

## MiniReview

# Health promoting bioactive phytochemicals from *Brassica*

Kumar, S. and Andy, A.

Directorate of Rapeseed-Mustard Research, Sewar, Bharatpur 321 303(Rajasthan),  
India

**Abstract:** *Brassica* vegetables are a family of 6 agriculturally important species consumed in high quantities throughout the world. Cruciferous vegetables are a good source of many health promoting and potentially protective phytochemicals including folic acid, phenolics, carotenoids, selenium, glucosinolates and vitamin C. Incorporating these potent plant based compounds in a daily food is a safe, effective and inexpensive way to guard against many of today's most common and lethal cancers of which nearly 30-40% are directly linked to improper diet and related factors. The present paper reviews the available literature on bioactive compounds from edible crucifers, their health promoting beneficial effects and possible role in imparting defence against diseases especially cancer.

**Keywords:** Bio active compounds, structure, distribution, activity, *brassicaceae*, rapeseed, mustard

### Introduction

Plant and animal foods, in addition to supplying essential nutrients for the mankind also possess a variety of bioactive substances like phenols, flavonoids, carotenes and organo sulphur compounds having anti proliferative activities (Plumb *et al.*, 1997).

Nearly 30-40% of cancers are directly linked to improper diet and related factors (Czapski, 2009). Epidemiological studies indicated positive association between intake of fruits and vegetables and reduced mortality from common cancers, heart and other degenerative diseases (Art *et al.*, 2005; Kaur *et al.*, 2001; Scalbert *et al.*, 2005; Vita, 2005). Cruciferous vegetables are a family of 6 agriculturally important species consumed in high quantities throughout the world (Fahey *et al.*, 2001). *Brassica oleracea* is the main vegetable species of this family including kale, cabbage, broccoli, Brussels sprouts, cauliflower whereas vegetable forms of *Brassica rapa* include turnip, Chinese cabbage and pak choi (Cartea *et al.*, 2011). Cruciferous vegetables are a good source of many health promoting and potentially protective phytochemicals including folic acid, phenolics, carotenoids, selenium, glucosinolates and vitamin C (Howard *et al.*, 1997; Femina *et al.*, 1998; Kushad *et al.*, 1999; Conaway *et al.*, 2001; Fimognari *et al.*, 2002) and offer powerful, broad-spectrum support for protecting against the ubiquitous cancer provoking agents encountered every day in our environment and have been reported to be associated with a lower incidence of certain cancers of lung, colon, breast, ovary and bladder (Higdon *et al.*, 2007; Fowke 2007; Juge *et al.*, 2007; Zhao *et al.*, 2007). Mustard leaves have been reported to possess many bioactive

substances and antioxidant properties (Kim *et al.*, 2003). Mustard leaves extract has also been observed to control glucose metabolism and reduced lipid per oxidation as well as the level of oxygen radicals ameliorating the damage caused by oxidative stress (Yokozawa *et al.*, 2003). Tiku *et al.*, (2008) also recorded the protective effect of *Brassica campestris* leaves against *in vivo* chromosomal damage and oxidative stress induced by gamma radiation and genotoxic chemicals. Incorporating these potent plant based compounds in a daily food is a safe, effective and inexpensive way to guard against many of today's most common and lethal cancers. Earlier reviews appeared dealing with biocidal, bio herbicidal, anti oxidant, anti cancer activities of glucosinolates and their products from *Brassicaceae* (Rosa *et al.*, 1997; Fahaey *et al.*, 2001; Zupalova and Vasak 2002; Halkier and Gershenzon 2006; Vig *et al.*, 2009). However, published information on other bioactive compounds from this family is very limited. The present paper reviews the available literature on bioactive compounds from edible crucifers, their health promoting beneficial effects and possible role in imparting defence against diseases especially cancer.

### Glucosinolates

#### Structure and hydrolysis

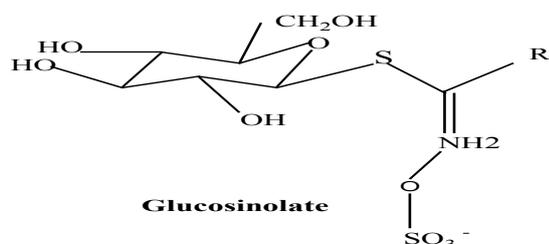
Glucosinolates are sulphur containing secondary metabolites. The structure of the glucosinolates consists of a  $\beta$ -D glucose moiety linked to a sulphated thiohydroximate (Figure1). The moiety linked to thiohydroximate or side chain varies and resulting into about 130 different types of glucosinolates (Fabre *et al.*, 2007). It could be aliphatic, aromatic or indolyl

\*Corresponding author.

Email: [satyanshu66@gmail.com](mailto:satyanshu66@gmail.com)

Tel: +91-2692271601; Fax: +91-2692271602

(Table 1). Desulphoglucosinolates, the penultimate compounds in the glucosinolates biosynthetic pathways are oximes having pka values about 9-12 (Jencks and Regenstein 1968; Underhill 1980). Due to low pka value of the sulphonic acid group, GSLs invariably occur in anionic form in nature (Larsen, 1981; Prestera *et al.*, 1996). Presence of glucose moiety and ionic forms makes glucosinolates hydrophilic and non-volatile compounds (Larsen, 1981).

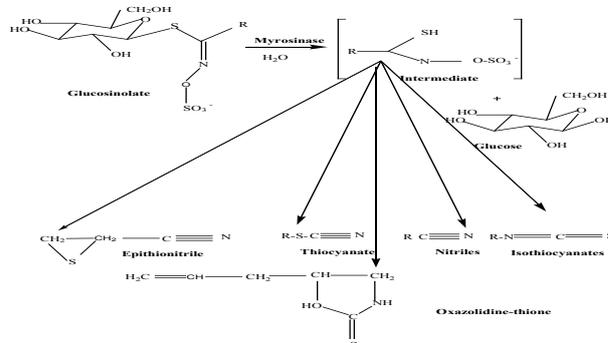


**Figure 1.** General structure of glucosinolate

**Table 1.** Different types of glucosinolates occurring in family Brassicaceae (Hansen *et al.*, 1995; Bellostas *et al.*, 2007)

S. No.	Systematic name	Trivial name	R
<b>I Aliphatic</b>			
1	2-propenyl (allyl)	Sinigrin	$\text{CH}_2=\text{CH}-\text{CH}_2$
2	3-butenyl	Gluconapin	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2$
3	4-pentenyl	Glucobrassicinapin	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2-\text{CH}_2$
4	4-methylthiobutyl	Glucorucin	$(\text{CH}_2)_4\text{S}(\text{CH}_3)$
5	3-methylsulphinyl propyl	Glucocerbin	$\text{CH}_3\text{SO}(\text{CH}_2)_3$
6	4-methylsulphinylbutyl	Glucoraphanin	$\text{CH}_3\text{SO}(\text{CH}_2)_4$
7	5-methylsulphinylpentyl	Glucosallysin	$(\text{CH}_3)\text{SO}(\text{CH}_2)_5$
8	3-methylthiopropyl	Glucobervirin	$(\text{CH}_3)\text{S}(\text{CH}_2)_3$
<b>II Hydroxy aliphatic</b>			
10	2-hydroxy-3-butenyl	Progoitrin	$\text{CH}_2=\text{CH}-\text{CHOH}-\text{CH}_2$
11	2-hydroxy-4-pentenyl	Napoleiferin	$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CHOH}-\text{CH}_2$
<b>III Cyclic</b>			
12	4-hydroxy benzyl	Sinalbin	
13	Phenethyl	Gluconasturtiin	
14	Benzyl	Glucotropaeolin	
<b>IV Heterocyclic</b>			
15	3-indolylmethyl	Glucobrassicin	
16	1-methoxy-3-indolylmethyl	Neoglucobrassicin	
17	4-hydroxy-3-indolyl methyl	4-hydroxy-glucobrassicin	
18	4-methoxy-3-indolyl methyl	4-Methoxy-glucobrassicin	

Glucosinolates are non-toxic *per se*. However, their hydrolytic bioactive products derived from cleavage by myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1 (Fenwick, 1983), a medium molecular weight protein, co-existing in tissues of *Brassica* species forming an unstable aglucone intermediate; the unstable aglucone then eliminates sulphate by Lossen rearrangement (Figure 2). Depending on factors such as pH, unstable intermediate spontaneously degrades to one of a number of products (Rouzaud *et al.*, 2004; Ugolini *et al.*, 2008]. Low pH tends to favour production of allyl cyanide, however, under neutral and alkaline pH conditions allyl isothiocyanate is formed as predominant breakdown product of sinigrin (Uda *et al.*, 1986). In presence of epithiospecifer protein, 1-cyano-2,-epithiopropene can also be formed (Cole, 1978). Epithionitriles and oxazolidine-2-thiones are formed from glucosinolates with a hydroxyl group at the 2-position of the side chain. Process of cooking vegetables destroys much of the naturally available myrosinase, however, glucosinolates can still be converted to isothiocyanates inside the body but much less efficiently than when myrosinase is available. The conversion can occur in the colon where native gut bacteria hydrolyze the glucosinolates (Getachum and Hung, 1999; Johanson, 2002).



**Figure 2.** Hydrolytic products of glucosinolates (Vig *et al.*, 2009)

### Distribution

More than 100 glucosinolates have been identified from 16 plant families such as *Cruciferae* (syn. *Brassicaceae*), *Bataceae*, *Bretschneideraceae*, *Pentadiplandraceae*, *Phytolaccaceae*, *Pittosporaceae*, *Resedaceae*, *Capparaceae*, *Moringaceae*, *Tropaeolaceae*, *Tovariaceae*, *Limnanthaceae*, *Caricaceae*, *Gyrotomonaceae*, *Salvadoraceae* and *Euphorbiaceae* (McGregor and Downey, 1975; Kjaer, 1980; Fahey *et al.*, 2001; Fabre *et al.*, 2007). They mainly occur in the *Brassicaceae*, which includes the crop plants such as rapeseed-mustard, cabbage, cauliflower, broccoli and Brussels sprouts (Table 2). They impart resistance/tolerance to diseases and pests and accumulated in

the organs contributing to resistance/tolerance at a particular developmental stage (Brown *et al.*, 2003; Grubb and Abel, 2006; Halkier and Gersherzon, 2006). Eight most significant glucosinolates have been identified so far from the Brassica genus. Ugolini *et al.*, (2008) reported that sinigrin was the major component in Indian, Ethiopian and black mustard whereas glucosinalbin was the predominant glucosinolates in yellow mustard (Table 3). Aliphatic glucosinolates are mostly present in Brassica genus. Indole glucosinolates are in traces in Brassica species (Zukalova and Vasak, 2002). Indolyl glucosinolates are major glucosinolates in the vegetative parts of Brassica species but are reported to be lacking in seed (Bergmann, 1970; Josefsson 1970). Sinalbin also has been reported to occur only in trace amounts in the seed of *Brassica napus* (Kjaer, 1980). Quantitative and qualitative changes in glucosinolates in Brassica plants depend on variety, various plant parts, developmental stage, agronomic management and climatic conditions (Tiedink *et al.*, 1988; Kushad *et al.*, 1999; Vallejo *et al.*, 2002, 2003; Borkowski *et al.*, 2008).

**Table 2.** Distribution of Different types of glucosinolates in oilseed *Brassica* and related specie (Appelqvist 1972; Hansson *et al.*, 2008 ; Ugolini *et al.*, 2008)

Glucosinolates (common name)	<i>B. juncea</i>	<i>B. napus</i>	<i>B. campestris</i>	<i>S. alba</i>
Sinigrin	+			
Gluconapin	+	+	+	+
Glucobrassicinapin		+	+	
Glucorucic			+	
Progoitrin		+	+	
Napoleiferin		+	+	
Glucoraphanin		+	+	
Glucosylsin		+	+	
Glucobrassicin		+	+	
Glucosinalbin				+
4-hydroxy Glucobrassicin	+	+		
4-methoxy-3-indolyl methyl		+		

The pod wall has been identified as the main site of the glucosinolates synthesis (Lein, 1972). However, in species like *Tropaeolum majus* (garden nasturtium), leaves have been found to be the primary site of glucosinolates (glucotropaeolin) synthesis (Lykkesfeldt and Moller, 1993). Synthesized glucosinolates are transported to different parts of the plant; however, nature of the transported forms is not clear. Identification of mechanism of transport could be useful to lower levels in specific tissues such as seed. Du *et al.*, (1998) administered <sup>14</sup>C tyrosine to developing seeds and siliquae walls of *Sinapis alba* and observed *de novo* synthesis of p-hydroxybenzylglucosinolate (sinalbin) in both and concluded that all the enzyme required for

glucosinolate synthesis were present in seeds unlike the seeds of *B. napus*. Total glucosinolates in leaves of Indian mustard at 60 days after sowing ranged from 19.9-32.7  $\mu$  moles / g (Kumar *et al.*, 2009).

**Table 3.** Major glucosinolates in oilseed Brassica and related species Ugolini *et al.*, (2008)

Crop	Botanical name	Major glucosinolates (%)
Indian mustard	<i>Brassica juncea</i>	Sinigrin (~90)
Karan rai (Ethiopian mustard)	<i>Brassica carinata</i>	Sinigrin (~97)
Black mustard	<i>Brassica nigra</i>	Sinigrin (~95)
Gobhi sarson	<i>Brassica napus</i>	Progoitrin (~70)
Taramira	<i>Eruca sativa</i>	Glucorucic (~95)
Yellow / white mustard	<i>Sinapis alba</i>	Glucosinalbin (~100)
Cabbage	<i>Brassica oleracea</i>	Progoitrin

### Activity

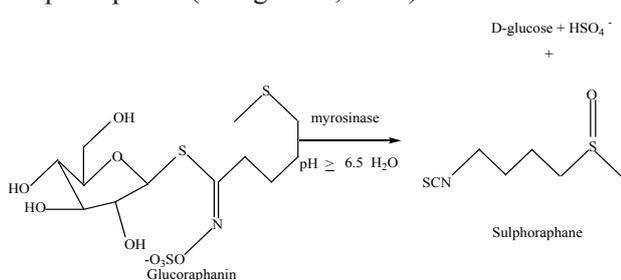
Glucosinolates and their derived products have been reported to have health beneficial effects by reducing the risk of certain cancers in humans (Zhang and Talay, 1994; Fahey *et al.*, 2001; Shapiro *et al.*, 2001; Mithen *et al.*, 2003), while other GSLs are detrimental for human and animal consumption (Rosa *et al.*, 1997). Epidemiological studies indicate that consumption of brassica vegetables is associated with a reduced incidence of cancers at a number of sites including the lung, stomach, colon and rectum (Conaway *et al.*, 2001). Glucosinolates, the thioglucosides, present in brassica vegetables are thought to contribute to this phenomenon. Dietary glucosinolates have been reported to block formation of endogenous or exogenous carcinogens for preventing initiation of carcinogenesis (Vig *et al.*, 2009).

Living organisms are continually exposed to a variety of naturally occurring chemicals. The ability of the carcinogens to exert their effects depends largely on the interaction between activating and deactivating enzymes. Imbalance, if any, will result in a change in the biological effect. Many compounds occurring naturally in the human diet moderate the biotransformation of several carcinogens, resulting in reduced tumour incidence (Wright, 1980). Glucosinolates and their hydrolytic products modulate the activity of xenobiotic metabolising phase I and II enzymes. Phase I enzymes generally increase the reactivity of lipophilic compounds. On the other hand, phase II enzymes increase the water solubility and facilitate removal of these metabolites from the body. For the protection of cell against DNA damage by carcinogens and reactive oxygen species, inhibition of phase I and induction of phase II enzymes are required. The genes for the phase II enzyme contain a specific sequence of DNA called antioxidant response element (ARE). Activities of

phase II enzyme have been reported to be enhanced by glucosinolates and their hydrolytic products (Holst and Williamson, 2004).

Isothiocyanates are an important group of breakdown products of glucosinolates and appear to act at a number of points in the tumour development by blocking the metabolism of carcinogenic compounds through biotransformation. They generally enhanced the activity of phase II enzymes and inhibited phase I enzymes (Tawfiq *et al.*, 1995; Fahey *et al.*, 1997; Smith *et al.*, 1990; Conaway *et al.*, 1996). Thus reduce the carcinogenic activity and enhance the detoxification and clearance of carcinogens. Further, they serve as suppressors during the promotion phase of neo-plastic process. Induction of apoptosis and action of signal transduction pathways within the cell activities of glucosinolates has also been reported (Smith *et al.*, 2004).

Benzyl-p-hydroxybenzyl- and 2-hydroxy but-3-enyl glucosinolates have been reported to induce mammalian phase 2 enzymes of detoxification (Tawfiq *et al.*, 1995; Fahey *et al.*, 1997; Smith *et al.*, 1990). Sulforaphane (Figure 3), enzymatic degradation product of glucosinolate glucoraphenin, activated gene expression, thereby, helping to clear carcinogenic substances from the body. Sulforaphane (SFN) also increased levels of mammalian phase 2 enzymes through antioxidant response element (ARE) mediated transcriptional activation (Zhang *et al.*, 1992, 1994; Talalay and Zhang 1996; Talalay *et al.*, 1995; Fimognari *et al.*, 2002; Hwang and Jeffery 2005; Khor *et al.*, 2006). SFN supported a healthy immune system by significantly enhancing the production of chemicals involved in immune response (Thejass and Kuttan, 2006). In a study in which animals were genetically bred to develop intestinal polyyps, a condition that led tumour formation, group of animals that were fed sulphoraphane had higher rates of apoptosis (cell suicide) and smaller tumour growing more slowly than animals not receiving sulphoraphane (Wang *et al.*, 2004).

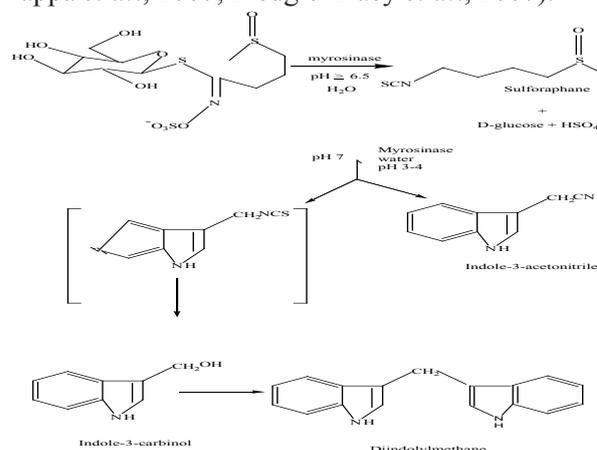


**Figure 3.** Sulforaphane from enzymatic hydrolysis of glucoraphenin (Fimognari *et al.*, 2002)

Watercress and broccoli are reported to be rich sources of phenethyl isothiocyanate (PEITC) which may block the cytochrome P450 mediated metabolic

activation of the common nitrosoamine to its potent carcinogenic forms (Palaniswamy *et al.*, 2003). Extracts of watercress and broccoli suppressed metalloproteinase-9, an enzyme closely associated with invasive potential of breast cancer (Conaway *et al.*, 1996). It also suppressed production of pro-inflammatory compounds such as nitric oxide (NO) and prostaglandins (Ribnický *et al.*, 2001). PEITC also inhibited induction of lung and oesophagus cancer in both rat and mouse tumour (Morse *et al.*, 1993; Stoner and Morse, 1996; Hecht, 1996; Stoner *et al.*, 1999).

Indole-3-carbinol (I3C) is produced from indole-3-glucosinolates like glucobrassicin through hydrolysis (Figure 4). Under acidic conditions indole-3-carbinol (I3C) and elemental sulphur are formed. Anti carcinogenic, antioxidant and anti-atherogenic activities of Indole-3-carbinol have been reported (Jongen, 1996). Further, indole-3-carbinol modulates the activities of both phase I and II enzymes. It suppressed cancer growth and induced programmed cell deaths in tumours of breast, prostate, leukaemia, cervix and colon because of its ability to favourably influence the human body's balance of estrogens (Aggarwal and Ichikawa, 2005; Ashok *et al.*, 2001; 2002; Yuan *et al.*, 1999). I3C also inhibited cancer cell growth by interfering the production of proteins involved in abnormal cellular reproduction and by promoting the production of tumour suppressor proteins (Firestone *et al.*, 2003; Sarkar *et al.*, 2003; Aggarwal and Ichikawa, 2005). I3C has also been reported to prevent cancer by interfering with angiogenesis, process of formation of new blood vessels that tumour require for their survival and spread (Wu *et al.*, 2005). 3-3'-diindolyl methane (DIM), a condensation product of I3C enhanced beneficial effects of I3C by influencing the expression of genes involved in carcinogenesis, cell survival and physiological behaviour (Li *et al.*, 2005; Pappa *et al.*, 2007; Pledgie-Tracy *et al.*, 2007).

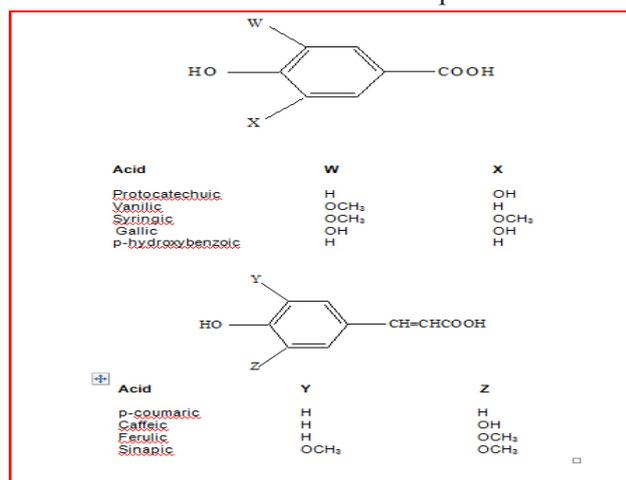


**Figure 4.** Indolyl 3-carbinol (I3C) and indole-3-acetonitrile from hydrolysis of indolyl glucosinolate (Jongen 1996)

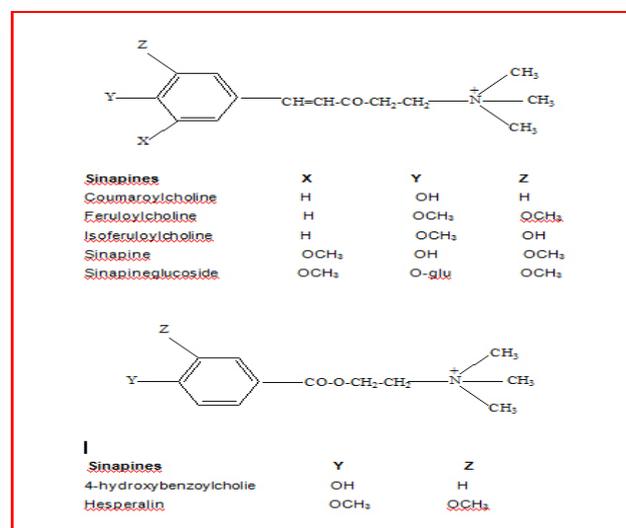
## Phenolic compounds

### Structure

Phenolic compounds are secondary metabolites and exist widely in plants. The predominant phenolics present in rapeseed-mustard are phenolic acids and condensed tannins. Phenolic acid present in the free, esterified and insoluble forms are derivatives of benzoic acid and cinnamic acids (Figures 5 a, b). Sinapine, choline ester of sinapic acid being the most abundant followed by sinapoyl glucose making up to 1-2% of the seed dry matter (Kozłowska *et al.*, 1990; Shahidi and Naczka, 1992) and markedly influenced by abiotic factors like soil and climate. The presence of sinapines, viz., sinapoycholine (sinapine), feruloylcholine, isoferuloylcholine, coumaroylcholine, 4-hydroxybenzoyl choline and 3,4- dimethoxybenzoyl choline has been reported in rapeseed (Larsen *et al.*, 1983). Sinapoyl glucose is precursor of sinapine and other minor sinapate esters. Sinapic acid is derived from hydrolysis of sinapine. It constitutes 71-75 % of the total free phenolic acids.



**Figure 5a.** Structures of phenolic acids found in rapeseed (Naczka *et al.*, 1998)



**Figure 5b.** Structures of sinapines found in rapeseed (Naczka *et al.*, 1998)

### Distribution

Sinapic acid is widely distributed in plants of *Brassicaceae* like *B. juncea* and *Lepidium sativum* (Andreasen *et al.*, 2002; Lu *et al.*, 2001; Chalas 2001). A number of phenolic choline esters, known as sinapines have also been reported. Rapeseed-mustard has been reported to contain more phenolic compounds than other oilseeds (Nowak *et al.*, 1992). Kumar *et al.*, (2009) reported significant varietal differences for total phenols (4.3-8.3 ppm gallic acid equivalent) and in vitro antioxidant activity in Indian mustard. In vitro antioxidant was positively correlated with total phenols and flavonoids content.

### Activity

Phenolics play an important role as defence compounds, however, their exact contribution is still not very clear (Puupponen-pimia *et al.*, 2005). Presence of sinapine and related phenolic compounds such as sinapic acid has been of concern to the oilseeds breeders and processors as they impart bitter taste, astringency and dark colour to repressed-mustard products (Sosulski, 1979; Ismail *et al.*, 1981; Blair and Reicnert, 1984; Sosulski and Dabrowski, 1984). These compounds also have anti oxidant activity depending on the type of phenolics-etherified, glycosylated and non-glycosylated (Andreasen *et al.*, 2001; Lu *et al.*, 2001; Chalas, 2001; Robbins 2003)

## Flavonoids

### Structure

Flavonoids are a group of poly phenolic compounds. Natural flavonoids usually occur as glycosides-glucose, rhamnose, rhamno glucosides and can be divided into subclasses according to the structure of C6-C3-C6 flavone skeleton. Kaempferol, quercetin, Isorhamnetin are aglucones. Rutin flavonol glycoside-C27 H30 O16 (quercetin-3-B-rutinoside) has been identified in the seeds of *B. campestris* .

### Distribution

Flavonoids are present in most plant tissues and often in vacuoles (Croteau *et al.*, 2002).The flavonoid composition of different edible Brassica species pak choi (*Brassica campestris*), broccoli (*Brassica oleracea*), cauliflower (*Brassica oleracea*), turnip tops (*Brassica rapa*) and tronchuda cabbage (*Brassica oleracea*) have been reported (Llorach *et al.*, 2003; Vallejo *et al.*, 2004; Romani *et al.*, 2006; Ferreres *et al.*, 2005; Rochfort *et al.*, 2006; Harbaum *et al.*, 2007; Ferreres *et al.*, 2008). Derivatives of flavonols kampferol and quercetin are the main flavonoids reported from brassica vegetables;

however, isorhamnetin and myricetin are less common. Flavonic glycoside has been detected in seeds of rapeseed cultivars (Durkee and Harborne, 1973). Flavonoids ranged from 0.8-2.3 ppm (quercetin equivalent) in 60 days old leaves of Indian mustard (Kumar *et al.*, 2009).

#### Activity

Bioactive properties such as free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory (Frankel 1995) and anti viral (Hodek *et al.*, 2002) action of flavonoids is known. Anti proliferative effects such as cancers, cardio vascular and inflammatory diseases of dietary flavonoids are recognized. Scavenging activity of hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals signifies the health promoting functions of flavonoids.

#### Phytic acid

##### Structure

Phytic acid (Inositol hexaphosphate Ins P<sub>6</sub>) is the storage form of phosphorus in many crop plants (Raboy 2001). It contains about 28% phosphorus in the form of phosphoric acid. Phytic acid contains six phosphate groups thereby; it can act as metal chelator (calcium, iron and nickel) to inhibit the generation of highly reactive oxygen species such as OH hydroxyl free radicals (Jabri *et al.*, 1995; Rimbach and Pallauf 1998; Grases *et al.*, 2001). Salt of phytic acid with minerals or protein complexes are known as phytates.

##### Distribution

Phytic acid, a dietary phytochemical occurs in many cereals, soybean / other legumes and fibre rich foods. Rapeseed has been reported to contain phytic acid. Mature seed is the main source of phytic acid (Naczka *et al.*, 1998).

##### Activity

Phytic acid has a number of biological activities (Singh *et al.*, 2004; Ma *et al.*, 1996). Anti-neoplastic activity on a variety of experimental models of carcinogenesis, however, is the most intriguing property (Midorikawa *et al.*, 2001; Vucernik and Shamsuddin 2003, Singh *et al.*, 2004b). It inhibited the metastasis of tumour (Weber *et al.*, 1995; Baten *et al.*, 1989; Shamsuddin *et al.*, 1989). Singh *et al.* (2003), Alabaster *et al.* (1996) and Takaba *et al.* (1997) reported inhibition of renal stone formation, colon carcinogenesis and cholesterol reduction in serum of experimental rats. Xu *et al.* (2008) demonstrated

significant neuro-protective effect of phytic acid in a cell culture model of Parkinson's disease. Because of chelation of multivalent cations (like Fe<sup>++</sup>), phytic acid is generally considered as anti nutritional, however, it is beneficial for the same property (Graf *et al.*, 1984; Reddy *et al.*, 1996; Shears 2001).

#### Brassinosteroids

##### Structure

Brassinosteroids are naturally occurring poly-hydroxy steroids. About 42 brassinosteroid configurates have been characterized (Fujioka and Sakurai, 1997). Natural brassinosteroids have a common 5- $\alpha$ -cholestan skeleton. Structural variation however, arises from the kind and orientation of functional group on skeleton. They are classified as C<sub>27</sub>, C<sub>28</sub> or C<sub>29</sub> brassinosteroids on the basis of substitution pattern of side chain (Fujioka and Sakurai 1997; Yokota, 1997). Brassinosteroids belong to C<sub>28</sub> steroids having a 24 alpha-methyl group in the side chain and C<sub>27</sub> brassinosteroids with no substitution at 24 (Fig. 6). C<sub>28</sub> brassinosteroids (24 beta-methyl, 24-ethylidene, 24-methylene-25 methyl). Campesterol or its analogues are considered to be biosynthetic precursors of brassinolide.

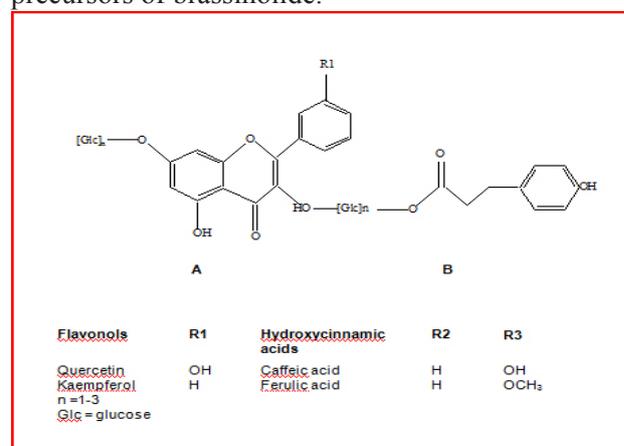


Figure 6a. Structures of flavonol glucosides present in Brassica ( Oslen *et al.*, 2009)

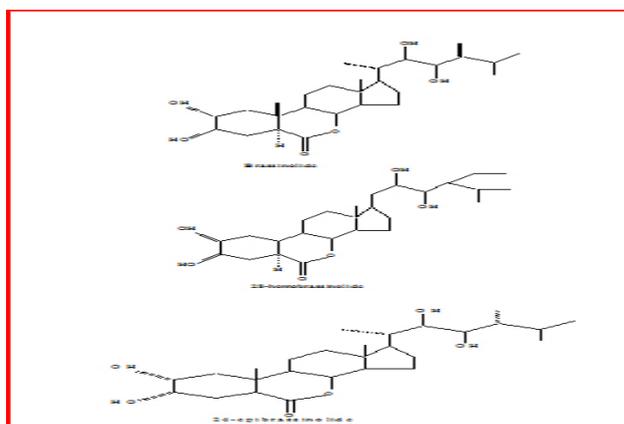


Figure 6b. Brassinosteroid isolated from Brassica (Fujioka, 1999).

### Distribution

Brassinosteroids, a group of steroidal substance, first isolated from the pollen of *Brassica napus* is considered as the sixth group of phytoharmones (Rao *et al.*, 2002). Distribution of brassinosteroids has been reported in pollen and seed of *Brassica napus* and *Brassica campestris*, respectively. Extremely low level (10<sup>-9</sup> g) of brassinosteroids is reported from plants. Its concentration varies with plant tissues. Pollen and immature seeds have higher concentration in comparison to shoots and leaves (Takatsuto, 1994). Brassinolide and castasteron are the two important brassinosteroids occurring in higher plants (Khripach *et al.*, 2000).

### Activity

Brassinosteroids are reported to act in response to stress factors in the process of plant growth and development such as cell division, elongation and differentiation, seed germination, vegetative growth, apical dominance, reproductive growth and senescence (Sasse *et al.*, 1998). Brassinosteroids are reported to have strong and unique biological activities at nano or micro molar level concentrations to plant tissues (Mandava, 1988; Sakurai and Fujioka, 1993). Brassinolide is the most biologically active among the naturally occurring brassinosteroids in many bioassay systems (Yokota, 1997). Wachsman *et al.* (2000, 2002) reported medical application of brassinosteroids. Substantial anti viral activity against pathogenic viruses including herpes simplex virus type 1 (HSV 1), RNA viruses and measles virus of 28-homocasterone and 24 epibrassinolide and synthetic analogues of these compounds were observed. In in vitro test 10 to 18 fold higher activities of the several brassinosteroids was observed to reference drug ribavirin towards HSV 1 and RNA viruses. Both brassinosteroids inhibited cell growth of breast and prostate cancer cell lines in a close dependent manner at micro molar concentrations (Wachsman *et al.*, 2000, 2002; Malikova *et al.*, 2008).

### Conclusion

Among the available bio active compounds in Brassica, only glucosinolates have been extensively investigated and vast information has been available regarding its role in minimizing the risk of cancer and several other diseases. However, studies should be carried out on the effect of genotype, environment and their interaction as well as crop management on the efficacy of these bioactive substances. Further, in depth planned and systematic clinical investigations

of these bio molecules are required to elucidate their role in imparting resistance against diseases and their mode of action. Presently, studies have been underway at this directorate to characterize prevalent rapeseed-mustard varieties for phenolics, flavonoids, ascorbic acid, glucosinolates, phytic acid and anti oxidant activities in seeds and leaves. Furthermore, use of glucosinolates, phenolics, flavonoids, phytic acid and other bio active components from *Brassica* in functional food, their bio-availability studies may provide additional evidences for the utilization of fascinating natural products.

### References

- Aggarwal, B.B. and Ichikawa, H. 2005. Molecular targets and anti cancer potential of indole-3-carbinol and its derivative. *Cell Cycle* 4: 1201-1215.
- Alabaster, O., Tang, Z., and Shivapurkar, N. 1996. Dietary fibre and the chemo preventive modelation of colon carcinogenesis. *Mutation Research* 350: 185-197.
- Andreasen, M.F., Landbo, A.K., Christensen, L.P., Hansen, A. and Meyer, A.S. 2001. Anti oxidant effect of phenolic rye (*Secale cereale* L.) extracts, monomeric hydroxycinnamates and ferulic acid dehydromers on human low density lipoproteins. *Journal of Agricultural and Food Chemistry* 49: 4090-4096.
- Appelqvist, L.A. 1972. Chemical constituents of rapeseed. In, *Rapeseed: cultivation, composition, processing and utilization*, p 150. Amsterdam, Elsevier.
- Art, I.C.W. and Hollman, P.C.H. 2005. Polyphenols and disease risk in epidemiologic studies. *American Journal of Clinical Nutrition* 81: 317S-325S.
- Ashok, B.T., Chen, Y., Liu, X., Bradlow, H.L., Mittelman, A. and Tiwari, R.K. 2001. Abrogation of estrogen-mediated cellular and biochemical effects by indole-3-carbinol. *Nutrition and Cancer* 41: 180-187
- Ashok, B.T., Chen, Y., Liu, X., Garikapaty, V.P., Seplovitz, R., Tschorn, J., Roy, K., Mittelman, A. and Tiwari, R.K. 2002. Multiple molecular targets of indole-3-carbinol: a chemo preventive anti estrogen in breast cancer. *European Journal of Cancer Prevention* 11: S86-S93.
- Bellostas, N., Kachlicki, P., Sorensen, J.C. and Sorensen, H. 2007. Glucosinolate profiling of seed and sprouts of *B.oleracea* varieties used for food. *Scientia Horticulture* 114: 234-242.
- Baten, A., Uelah, A., Tomaic, V.J. and Shamsuddin, A.M. 1989. Inositol phosphate induced enhancement of natural killer cell activity correlates with tumor suppression. *Carcinogenesis* 10: 1595-1598.
- Bergmann, F. 1970. The biosynthesis of glucosinolates in the course of ontogenesis of *Sinapis alba* L. *Z.Pflanzenphysiol* 62: 362-375.
- Blair, R. and Reichert, R.D. 1984. Carbohydrates and phenolic constituents in a comprehensive range of rapeseed and canola fractions: nutritional significance for animals. *Journal of the Science of Food and*

- Agriculture 35: 29-35.
- Brown, P.D., Tokuhisa, J.G., Reichelt, M. and Gershenzon, J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* 62: 471-481.
- Borkowski, J., Szajdek, A., Borkowska, E.J., Ciska, E. and Zielinski, H. 2008. Content of selected bioactive components and anti oxidant properties of broccoli (*Brassica oleracea L.*) *European Food Research and Technology* 226: 459-465.
- Cartea, M.E., Francisco, M., Soengas, P. and Velasco, P. 2011. Phenolic compounds in Brassica vegetables. *Molecules* 16: 251-280.
- Chalas, J., Claise, C., Edeas, M., Messaoudi, C., Vergnes, L., Abella, A. and Lindenbaum, A. 2001. Effect of ethyl esterification of phenolic acids on low-density lipoprotein oxidation. *Biomedicine and Pharmacotherapy* 55: 54-60.
- Czapski, J. 2009. Cancer preventive properties of Cruciferous Vegetables. *Vegetable Crops Research Bulletin* 70: 5-18.
- Cole, R.A. 1978. Epithiospecifer protein in turnip and changes in products of autolysis during ontogeny. *Phytochemistry* 17: 1563-1565.
- Conaway, C.C., Getachun, S.M., Liebes, L.L., Pusateri, D.J., Tophan, D.K.W., Botero-Omary, M. and Chung, F.L. 2001. Disposition of glucosinolates and sulphoraphanes in human after ingestion of steam and fresh broccoli. *Nutrition and Cancer* 38:168-178.
- Conaway, C.C., Jiao, D. and Chung, F.L. 1996. Inhibition of rat liver cytochrome p 450 isozymes by isothiocyanates and their conjugates : a structure activity relationship study. *Carcinogenesis* 17: 2423-2427.
- Croteau, R., Kutchan, T.M. and Lewis, N.G. 2000. Natural products (secondary metabolites). In: Buchman V V, Grisse W and Jones R L (Eds). *Biochemistry and Molecular Biology of Plants*. Rockville , MD, USA, American Society of Plant Physiologists pp. 1250-1318
- Du, L. and Halker, B. 1998. Biosynthesis of glucosinolates in the developing silique walls and seeds of *Sinapis alba*. *Phytochemistry* 48: 1145-1190.
- Durkee, A. and Harborne, J. 1973. Flavonol glycosides in Brassica and *Sinapis* . *Phytochemistry* 12: 1085-1089.
- Fabre, N., Poinot, V., Debrauwer, L., Vigor, C., Tulliez, J., Fouraste, I. and Moulis, C. 2007. Characterization of glucosinolates using electro spray ion trap and electro spray quadrupole time of flight mass. *Phytochemical Analysis* 18: 306-319.
- Fahey, J.W., Zhang, Y.S. and Talalay, P. 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protects against chemical carcinogens. *Proceedings of the National Academy of Science, USA* 94: 10367-10372.
- Fahey, J.W., Zalcmann, A.T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56: 5-51.
- Femina , A., Robertson, J.A. and Waldron, K.W. 1998. Cauliflower (*Brassica oleracea L.*), globe artichoke (*Cynarascolymous L.*) and Chicory witloof (*Cichorium intybus*) processing by-products as source of dietary fibre. *Journal of the Science of Food and Agriculture* 77: 511-518.
- Fenewick, G.R., Heaney, R.K. and Mullin, J. 1983. Glucosinolates and their break down products in food and food plants. *CRC Critical Reviews in Food Science and Nutrition* 18: 123-201.
- Ferreres, F., Valentao, P., Pereria, J.A., Bento, A., Noites, A., Seabra, R.M. and Andrade, P.B. 2008. HPLC-DAD –MS/MS-ESI screening of phenolic compounds in *Piers brassicae L.* reared on *Brassica rapa* var. *rapa L.* *Journal of Agricultural and Food Chemistry* 56: 844-853.
- Ferreres, F., Valentao, P., Llorach, R., Pinheiro, C., Cardoso, U., Pereira, J.A., Sousa, C., Seabra, R.M. and Andrade, P.B. 2005. Phenolic compounds in external leaves of tronchuda cabbage ( *Brassica oleracea L.* var. *Costata DC* ). *Journal of Agricultural and Food Chemistry* 53: 2901- 2907
- Fimognari, C., Nusse, M., Cesari, R., Iori, R., Cantelli-Forti, G. and Hrelia, P. 2002. Growth inhibition, cell cycle arrest and apoptosis in human T-cell leukaemia by the isothiocyanates sulphoraphane. *Carcinogenesis* 23: 581-586.
- Firestone, G.L. and Bjeldanes, L.F. 2003. Indole-3-carbinol and 3',3'-diindolylmethane anti-proliferative signalling pathways control cell cycle gene transcription in human breast cancer cells by regulating promoter sp-1 transcription factor interactions. *Journal of Nutrition* 133: 2448S-2455S.
- Fowke, J.H. 2007. Head and neck cancer: a case for inhibition by isothiocyanates and indols from Cruciferous vegetables. *European Journal of Cancer Prevention* 16: 348-356.
- Fujioka, S. 1999. Brassionsteroids-steroidal plant hormones (Eds. Sakurai A., Yokota T., Clouse S.D.). Springer, Tokyo, pp. 21-45.
- Fujioka, S., and Sakurai, A. 1977. Biosynthesis and metabolism of brassinosteroids. *Physiologia Plantarum* 100: 710-715.
- Getahun, S.M. and Chung, F.L. 1999. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. *Cancer Epidemiology, Biomarkers and Prevention* 8: 447-451.
- Graf, E., Mahoney, J.R., Bryant, R.G. and Eaton, J.W. 1984. Iron catalyzed hydroxyl radical formation stringent requirement for free iron coordination site. *Journal of Biological Chemistry* 259: 3620-3624.
- Grases, F., Simonet, B.M., Prietro, R.M. and March JG. 2001. Phytate levels in different rat tissues : influence of dietary phytate. *British Journal of Nutrition* 86: 225-231.
- Grubb, C.D. and Abel S. 2006. Glucosinolate metabolism and its control. *Trends in Plant Science* 11: 89-100.
- Halkier, B.A. and Gershenzon, J. 2006. Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology* 57: 303-333.

- Hansen, M., Moller, P., Sorensen, H. and Trejo, M.C. 1995. Glucosinolate in Broccoli stored under controlled atmosphere. *Journal of American Society of Horticulture* 120: 1069-1074.
- Hansson, D., Morra, M.J., Borek, V., Snyder, A.J., Johnson-Maynard, J.L. and Thill, D.C. 2008. Ionic thiocyanate (SCN<sup>-</sup>) production, fate and phytotoxicity in soil amended with *Brassicaceae* seed meals. *Journal of Agricultural and Food Chemistry* 56: 3912-3917.
- Harbaum, B., Hubbermann, E.M., Wolff, C., Herges, R., Zhu, Z. and Schwarz, K. 2007. Identification of flavonoids and hydroxycinnamic acids in pak choi varieties (*Brassica campestris* L ssp. chinensis var. communis) by HPLC-ESI-MSn and NMR and their quantification by HPLC-DAD. *Journal of Agricultural and Food Chemistry* 54: 4855-4860.
- Hecht, S.S. 1996. Chemoprevention of lung cancer by isothiocyanates. *Advances in Experimental Medicine and Biology* 401: 1-11.
- Higdon, J.V., Delage, B., Williams, D.E. and Dashwood R.H. 2007. Cruciferous vegetable and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacology Research* 55: 224-236.
- Hodek, P., Trebil, P. and Stiborova, M. 2002. Flavonoids - potent and versatile biologically active compounds interacting with cytochromes. *Chemistry Biology Interact actions* 139: 1-21.
- Holst, B. and Williamson, G. 2004. A critical review of the bioavailability of glucosinolates and related compounds. *Natural Product Reports* 21: 425-447.
- Howard, L.A., Jeffery, E.H., Wallig, M.A. and Klein, B.P. 1997. Retention of phytochemicals in fresh and processed broccoli. *Journal of Food Science* 62: 1098-1100.
- Hwang, E.S. and Jeffery, E.H. 2005. Induction of quinone reductase by sulforaphane N-acetyl cystein conjugate and in murine hepatoma cells. *Journal of Medicinal Food* 8: 198-203.
- Ismail F., Vaisey-Genser, M., Fyfe, B. 1981. Bitterness and astringency of sinapine and its components. *Journal of Food Science* 46: 1241-1244.
- Jabri, E., Carr, M.B., Hausinger, R.P. and Karplus, P.A. 1995. The crystal structure of urease from *Klebsiella aerogenes*. *Science* 268: 998-1004.
- Jencks, W. P and Regenstein, J. 1968. Ionization constants of acids and bases. In: Sober, H. ed. *Handbook of biochemistry; selected data for molecular biology*. CRC, J150-188.
- Johnson I.T. 2002. Glucosinolates: bio availability and importance to health. *International Journal of Vitamin and Nutrition Research* 72: 26-31.
- Jongen, W.M.F. 1996. Glucosinolates in brassica : occurrence and significance as cancer modulating agents. *Proceedings of the Nutrition Society* 55: 433-446.
- Josefsson, E. 1970. Pattern, content and biosynthesis of glucosinolates in some cultivated Cruciferae. Swedish Seed Association Publication pp.42.
- Juge, N., Mithen, R.F. and Traka M. 2007. Molecular basis for chemoprevention by sulforaphane : a comprehensive review. *Cellular and Molecular Life Sciences* 64: 1105-1127.
- Kaur, C. and Kapoor, H. C. 2001. Anti oxidants in fruits and vegetables- the millennium's health. *International Journal of Food Science and Technology* 36: 703-725.
- Khor, T.O., Hu, R., Shen, G., Jeong, W.S., Hebbar, V., Chen, C., Xu, C., Nair, S., Reddy, B., Chada, K. and Kong, A. N. T. 2006. Pharmacogenomics of cancer chemo preventive isothiocyanate compound sulforaphane in the intestinal polyps of Apc Min/+ mice. *Biopharm Drug Dispos* 27: 407-420.
- Khripach, V., Zhabinski, V. and Degroot, A. 2000. Twenty years of Brassionsteroids: steroidal plant hormones warrant better crops for the XXI century. *Annals of Botany* 86: 441-447.
- Kim, H. Y., Yokozawa, T., Cho, E. J., Cheigh, H. S., Choi, J. S. and Chung, H. Y. 2003. *In vitro* and *in vivo* antioxidant effects of mustard leaf (*Brassica juncea*). *Phytotherapy Research* 17: 465-471
- Kjaer, A. 1980. Naturally derived isothiocynates (mustard oils) and their parent glucosides. In: W. Hertz, H. Griseback and G.W. Kirby eds. *Progress in the Chemistry of Organic natural products*. pp. 122-176. Springer Verlag, New York, N.Y. (USA).
- Kozłowska, H., Naczka, M., Shahidi, F. and Zadernowski, R. 1990. In: *Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology*, (Shahidi F (Ed.) Van Nostrand Reinhold, United States of America (1990) pp. 193-210.
- Kumar, S., Chauhan, J S, Singh, K H and Mishra, A. P. 2010. Phytochemical characters and *in vitro* anti oxidant activity of Indian mustard (*Brassica juncea* L.) leaves for nutraceutical studies. *Indian Journal of Nutrition and Dietetics* 47: 30-34.
- Kushad, M. K., Brown, A. F., Kurillic, A. C., Juvik, J.A., Klein, B.P., Wallig, M.A., Jeffery E.H. 1999. Variation in glucosinolates in vegetable crops of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry* 47: 1541-1548.
- Larsen, P.O. 1981. Glucosinolates. In: Stumpf, P.K., Conn, E.E., eds. *The biochemistry of plants*, vol. 7. New York: Academic Press, 501-525
- Larsen, L., Olsen, O., Ploeger, A. and Sorenson, H. 1983. Phenolic choline esters in rapeseed: possible factors affecting nutritive value and quality. In: *Proceedings 6th International Rapeseed Congress, Paris, 1577-1582*.
- Lein, K. A. 1972. Genetische und Physiologisch Untersuchungen Zur bildung von glucosinolaten in rapassamen : Lokalisierung des hauptbiosyntheseortes durch pflanzfropfungen. *Zeitschrift fur Pflanzenphysiologie* 67: 333-342
- Li, Y., Chinni, S.R. and Sarkar, F. H. 2005. Selective growth regulatory and pro-apoptotic effects of DIM is mediated by AKT and NF-kappa  $\beta$  pathways in prostate cancer cells. *Frontiers in Bioscience* 10: 236-243.
- Llorach, R., Gil-Izquierdo, A., Ferreres, F and Tomas-Barberan, F.A. 2003. HPLC-DAD-MS/MS ESI

- characterization of unusual highly glycosylated acylated flavonoids from cauliflower (*Brassica oleracea L. var. botrytis*) agro industrial by-products. *Journal of Agricultural and Food Chemistry* 51: 3895-3899.
- Lu, C., Yao, S. and Lin, N. 2001. Studies on reactions of oxidizing sulfur-sulfur three electron bond complexes and reducing alpha amino radicals derived from OH reaction with methionine in aqueous solution. *Biochimica et Biophysica Acta* 1525: 89-96.
- Lykkesfeldt, J. and Moller, B.L. 1993. Synthesis of benzyl glucosinolates in *Tropaeolum majus L.*: isothiocyanates as potent enzyme inhibitors, *Plant Physiology* 102: 609-613.
- Ma, Q., Hoper, M., Halliday, I. and Rowland, B.J. 1996. Diet and experimental colorectal cancer. *Nutrition Research* 16: 4792-4797.
- Malikova, J., Swaczynova, J., Kolar, Z. and Strnad, M. 2008. Anti cancer and anti-proliferative activity of natural brassionsteroids. *Photochemistry* 69: 418-426.
- Mandova, N.B. 1988. Plant growth promoting brassionsteroids. *Annual Review of Plant Physiology and Plant Molecular Biology* 39: 23-52.
- McGregor, D.I., Downey and R.K. 1975. A rapid and simple assay for identifying low glucosinolates rapeseed. *Canadian Journal of Plant Science* 55: 191-196.
- Mithen, R., Faulkner, K., Magrath, R., Rose, P., Williamson, G. and Marquez J. 2003. Development of isothiocyanate enriched broccoli and its enhanced ability to induce phase2 detoxification enzymes in mammalian cells. *Theoretical and Applied Genetics* 106: 727-734.
- Morse, M.A., Zu, H., Galati, A.J, Schmidt, C.J. and Stoner, G.D. 1993. Dose-related inhibition by dietary phenethyl isothiocyanate of esophageal tumorigenesis and DNA methylation induced by N-nitrosomethylbenzylamine in rats. *Cancer Letters* 72: 103-110.
- Naczk, M.R., Amarowicz, R., Sullivan, A. and Shahidi, F. 1998. Current research developments on polyphenolics of rapeseed/canola: a review. *Food Chemistry* 62: 489-502.
- Oslen, H., Aaby, K. and Borge, G.I.A. 2009. Characterization and quantification of flavonoids and hydroxycinnamic acids in curly Kale (*Brassica oleracea L. Con var. acephala Var. sabellica*) by HPLC-DAD-ESI-MS. *Journal of Agricultural and Food Chemistry* 57: 2816-2825.
- Palaniswamy, U.R., McAvoy, R.J., Bible, B.B. and Stuart, J.D. 2003. Ontogenic variations of ascorbic acid and phenethyl isothiocyanate concentrations in watercress (*Nasturtium officinale* R.Br.) leaves. *Journal of Agricultural and Food Chemistry* 51: 5504-5509.
- Pappa, G., Strathmann, J., Lowinges, M., Bartsch, H. and Gerhausew, C. 2007. Quantitative combination effects between sulforaphane and 3,3'-diindolylmethane on proliferation of human colon cancer cells in vitro. *Carcinogenesis* 28: 1471-1477.
- Pledge-Tracy, A., Sobolewski, M. and Davidson, N. E. 2007. Sulforaphane induces cell type-specific apoptosis in human breast cancer cell lines in vitro. *Molecular Cancer Therapy* 6: 1013-1021.
- Plumb G.W., Price, K.R., Rhodes, M.J., and Williamson, G. 1997. Antioxidant properties of the major polyphenolic compounds in broccoli. *Journal of Agricultural and Food Chemistry* 49: 2679-2683.
- Prester, T., Fahey, J.W., Holtzclaw, W.D., Abeygunawardana, C., Kalchinski, J.L. and Talalay, P. 1996. Comprehensive chromatographic and spectroscopic methods for the separation and identification of intact glucosinolates. *Analytical Biochemistry* 239: 168-179.
- Puupponen-Pimia, R., Nohynek, L., Schmidlin, Kahkonen, M., Heinonen, M., Maatta-Riihinen, K. and Oksman-Caldentey K.M. 2005. Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology* 98: 991-1000
- Raboy, V. 2001. Seeds for a better future: low phytic grains help to overcome malnutrition and reduce pollution. *Trends in Plant Science* 6: 458-462.
- Rao, S.S.R., Vidya Vardhini, B., Sujatha, E. and Anuradha A. 2002. Brassionsteroids –a new class of phyto hormones. *Current Science* 82: 1239-1245.
- Reddy, M.V., Hurrell, R. F., Juillerat, M.A. and Cook J.D. 1996. The influence of different protein sources on phytate inhibition of non haem-iron absorption in humans. *American Journal of Clinical Nutrition* 63: 203-207.
- Ribnicky, D.M., Poulev, A., Henry, E. and Raskin, J. 2001. Seed of *Barbarea verna* as a rich source of phenethyl isothiocyanate to provide natural protection from environmental and dietary toxins. *Journal of Nutraceuticals, Functional and Medicinal Foods* 3: 43-65.
- Rimbach, G. and Pallanf, J. 1998. Phytic acid inhibits free radical formation *in vitro* but does not affect liver oxidant or anti oxidant status in growing rats. *Journal of Nutrition* 128: 1950-1955.
- Robbins, R.J. 2003. Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry* 51: 2866-2887.
- Romani, A., Vignolini, P., Isolani, L., Ieri, F. and Heimler, D. 2006. HPLC-DAD/MS characterization of flavonoids and hydroxycinnamic derivatives in turnip tops (*Brassica rapa L. subsp. sylvestris L.*). *Journal of Agricultural and Food Chemistry* 54: 1342- 1346.
- Rosa, E.A.S., Heaney, R.K., Fenewick, G.R. and Portas, C.A.M. 1997. Glucosinolates in crop plants. *Horticultural Reviews* 19: 99-215.
- Rouzand, G., Young, S.A. and Duncan, A. 2004. Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. *Cancer Epidemiology, Biomarkers and Prevention* 13: 125-131.
- Sakurai, A. and Fujioka, S. 1993. The current status of physiology and biochemistry of brassionsteroids. *Plant growth regulators* 13: 147-159.
- Sarkar, F. H., Rahman, K. M. and Li, Y. 2003. Bax translocation to mitochondria is an important event in inducing apoptotic cell death by indole-3-carbinol

- (I3 C) treatment of breast cancer cells. *Journal of Nutrition* 133 (Suppl.): 2434S–2439S.
- Sasse, J.M., Griffiths, P.G., Gaff, D.F., Yokota, T. and Cameron, D.W. 1998. Brassinosteroids of a resurrection grass. 16th International conference on Plant Growth Substances. Chiba (Japan), 131.
- Scalbert, A., Johnson, I.T. and Saltmarsh, M. 2005. Polyphenols: anti oxidants and beyond. *American Journal of Clinical Nutrition* 81 (suppl.): 215S- 217S.
- Shamsuddin, A.M., Uelah, A. and Chakravarthy A.K. 1989. Inositol and Inositol hexaphosphate suppress cell proliferation and tumour formation in CD-1 mice. *Carcinogenesis* 10:1461-1463.
- Shapiro TA, Fahey JW, Wade KL, Stephenson KK, Talalay P. 2001. Chemo preventive glucosinolates and isothiocyanates of Broccoli sprouts: metabolism and excretion in urine *Cancer Epidemiology Biomarkers and Prevention* 10: 501-508.
- Shears S.B. 2001. Assessing the omnipotence of inositol hexakisphosphate. *Cellular Signalling* 13: 151-158.
- Shahidi, F. and Naczki, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of American Oil Chemists' Society* 69: 917-924
- Singh, R.P., Agarwal, C. and Agarwal R. 2003. Inositol hexaphosphate inhibits growth and induces G1 arrest and apoptotic death of prostate carcinoma DU 145 cells: modulation of CDKI-CDK- cyclin and pRb-related protein-E2F complexes. *Carcinogenesis* 24: 555-563.
- Singh, R.P., Sharma, G., Mallikarjuna, G.V., Dhanalakshmi, S., Agarwal, C. and Agarwal, R. 2004a. In vitro suppression of hormone refractory prostate cancer growth by inositol hexaphosphate induction of muslin like growth factor binding protein-3 and inhibition of vascular endothelial growth factor. *Clinical Cancer Research* 10: 244-250.
- Smith, T.J., Guo, Z., Thomas, P.E., Chung, F.L., Morse, M.A., Elkind, K. and Yang, C.S. 1990. Metabolism of 4- (methylnitrosoamino)-1-(3, pyridyl)-1-butanone in mouse lung microsomes and its inhibition by isothiocyanates. *Cancer Research* 50: 6817-6822
- Smith, T.K., Lund, E.K., Parker, M.L., Clarke, R.J. and Johnson, I.T. 2004. Allyl isothiocyanate causes mitotic block, loss of cell adhesion and disrupted cytoskeletal structure in HT 29 cells. *Carcinogenesis* 25: 1409-1415
- Sosulski, F. 1979. Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: a review. *Journal of American Oil Chemists' Society* 56: 711-715.
- Sosulski, F.W. and Dabrowski, K.J. 1984. Composition of free and hydrolysable phenolic acids in the flours and hulls of ten legume species. *Journal of Agricultural and Food Chemistry* 32: 1311-133.
- Stoner, G.D., Morse, M.A. 1996. Isothiocyanates as inhibitors of esophageal cancer. *Advances in Experimental Medicine and Biology* 401: 13-23.
- Takaba, K., Hirose, M., Yoshida, Y., Kimura, J. Ito, N. and Shirai T. 1997. Effects of n-tritriacontane-16, 18-dione, curcumin, chlorophyllin, dihydroguaiaretic acid, tannic acid and phytic acid on the initiation stage in a rat multi organ carcinogenesis model. *Cancer Letters* 113: 39-46.
- Takatsuto, S. 1994. Brassinosteroids: distribution in plants, bioassay and microanalysis by gas chromatography. *Journal of Chromatography* 658: 3-5.
- Talalay, P., Fahey, J.W., Holtzclaw, W.D., Prester, T. and Zhang, Y. 1995. Chemo protection against cancer by phase 2 enzyme induction. *Toxicology Letters* 82: 173-179.
- Talalay, P. and Zhang, Y. 1996. Chemo protection against cancer by isothiocyanate and glucosinolates. *Biochemical Society Transactions*. 24: 806-810.
- Tawfiq, N., Heaney, R.K., Plumb, J.A., Fenewick, G.R., Musk, S.S.R. and Williamson, G. 1995. Dietary glucosinolates as blocking agents against carcinogenesis: glucosinolates breakdown products assessed by induction of quinone reductase activity in murine hepa1C1c 7 cells. *Carcinogenesis* 16: 1191-1194.
- Thejass, P. and Kuttan, G. 2006. Augmentation of natural killer cell and antibody dependent cellular cytotoxicity in BALB/c mice by sulforaphane, a naturally occurring isothiocyanate from broccoli 1L-2 and IFN-gamma. *Immunopharmacology and Immunotoxicology* 28: 443-457.
- Tiedink, H.G.M., Davies, J.A.R., Van Broekhoven, L.W., Vander Kamp, H.J. and Jongen, W.M.F. 1988. Formation of mutagenic N-nitroso compounds in vegetable extracts upon nitrile treatment: a comparison with glucosinolate content. *Food and Chemical Toxicology* 26: 947-954.
- Tiku, A. B., Abraham, S. K. and Kale, R. K. 2008. Protective effect of the Cruciferous vegetables mustard leaves (*Brassica campestris*) against in vivo chromosomal damage and oxidative stress induced by gamma radiation and genotoxic chemicals. *Environmental and Molecular Mutagenesis* 49: 335-342.
- Uda, Y., Kurata, T. and Arakawa, N. 1986. Effects of pH and ferrous ions on the degradation of glucosinolates by myrosinase. *Agricultural and Biological Chemistry* 50: 2735-2780.
- Underhill, E.W. 1980. In, *Encyclopedia of plant physiology*, (Bell, E.A., Charlwood, B.V., Archer, S.L, eds) . New series, vol.8. Berlin, Heidelberg, New York: Springer-Verlag (1980), 490-511.
- Vallejo, F., Tomas-Barberan, F.A. and Garcia-Viguera, C. 2002. Potential bioactive compounds in health promotion from broccoli cultivars grown in Spain. *Journal of the Science of Food and Agriculture* 82: 1293-1297.
- Vallejo, F., Tomas-Barberan, F.A., Banavent-Garcia, A.G. and Garcia-Viguera C. 2003. Total and individual glucosinolate contents in inflorescences of eight broccoli cultivars grown under various climatic and fertilization conditions. *Journal of the Science of Food and Agriculture* 83: 307-313.
- Vallejo, F., Tomas-Barberan, F.A. and Ferreres, F. 2004. Characterization of flavonols in broccoli (*Brassica*

- oleracea* L.var.italica) by liquid chromatography –UV diode array detection- electro spray ionisation mass spectrometry. Journal of chromatography A 1054:181-193.
- Vig, A.P., Rampal G., Singh, T.S. and Arora S. 2009. Bio-protective effects of glucosinolates- A review. LWT-Food Science and Technology 42 1561-1572.
- Vita J.A. 2005. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. American Journal of Clinical Nutrition 81 (suppl.): 292 S- 297 S.
- Vucenik, I. and Shamsuddin, A.M. 2003. Cancer inhibition by Inositol hexa phosphate (IPb) and inositol: from laboratory to clinic: Journal of Nutrition 133: 3778 S-3784S.
- Wachsman, M.B., Lopez, E.M., Ramirez, J. A., Galagovsky, L.R. and Coto. C.E. 2000. Anti viral effect of brassinosteroids against herpes virus and arena viruses. Antiviral Chemistry and Chemotherapy. 11: 71-77
- Wachsman, M.B., Ramirez J. A., Galagovsky, L.R. and Coto C.E. 2002. Anti viral activity of brassinosteroids derivatives against measles virus in cell cultures. Antiviral Chemistry and Chemotherapy 13: 61-66.
- Wang, L. J., Giovannucci, E.L., Hunter, D., Neubergh, D., Su, L. and Christiani, D.C. 2004. Dietary intake of Cruciferous vegetables glutathione -S- transferase (GST) polymorphisms and lung cancer risk in a Caucasian population. Cancer Causes Control 15: 977-985.
- Weber, G., Singhal, R.L., Prajda, N., Yeh, Y.A., Look, K.Y. and Slege, G.W. Jr. 1995. Regulation of signal transduction. Advance in Enzyme Regulation 35: 1-21.
- Wright, A.S. 1980. The role of metabolism in chemical mutagenesis and chemical carcinogenesis. Mutation Research 75: 215-223.
- Wu, H. T., Lin, S.H. and Chen, Y. H. 2005. Inhibition of cell proliferation and in vitro markers of angiogenesis by indole-3-carbinol a major indole metabolite present in Cruciferous vegetables. Journal of Agricultural Chemistry 53: 5164-5169.
- Xu, Q., Kanthasamy, A.G. and Reddy, M.B. 2008. Neuro protective effect of natural iron chelator, phytic acid in a cell culture model of Parkinson's disease. Toxicology 245: 01- 108.
- Yokota, T. 1997. The structure, biosynthesis and function of brassinosteroids. Trends in Plant Science 2: 137-143.
- Yokozawa, T., Kim, H. Y., Cho, E. J., Yambi, Y. and Choi, J. S. 2003. Protective effects of mustard leaf (*Brassica juncea*) against diabetic oxidative stress. Journal of Nutrition Science and Vitaminology 49: 87-93.
- Yuan, F., Chend, D.Z., Liu, K., Sepkovic, D.W., Bradlow, H.L. and Auburn, K. 1999. Anti estrogenic activities of indole-3-carbinol in cervical cell: Implication for prevention of cervical cancer. Anticancer Research 19: 1673-1680.
- Zhang, Y., Kensler T.W., Cho, C.G., Posner, G.H and Talalay, P. 1994. Anti carcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanate. Proceedings of the National Academy of Science, USA. 91:3147-3150.
- Zhang, Y. and Talalay, P. 1994. Anticarcinogenic activities of organic isothiocyanate : chemistry and mechanisms. Cancer Research 54 (Suppl.) 1976s-1981s.
- Zhao, H., Lin, J., Grossman, H.B., Hernandez, L. M., Dinney, C.P and Wu, X. 2007. Dietary isothiocyanates GSTM 1, GSTT 1, NAT 2 polymorphism and bladder cancer risk. International Journal of Cancer 120:2208-2213.
- Zukalova, H. and Vasak, J. 2002. The role and effects of glucosinolates of Brassica species- a review. Rostlinna Vyroba 48 (4): 175-180.