancers.

It is presumed that most if not all of his protective effect is due to vitamin C. This suggests that citrus fruits contain not one but multiple cancer chemopreventive agents. Citrus fruits including oranges, lemons, limes and grapefruits are a principal source of such important nutrients. They contain vitamin C, folate and dietary fibre and other bioactive components such as carotenoids and flavonoids, which are suggested to be responsible for the prevention of degenerative disease. Citrus fruits are particularly high in a class of phytochemicals known as the limonoids 3. Therefore, citrus fruits could be categorised as functional foods containing components shown to have health promoting and anticancer activities. These components include the bitter substances limonoids, ascorbic acid (vitamin C), carotenoids (especially β-carotene), folate, flavonoids and dietary fibres and have been shown to prevent a variety of cancers and cardiovascular diseases (Kurowska and Manthey, 2004; Silalahi, 2002).

This terpene subclass, found in citrus fruit peels, appears to be specifically directed to protection of lung tissue. In one study, a standardized extract of d-limonene, pinene and eucalyptol was effective in clearing congestive mucus from the lungs of patients with chronic obstructive pulmonary disease. Additionally, limonoids may be specific chemopreventive agents. In animal studies, results suggest that the chemotherapeutic activity of limonoids can be attributed to induction of both Phase I and Phase II detoxification enzymes in the liver. Cancer is caused by an abnormal overgrowth of cells with >100 cancer subtypes depending on which cell grows. In many cancers, the cells clump together to form solid tumors but in some the cells are dispersed around the blood stream (leukemia) or the lymphatic system (lymphoma). Prognosis of cancer has improved greatly in modern times owing to treatment advances and early detection programs. However, although survival rates have improved, cancer still remains the 2nd top cause of death, second only to heart disese in the USA.

Misdiagnosis of cancer is naturally possible but not common if professional medical advice is sought because physicians will usually perform comprehensive diagnostic testing if cancer is a possibility. Diagnostic tests have also improved in accuracy including newer tumor marker blood tests. Self-diagnosis of cancer is usually incorrect and quite common is for people to fear that they have cancer based on a symptom (e.g., weight loss, persistent cough, lumps or frequent urination), only to find out they have other less severe conditions (Loo, 2003).

On the other hand, sadly common is for people to have cancer but be unaware of it because many types have a slow insidious onset without early symptoms. For this reason, regular screening for particular types of cancer is valuable in preventing severe cancer cases. There are a number of cancer treatments including surgery, radiation therapy, anticancer drugs and biological therapy. Patients with cancer often are treated by a team of specialists, which may include a medical

oncologist, a surgeon, a radiation oncologist and others. The doctors may decide to use one type of treatment alone or a combination of treatments. The choice of treatment depends on the type and location of the cancer, the stage of the disease, the patient's general health and other factors. Limonoids in large concentrated therapeutic doses (500 mg) in humans have been reported to support detoxication of hormones and related substances that can have a negative effect on cellular DNA and cell proliferation (Block, 1991; Milner, 1994; Tian *et al*., 2001).

The future course of their study focus may well evolve into their use against cancer, bacteria, viruses and fungi. Antitumor activities of certain limonoids have been showed inhibition of MCF-7, CYP1A1, CYP1B1 and CYP3A4 and enhancing apoptosis through downstream induction of Caspase 3/7 in cell culture. Furthermore, animal studies have been demonstrated induction of glutathione S-transferase and enhancing apoptosis. In another study, long term exposure of limonoids seems to suggest that high levels of limonoids caused potentially unsafe changes. Considering the potential benefits of citrus bioactive components and little toxicity to human health, studies have been conducted to enhance bioactive compounds through several preharvest and postharvest storage conditions. Limonoids are either stable or increased at marginal levels. Compounds through several preharvest and postharvest storage conditions. Limonoids are either stable or increased at marginal levels (Block, 1991; Milner, 1994; Tian *et al*., 2001; Lam and Hasegawa, 1989; Poulose *et al*., 2005).

## CONCLUSION

Cancers are neuroblastomas often found in the neck, chest, spinal cord or adrenal gland. Limonoids found in citrus fruits have been able to stop the progression of neuroblastomas in the lab. It also produces no side effects unlike the currently used toxic cancer drugs. Further research is needed to determine how the substance achieves this effect and how it may be used in human cancers. Both flavenoids and limonoids may be capable of stopping, slowing or killing cancer cells. Ultimately these compounds could be used as food additives as a preventative measure. Limonoids have effects of natural cancer inhibitor.

Therefore, limonoids can prevent tumorogenesis and development of cancer cells. Recent research suggests that some natural chemical compounds found in fruit may help prevent certain types of cancer . The compounds are known as limonoids and were tested on rats. The results showed that limonoids caused an increase in the secretion of an anti-cancer enzyme in the rats' stomachs providing them with added protection against stomach tumors and stomach cancers. Limonoids are the natural compounds that create the slightly bitter taste in some fruits including oranges, lemons, limes and grapefruit. Citrus fruits are full of flavonoids, a type of antioxidant that has anti-inflammatory and anti-tumor properties.

Flavonoids inhibit tumor cell growth and can activate important detoxifying enzymes. These polyphenols called limonoids inhibit tumor formation by stimulating a detoxifying enzyme, which catalyzes the formation of less toxic and more water-soluble compounds that can be easily excreted from the body. Polyphenols cause the bitter taste in citrus fruits and tanginess in many fruits and teas.

## REFERENCES

Block, G., 1991. Vitamin C and cancer prevention: The epidemiologic evidence. Am. J. Clin. Nutr., 53: 270s-282s.

Guthrie, N., S. Hasegawa, G. Manners and T.A. Vandenberg, 2000. Inhibition of Human Breast Cancer Cells by Citrus Limonoids. In: Citrus Limonoids: Functional Chemicals in Agriculture and Foods, Berhow, M., S. Hasegawa and G. Manners (Eds.). American Chemical Society, United States, pp: 164-174.

Hasegawa, S., P. Ou, C.H. Fong, Z. Herman, C.W. Coggings and D.R. Atkin, 1989. Changesin limonoate A-ring lactone and limonin 17-$-D-glucopyranoside content of navel oranges during fruit growth and maturation. J. Agric. Food Chem., 39: 262-265.

Jacob, R., S. Hasegawa and G. Manners, 2000. The potential of citrus limonoids as anticancer agents. Perishables Handl. Q., 8: 6-8.

Kurowska, E.M. and J.A. Manthey, 2004. Hydrolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. J. Agric. Food Chem., 52: 2879-2886.

Kweon, S.M., Z.W. Lee, S.J. Yi, Y.M. Kim, J.A. Han, S.G. Paik and S.S. Ha, 2004. Protective role of tissue transglutaminase in the cell death induced by TNF-alpha in SH-SY5Y neuroblastoma cells. J. Biochem. Mol. Biol., 37: 185-191.

Lam, L.K. and S. Hasegawa, 1989. Inhibition of benzo(a)pyrene-induced forestomach neoplasia in mice by citrus limonoids. Nutr. Cancer, 12: 43-47.

Lam, L.K.T., J. Zhang and S. Hasegawa, 1994. Citrus limonoid reduction of chemically induced tumorigenesis. Food Technol., 1994: 104-108.

Loo, G., 2003. Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation. J. Nutr. Biochem., 14: 64-73.

Maier, V.P., R.D. Bennett and S. Hasegawa, 1977. Limonin and Other Limonoids. In: Citrus Science and Technology, Nagy, S., P.E. Shaw and M.K. Veldhuis (Eds.). Vol. 1., AVI Publishing Company, Inc., USA., pp: 355-396.

Miller, E.G., R. Fanous, F. Rivera-Hidalgo, W.H. Binnie, S. Hasegawa and L.K.T. Lam, 1989. The effect of citrus limonoids on hamster buccal pouch carcinogenesis. Carcinogenesis, 10: 1535-1537.

Miller, E.G., A.P. Gonzales-Sanders, A.M. Courillon, J.M. Wright, S. Hasegawa and L.K.T. Lam, 1992. Inhibition of hamster buccal pouch carcinogenesis by limonin 17-beta-D-glucopyranoside. Natr. Cancer, 17: 1-7.

Miller, E.G., J.L. Porter, W.H. Binnie, I.Y. Guo and S. Hasegawa, 2004. Further studies on the anticancer activity of citrus limonoids. J. Agric. Food Chem., 52: 4908-4912.

Milner, J.A., 1994. Reducing the Risk of Cancer. In: Functional Foods. Designer Foods, Pharma Foods, Nutraceuticals, Goldberg, I. (Ed.). Chapman and Hall, New York, London.

Ozaki, Y., S. Ayano, N. Inaba, M. Miyake, M.A. Berhow and S. Hasegawa, 1995. Limonoid glucosides in fruit, juice and processing by-products of Satsuma Mandarin (*Citrus unshiu* Marcov.). J. Food Sci., 60: 186-189.

Patil, B., G.K. Jayaprakasha and E.D. Harris, 2007. Impact of citrus limonoids on human health. Acta Hortic., 744: 127-134.

Poulose, S.M., E.D. Harris and B.S. Patil, 2005. Citrus limonoids ýnduce apoptosis in human neuroblastoma cells and have radical scavenging activity. J. Nutr., 135: 870-877.

Roy, A. and S. Saraf, 2006. Limonoids: Overview of significant bioactive triterpenes distributed in plants kingdom. Biol. Pharma. Bull., 29: 191-201.

Silalahi, J., 2002. Anticancer and health protective properties of citrus fruit components. Asia Pacific. J. Clin. Nutr., 11: 79-84.

So, F.V., N. Guthrie, A.F. Chambers, M. Moussa and K.K. Carroll, 1996. Inhibition of breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices. Nutr. Cancer, 25: 167-181.

Tanaka, T., H. Kohno, K. Kawaqbata, S. Honjo and M. Miyake and K. Wada, 2000a. Citrus Limonoids Obacunone and Limonin Inhibit the Development of a Precursor Lesion, Aberrant Crypt Foci for Colon Cancer in Rats. In: Citrus Limonoids: Functional Chemicals in Agriculture and Foods, Berhow, M., S. Hasegawa and G. Manners (Eds.). American Chemical Society, USA., pp: 145-163.

Tanaka, T., H. Kohno, Y. Tsukio, S. Honjo, M. Tanino, M. Miyake and K. Wada, 2000b. Citrus limonoids obacunone and limonin inhibit azoxymethane-induced colon carcinogenesis in rats. Biofactors, 13: 213-218.

Tanaka, T., M. Maeda, H. Kohno, M. Murakami, S. Kagami, M. Miyake and K. Wada, 2001. Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. Carcinogenesis, 22: 193-198.

Tian, Q., E.G. Miller, A. Hassan, L. Tang and B.S. Patil, 2001. Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutr. Cancer, 40: 180-184.