Review

Therapeutic potentials of *Brassica juncea*: an overview

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ABSTRACT

Diverse medicinal uses of different types of products obtainable from *Brassica juncea* have been known for centuries. Most such traditionally known uses of the plant have been centered on its seeds and oils obtainable from them. During more recent decades diverse bio-active molecules and their therapeutically interesting pharmacological properties of its green edible leaves have also been described, and they are now often considered to be effective substitutes for other so called "healthy" Brassica vegetables. However, little concentrated effort has yet been made to obtain a pharmacologically better defined phyto-pharmaceutical from this easily cultivable plant of commercial interest in many underdeveloped and developing countries. The main aim of this overview is to point out some possibilities for designing and developing such products from the plant for combating the rapidly spreading obesity epidemic in the developed countries and some other countries. Efforts to achieve such goals could as well be an economically more feasible, and culturally more acceptable, starting point for better understanding the potential health benefits of other vegetarian foods.

INTRODUCTION

Brassica juncea, also known as Indian mustard, Chinese mustard, oriental mustard, leaf mustard, or mustard green, is a species of mustard family of Brassicaceae (Cruciferae) plants (Fig. 1). Its primary center of origin is central Asia (northwest India), with secondary centers in central and western China, eastern India, Burma, and through Iran to the Near East. The principle growing countries are Bangladesh, Central Africa, China, India, Japan, Nepal, and Pakistan, as well as southern Russia north of the Caspian Sea. It is considered also as a principle weed in Canada, a common weed in Argentina and Australia, and a weed in Fiji, Mexico, and the United States. Indian mustard is widely distributed as a cultivar and transgenic escape in subtropical and temperate climates. Seeds of this plant are widely used in America, Japan, China and other countries and regions as a traditional pungent spice, a source of edible oil and protein, and a type of complementary or alternative medicine. The leaves are used in a range of folk medicines as stimulants, diuretics and expectorants as well as a spice (Farrell, 1985). In Korea, it is used for both food itself and the major ingredient of kimchi, a traditional fermented vegetable food, and kimchi including mustard leaf has recently attracted a lot of attention as a functional food for health maintenance and disease prevention (Kim et al., 2003). The essential oil of *Brassica juncea* seeds, also referred to as mustard oil, has also been used in cosmetics for hair control (Yu et al, 2003).

The major pungent chemical constituent of such commercialized oils is Allyl isothiocyanate which is formed from its precursor during the processing of the seeds (Yu et al., 2003). This isothiocyanate is now considered to be the most important cancer chemo-preventive phytochemical with other potential health benefits (Okulicz, 2010; Zhang et al., 2010) and antimicrobial agent against a variety of organisms (Luciano and Holley, 2009). Structurally diverse glucosinolates and other precursors of isothiocyanates are encountered not only in *Brassica juncea* leaves (Hill et al., 1987), but also in diverse other edible cruciferous vegetables well recognized for their health benefits (Higdon et al., 2007). Amongst many such vegetables, the glucosinolates contents of *Brassica juncea* leaves are reported to be the highest (McNaughton and Marks, 2003). In general, contents of these phyto-chemicals in seeds of Brassicacea family grown in tropical environment are higher than of those grown in temperate regions (Tripathi et al., 2007).

Brassica juncea is known to produce several other classes of bioactive phyto-chemicals including glycosides, flavonoids, phenolic compounds, sterols and triterpene alcohols, proteins and carbohydrates (Appelqvist et al., 1973; Das et al., 2009; Fabre et al., 1997; Jung et al., 2009; Li et al., 2000; Sang et al., 1984; Yokozawa et al., 2002). The potential importance of such secondary metabolites of the plant in diverse therapeutically interesting bio-activities of preparations obtainable from its seeds and leaves has often been pointed out in more recent years (Table 1, 2, and 3). Taken together, available preclinical information on this easily cultivable and edible plant strongly suggest that it could as well be a sustainable source for affordable nutraceuticals, and/or drugs, potentially useful for the prevention and cure of diverse types of non-communicable diseases of the 21st century. A proper understanding of the pharmaceutically relevant properties of its bio-active components, and of possible interactions between them, is an

Fig. 1. *Brassica juncea* (L.) Czern and Coss (Indian mustard, Rai)

essential prerequisite for such ventures. Unfortunately, no systematic, concentrated efforts have yet been made to more rationally clarify the situation. The main aim of this communication is to summarize the currently available preclinical knowledge on the diverse parts of the plant, and to point out some unique therapeutic possibilities potentially offered by its edible leaves.

Plant description

The genus Brassica contains over 150 species that are cultivated worldwide as oilseed crops and/or vegetables. *Brassica juncea* is one such economically important plant well known in India for centuries for its nutritive and medicinal values (Ram Manohar et al., 2009). The leaves as well as the seeds of this mustard variety are edible, and diverse medicinal uses of its seeds are also well known in other countries. During more recent years it has also been cultured to produce a greater variety of benefits, including selenium, chromium, iron, and zinc food supplements. In general, the plant is taxonomically defined as follows:

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida - Dicotyledons
Subclass	Dilleniidae
Order	Capparales
Family	Brassicaceae - Mustard family
Genus	Brassica L. - Mustard
Species	*Brassica juncea* (L.) Czern. and Coss. - Indian mustard

Potential bioactive constituents

Together with glucosinolates, numerous polyphenolic secondary metabolites of *Brassica juncea* are often considered to be its major therapy relevant bioactive components (Cartea et al., 2011; Jahangir et al., 2009). However, medicinal phytochemistry and structure activity relationships of these and other extractable components of the herb still remain to be properly defined. Table 1 summarizes different chemical classes of its better characterized bioactive constituents, and some others identified ones will be described later.

Glucosinolates

Glucosinolates belong to the class of organic compounds which are characterized by a glucose-derived functional group attached to a sulphonated oxime through a side chain which may be either aliphatic, aromatic or heterocyclic (Chew, 1988). Some examples of glucosinolate are given in Fig. 2a Aliphatic Glucosinolate and 2 (b) Aromatic Glucosinolate. More than 200 individual glucosinolates have already been identified in diverse Brassicaceae plants, and many of them are also known to be present in *Brassica juncea*. In general, glucosinolates are water-soluble anions, which in the presence of the enzyme myrosinase and water generate isothiocyanates, thiocyanates or nitriles (Morra, et al., 1994). The enzymatic hydrolysis of glucosinolates by membrane-bound thioglucosidase produces

Table 1. Isolated constituents of *Brassica juncea* and their pharmacological activities

| No.	Isolated constituents	Activities	References
1.	**Glucosinolates** Sinigrin (allyl glucosinolate)	Goitrogenic	Yu et al, 2003; Carlson et al., 1987; Tripathi et al., 2001; Goetz and Schraudolf, 1983; Schreiner et al., 2009.

2.	**Isothiocyanates**
	   I. Allyl isothiocyanate
	
	   II. Phenyl isothiocyanate
		Fungicidal activity, antitumor activity, Antimicrobial, Anti-tumour and anti-oxidant activity	Mayton et al., 1996; Luciano and Holley, 2009; Kumar et al., 2009; Manesh and Kuttan, 2003.
			 Thejass and Kuttan, 2007.

3.	**Phenolic compounds** (Sinapic Acid, Sinapine)	Anxiolytic activity, antioxidant, Cognition-improving activity	Yoon et al., 2007; Karakida et al., 2007; Zou et al., 2002.
4.	**Fatty Acids** (α-linolenic acid)	Astrocyte developing activity and other health benefits	Joardar et al., 2007; Chew, 1988.
5.	**Kaempferol glycosides** (Kaempferol 7-O-β-D-glucopyranosyl-(1→3)-[β -D-glucopyranosyl-(1→6)]-glucopyranoside, Kaempferol-3-O-(2-O-feruloyl- β -D-glucopyranosyl-(1→2) - β- D- glucopyranoside)- 7 -O -β-D-glucopyranoside, Kaempferol-3-O-β -D- glucopyranosyl-(1→2)-O-β-D-glucopyranoside-7-O-β D-glucopyranoside and 1-O-sinapoyl glucopyranoside)	Antioxidant activity	Jung et al., 2009; Kim et al., 2002.
6.	**Other Flavonoid compound** Isorhamnetin 3,7-di-O-ß-D-glucopyranoside (Isorhamnetin diglucoside)	Antioxidant effects	Yokozawa et al., 2002, 2003.

7.	**Proteins**
	   I. Napins
	
	   II. Juncin
		Antifungal, Allergenicity Antifungal	Appelqvist et al., 1973; Dasgupta et al., 1995; Jyothi et al., 2007; Ye and Ng, 2009.
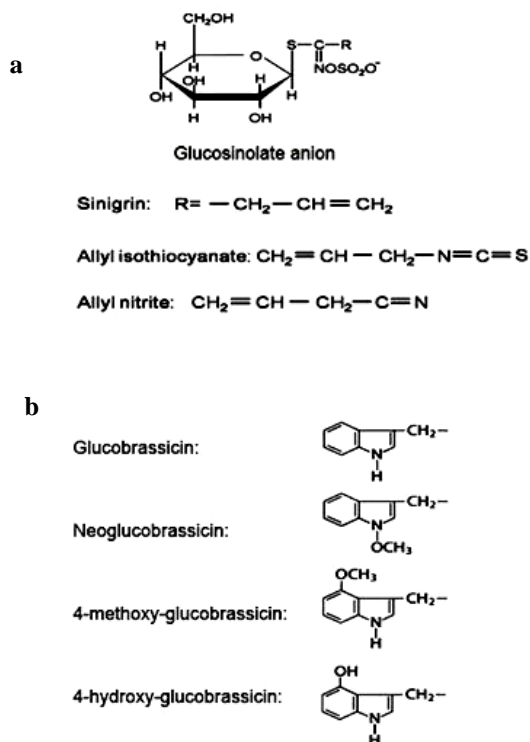
Fig. 2. Glucosinolate present in *Brassica juncea*. (a) aliphatic glucosinolate and (b) aromatic glucosinolate (indole glucosinolate).

also numerous compounds including isothiocyanates, nitriles, thiocyanates, epinitriles, and glucose. The end product of this hydrolytic reaction is determined by the substituent-groups of the glucosinolates and the physical and chemical conditions under which hydrolysis takes place (Mayton et al., 1996). Glucosinolates with aliphatic side chains are called aliphatic gluosinolates and are commonly found in Brassica spp. The aliphatic glucosinolate profile of *Brassica juncea* consists mainly of 3-butenyl and 2-propenyl (Fenwick et al., 1983). Like in other plants of the family, sinigrin was also identified as a major glucosinolate in the seeds and leaves of *Brassica juncea* (Carlson et al., 1987; Hill et al., 1987; Sang et al., 1984). Sinigrin (2-propenyl or allylglucosinolate) and glucoraphanin (4-methylsulfinylbutylglucosinolate) are precursors of the anticancer compounds ally-isothiocyanates (Manson et al., 1997; Smith et al., 1998) and sulforaphane (4-methylsulfinylbutyl isothiocyanate) (Fahey et al., 1997; Nestle, 1997), respectively. Sinigrin on hydrolysis by myrosin (myrosinase) yields allyl isothiocyanate, glucose, and potassium bisulfate (Fig. 3). Allyl isothiocyanate is volatile, and the yields from *Brassica juncea* vary between 0.25 to 1.4%. Indole glucosinolate has been isolated from the Brassica species (Carlson et al., 1987; Goetz and Schraudolf, 1983). Some examples of indole glucosinolate are glucobrassicin, neoglucobrassicin, 4-methoxy-glucobrassicin and 4-hydroxy-glucobrassicin (Schreiner et al., 2009).

Three native glucosinolates have been isolated from the seeds of *Brassica juncea* (Fig. 4). The main one is *p*-hydroxybenzyl glucosinolate, with the two others being 9-(methylsulfonyl) nonyl glucosinolate and 8- (methylsulfonyl) octyl glucosinolate (Fabre et al., 1997). It must be noted though that the contents as well as the nature of glucosinolates and other bioactive secondary metabolites of all Brassicaceae plants vary considerably with diverse agronomic practices (Bjorkman et al., 2011). Moreover, it has been demonstrated also that the contents of its glucosinolates and other

phytoalexins, as well their metabolites, can be drastically altered by pathogen attacks on the plant (Pedras et al., 2002; Schreiner et al., 2009). These findings clearly point out the necessity of appropriately standardized agricultural practices for obtaining the maximum medicinal benefits potentially offered by the plant.

Flavonoids and their glycosides

The most abundant polyphenols in Brassica species are the flavonoids (mainly flavonols, but also anthocyanins) and the hydroxycinnamic acids. Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge. Flavonols are often the most widespread flavonoids of mustard greens. Quercetin, Kaempferol and Isorhamnetin, the main flavonols in Brassica crops, are most commonly found as O-glycosides (Fig. 5). Conjugation occurs most frequently at the 3 position of the C-ring, but substitutions can also occur at the 5, 7, 4´, 3´ and 5´ positions (Aron and Kennedy, 2008; Crozier et al., 2006; Hollman and Arts, 2000). Within the colored flavonoids, anthocyanins are the most important group of plant pigments, also considered as multifunctional components of food due to their antioxidant activities and other beneficial biological properties (McDougall et al., 2007; Moreno et al., 2010; Sadilova et al., 2006) The most common anthocyanins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin, with cyanidin the most common in Brassica crops (Lo Scalzo et al., 2008; Moreno et al., 2010; Tatsuzawa et al., 2006). A new rare Kaempferol-7-O-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-glucopyranoside was isolated from *Brassica juncea* along with the already known kaempferol-3-O-(2-O-feruloyl-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside)-7-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranoside-7-O-β-D-glucopyranoside and 1-O-sinapoyl-glucopyranoside (Kim et al., 2002). More recently, kaempferol-3-O-(2-O-sinapoyl)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside-7-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside-7-O-β-d-glucopyranosyl-(1→6)-β-D-glucopyranoside, and kaempferol-3-O-(2-O-sinapoyl)-β-D-glucopyranosyl-(1→2)-β-D-gluco-pyranoside-7-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and several other kaempferol glycosides were isolated from the leaves of
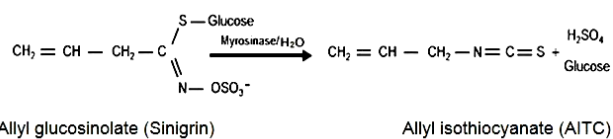

Fig. 3. Volatile hydrolytic product (allyl isothiocyanate) of sinigrin in presence of myrosinase enzyme.
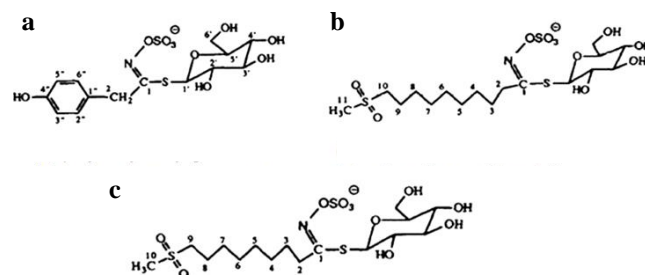

Fig. 4. Glucosinolates isolated from seeds of *Brassica juncea*. (a) *p*-hydroxybenzyl glucosinolate, (b) 9-(methylsulfonyl) nonyl glucosinolate and (c) 8- (methylsulfonyl) octyl glucosinolate.
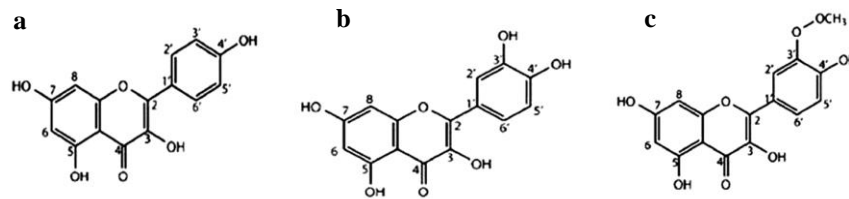
Fig. 5. Flavonols as O-glycosides present in Brassica crops. (a) quercetin, (b) kaempferol, and (c) isorhamnetin.
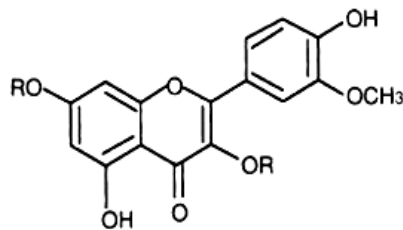

Fig. 6. Mustard leaf contains isorhamnetin 3, 7-di-O-β-D-glucopyranoside (Isorhamnetin diglucoside) as a major flavonolic secondary metabolite (R=β-D-glucopyranoside).

Fig. 7. Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) is as main phenolic compounds present in *Brassica juncea* having bitterness and astringency in nature with antioxidant properties.

Brassica juncea, (Jung et al., 2009).

The mustard leaf also contains Isorhamnetin 3, 7-di-O-β-D-glucopyranoside (Isorhamnetin diglucoside), which is suggested to be its major flavonolic secondary metabolite (Yokozawa et al., 2002). A more recent independent comparative study on the flavonoid content of 91 vegetables is in agreement with this hypothesis, and this study also reveals a unique flavonol aglycone spectrum of *Brassica juncea*, not present in any vegetables including other plants which belong to the Brassicaceae family (Yang et al., 2008). In this study, the total flavonoid content of *Brassica juncea* was not the highest, but the spectrum of flavonoids observed for this plant was not comparable to any other plants of the Brassicaceae family examined. Such is especially the case for *Brassica juncea* leaves, i.e. the main source of edible vegetable from the plant (Cartea et al., 2011). This last mentioned report can be consulted for obtaining more detailed information on the flavonoids and other phenolic components of the Brassica vegetables. It must be noted though, that Isorhamnetin (Fig. 6) and its conjugates are also the human metabolites Quercetin and other naturally more abundant flavonoids (Manch et al., 2004).

Other Phenolic compounds
Mustard meal is a good source of phenolic compounds. These compounds were previously considered undesirable because the presence of phenolic compounds can cause bitterness and astringency and dark colors in protein products, but they are now emerging as value added products with antioxidant properties. Sinapic acid (SA) (MW-224.2 Da), the main phenolic compound in mustard meal (Fig. 7), constitutes over 73% of free phenolic acids and about 80-99% of the total phenolic acids mainly occurring as esters of sinapic acid, sinapine (MW-275 Da), and glucosides. Sinapic acid and sinapine are the major water-soluble antioxidant components in the mustard meal (Das et al., 2009).

More than a dozen other phenolic acid conjugates have also been encountered in *Brassica juncea* leaves (Cartea et al., 2011) and the spectrum of polyphenolics and their conjugates encountered in them is als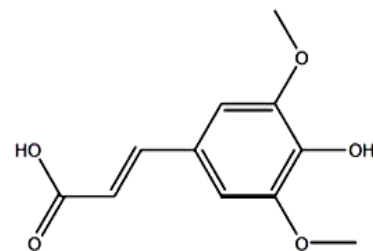o very unique and broad. Similar to the other structural classes of secondary metabolites, the spectrum of their polyphenolic ones depends also on the agricultural conditions used to cultivate them, and on their harvesting stages.

Proteins
The two major seed storage proteins of Brassica species are napin (2S albumin), constituting about 45–50% of the total proteins, and cruciferin (12S globulin), constituting about 25% of the total proteins (Appelqvist et al., 1973). Mature napin from *Brassica juncea* consists of two polypeptides, a small subunit of 29 amino acids (molecular weight of 4442) and a large subunit of 86 amino acids (molecular weight of 10300), held together by disulfide bonds derived by proteolytic cleavage from a single polypeptide precursor (Dasgupta et al.,1995). The precursor of napin is a trypsin inhibitor (Mandal et al., 2002). The presence of disulfides contributes to the stability and compactness of napin. Napins, all alpha proteins, are characterized by a high content of α-helix and are basic in nature. These are reported to be antifungal in nature (Jyothi et al., 2007). An 18.9 kDa antifungal protein designated juncin was isolated from seeds of the *Brassica juncea* var. Integrifolia (Ye and Ng, 2009). *Brassica juncea* glyoxalase I (S-lactoylglutathione-lyase, EC 4.4.1.5) is a 56 kDa, heterodimeric protein. It requires magnesium (Mg^{2+}) for its optimal activity (Deswal and Sopory, 1999, 1998). It also includes globulins and mucilage (Leung, 1980).

Fixed oils
Mustard seed oil consists mainly of glycerides: erucic, eicosanoic, arachidic, nonadecanoic, behenic, oleic, and palmitic acids in addition to α-linolenic acid and arachidonic acid (Joardar and Das, 2007). In general, this oil is enriched in erucic acid, which according to some authors could also have adverse effects in high doses. Therefore, attempts are now being made in some laboratories to obtain *Brassica juncea* seeds with lower contents of this and other possible "undesirable" constituents of mustard oils. The economic value of the plant is mainly due to its widespread uses to produce fixed oils from its seeds, which still remains to be the main
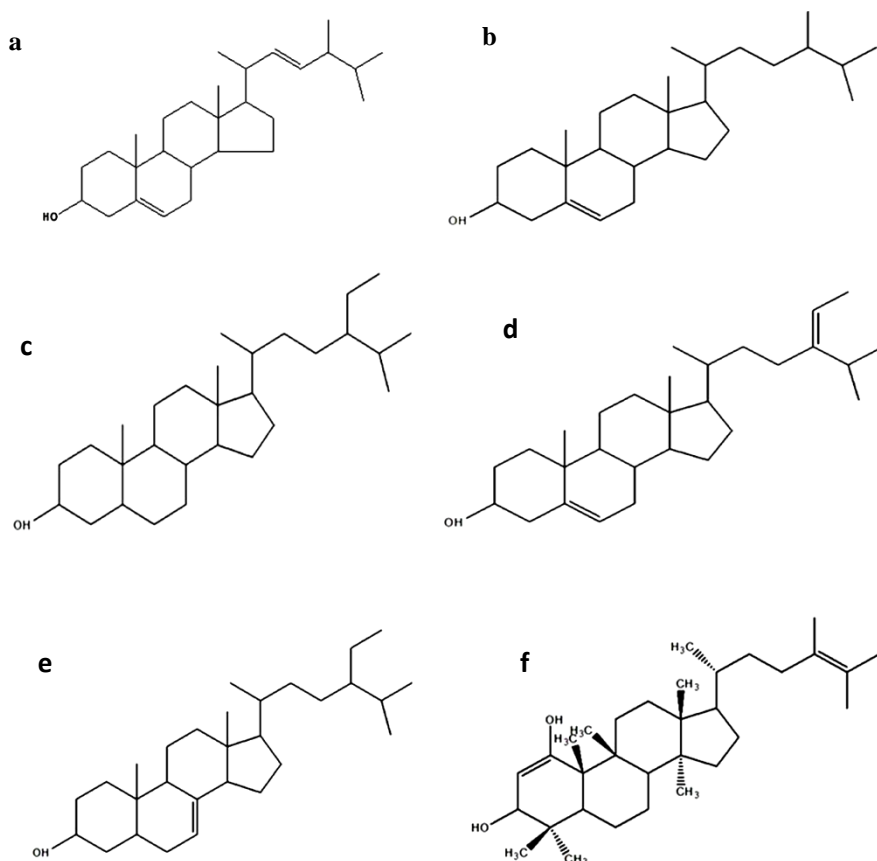
Fig. 8. Phytosterols in mustard oil (a) brassicasterol, (b) campesterol, (c) β-sitosterol, (d) Δ^5-avenasterol, (e) Δ^7-stigmasterol, and triterpene alcohol as (f) cyclobranol.

and a trace of Δ^7-stigmasterol. Its triterpene alcohol was cyclobranol (Li et al., 2000). Chemical structures of these phytosterols are given in Fig. 8. A long chain alcohol 4-decanol has been identified as an antimutagenic constituent of the mustard leaf (Kim et al., 1993). In general, the beneficial effects of phytosterols and the long chain alcohols present in mustard seeds are now often discussed as potential active components of "healthy vegetables" useful for combating hypercholesterolemia.

Nutritive constituents
Mustard greens are high in vitamins and minerals. Following are the approximate estimates per 100 g edible portion: 62 kJ energy, 93.8 g H_2O, 2.3 g protein, 0.3 g fat, 0.7 g total sugar, 1.8 g fiber,0.14 g total organic acid, 1.6 g ash, 130 mg Calcium (Ca), 11 mg Magnesium (Mg), 0.7 mg Iron (Fe), 3 mg Sodium, 450 mg Potassium, 0.1 mg Zinc (Zn), 100 mg vitamin C, 1550 µg β-carotene equivalent, 0.06 mg thiamine, 0.09 mg riboflavin, and 0.6 mg niacin (Wills et al., 1984). Some analogous values for healthy mustard green leaves are (in mg/g dry weigh): Sugar 5.50, Starch 7.50, Protein 6.80, Lipid 78, Phenol 8.0, Amino acid 0.65 and Ascorbic acid 0.85 (Singh et al., 2011).

Pharmacology and toxicology
Brassica juncea seeds are widely used in almost all traditionally known Indian Systems of Medicine. Observations that its essential oil causes irritation and inflammation led to its experimental uses as a tool useful for better understanding the biological processes involved in such processes. Several observations made during the first half of the 20th century provided valuable contributions towards our current understanding of the processes involved in vascular and neurogenic inflammation. It is now well recognized that the glucosinolates and isothiocyanates present in mustard seeds and its oils are involved in their cancer preventive effects, and that these components are orally absorbed from vegetables as well (Bhattacharya et al., 2010; Shapiro et al., 1998). Furthermore, realization of the fact that edible mustard oil is a rich source of polyunsaturated fatty acids and phytosterols has also led to the speculation that it could also have cardio-protective effects and other health benefits. Observations made during an

source of edible vegetable oils in many countries and cultural groups. Mustard seeds and oil are now also being explored for producing bio-fuels and diverse other commercial purposes (Jham et al., 2009).

Essential oil
Isothiocyanates are known to be the main group of constituents in the essential oils of *Brassica juncea*. It includes allyl isothiocyanate (54.8 - 68.8%), 3-butenyl isothiocyanate (4.8 - 5.9%) and phenethyl isothiocyanate (2.4 - 3.4%). They represent more than 62.9% of the total essential oil. The sulfides are present in relatively small amounts (14.8 - 23.4%). They include Diallyl trisulfide (7.8 - 9.7%), diallyl sulphide (3.2 - 5.5%) and diallyl disulfide (2.7 - 4.1%) (Yu et al., 2003).

Phytosterols and alcohols
Among several edible fats and oils analyzed, mustard oil contained the highest concentration (64 mg/g) of Phytosterols (Sabir et al., 2003). Mustard seed oil's nonsaponiafiable sterol fraction has been reported to contain 19.2% brassicasterol (9.1% esterified), 23.6% free campesterol (34.0% esterified), 57.2% β-sitosterol (55.2% esterified), 1.7% esterified Δ^5-avenasterol,

Table 2. Pharmacological activities of different parts of *Brassica juncea* (Rai)

No.	Parts of plant	Activity reported	References
1.	Seeds	Anti-diabetic/ Antihyperglycemic, Anti-oxidant, Antiatherogenic Antifungal activity, Allergenicity, Antitumor activity	Grover et al., 2002; Zou et al., 2002; Ye and Ng, 2009; Khan et al., 1996A, 1996B,1997; Jyothi et al., 2007; Yadav et al., 2004; Monsalve et al., 1993.
2.	Leaves	Anti-oxidant, Fungicidal Activity, antiatherogenic effect.	Kim et al., 2003; Yokozawa et al., 2002; Mayton et al., 1996; Jo et al., 1993; Lee et al., 2010.

Table 3. Pharmacological activities of *Brassica juncea* (Rai)

No.	Activity reported	References
1.	Anti-diabetic/ Antihyperglycemic/hypoglycemic activity	Grover et al., 2002; Khan et al., 1995B; Yadav et al., 2004; Grover et al., 2001; 2003.
2.	Anxiolytic activity	Yoon et al., 2007.
3.	Inflammatory activity	Inoue et al., 1997; Fiorentino et al., 1999; Zhang et al., 2006.
4.	Astrocyte developing activity	Joardar et al., 2007.
5.	Anti-oxidant/Peroxynitrite Scavenging Activity	Kim et al., 2003; Jung et al., 2009; Yokozawa et al., 2002, 2003; Zou et al., 2002; Khan et al., 1997.
6.	Haematological and histological studies	Khan et al., 1995A, Tripathi et al., 2008.
7.	Antiatherogenic/Lipid profile	Khan et al., 1996A,1996B,1997; Jo et al., 1993.
8.	Antimicrobial activity	Ye and Ng, 2009; Mayton et al., 1996; Luciano and Holley, 2009; Lin et al., 2000; Guan et al., 2008. Li et al., 2000.
9.	Antitumor activity	Kumar et al., 2009; Khan et al., 1996B; Manesh and Kuttan, 2003; Thejass and Kuttan, 2007.
10.	Cerebral protective and cognition-improving activity	Karakida et al., 2007
11.	Allergenicity	Jyothi et al., 2007; Monsalve et al., 1993.
12.	Defense against insects	Blau et al., 1978; David and Gardiner, 1966; Nault and Styer, 1972; McCloskey and Isman, 1993; Wadleigh and Yu, 1988.
13.	Goitrogenic	Tripathi et al., 2001.

epidemiological study in India (Rastogi et al., 2004) provide evidence supporting these ideas. Since numerous other epidemiological studies have revealed the diverse health benefits of cruciferous green vegetables, reports on therapeutically interesting bioactivities of its leaf extracts have also now started appearing. Tables 2 and 3 summarize the major known bioactivities of the leaves and seeds of the plant and their active constituents. Potential uses of such information for obtaining pharmacologically standardized extracts of the plant for therapeutic purposes will be outlined in the following paragraphs.

Metabolic disorders
Diabetes and hyperlipidemia are two major life threatening metabolic disorders often encountered in obese patients with sedentary lifestyles. The close association between these two disorders has now led to the medical terminologies like diabesity, insulin resistance and medical syndrome. Available information on pharmacological activity profiles of diverse types of *Brassica juncea* derived products strongly suggests their therapeutic potential against such disorders. However, no definitive statements on the nature of phyto-constituents involved in their observed effects can yet be made. This is not only because of the diverse types of extracts and experimental designs were used in different studies, but also due to the fact that none of the animal models used to date for such studies truly represent the complex pathologies involved in metabolic disorders, and depend on the experimental conditions used. For example, a recent report (Thirumali et al., 2011) describes dose dependant (250, 350 and 450 mg/kg/day) beneficial effects of an aqueous mustard seed extract against hyperglycemia and insulin deficiency in streptozotocin induced diabetic rats; whereas in an earlier study no hypoglycemic effect of the seeds was observed in an analogous rat diabetes model (Grover et al., 2002). Since *Brassica juncea* caused the reduction in glucose levels in moderate diabetes but not in severely diabetic rats, it seems that the antihyperglycemic activity of *Brassica juncea* depends upon the presence of the functional β-cells to release the insulin. *Brassica juncea* seeds probably prevented the destruction of β-cells of islets in the pancreas by its antioxidant effect. This is an interesting finding and suggests the likelihood of *Brassica juncea* having antioxidant and free radical scavenger activities (Grover et al., 2002). Khan et al. (1995b) demonstrated that *Brassica juncea* in normal rats increases glucose utilization (increase in glycogenesis as evidenced by the increased activity of glycogen synthase) and decreases

glycogenolysis and gluconeogenic enzymes (evidenced by the decreased activity of glycogen through phosphorylase and gluconeogenic enzymes). However, in the diabetic rats, *Brassica juncea* did not influence enzyme activity, indicating a positive role only in the pre-diabetic state. For control of severe diabetes it alone cannot be useful and may be of no use in insulin dependent diabetes. In another study, *Brassica juncea* significantly prevented the development of insulin resistance in rats fed fructose-enriched diet. The feeding of a fructose rich diet for 30 days resulted in rises in blood glucose by 29.4%, insulin by 101.2% and cholesterol by 26.7%, indicating the development of insulin resistance. However, the feeding of a fructose diet containing 10% *Brassica juncea* seeds powder for 30 days significantly decreased fasting serum glucose, insulin and cholesterol levels but did not normalize them. Thus, the results suggest that *Brassica juncea* can play a role in the management of pre-diabetic state of insulin resistance and its use in higher quantity as a food ingredient should be promoted in patients prone to diabetes (Yadav et al., 2004). In one study, the effects of *Brassica juncea* and *Murraya koeingii* in attenuating parameters of diabetic nephropathy, i.e. urine volume, serum creatinine, and urinary albumin (UAE) levels have been studied. *Murraya koeingii* and *Brassica juncea* failed to reduce polyuria significantly. UAE levels are a marker of glomerular injury and considered a harbinger of progressive nephropathy. Diabetic animals had significant increases in UAE levels consistent with the earlier reports (Grover et al., 2001). The feeding of *Murraya koeingii* and *Brassica juncea* decreased UAE levels, but the effect was not statistically significant. *Brassica juncea* showed significant reductions in serum creatinine values. Since *Murraya koeingii* and *Brassica juncea* showed only weak anti-hyperglycemic activity in a severe hyperglycaemic state, they can best be utilized only as a dietary supplement among pre-diabetics or mild diabetic patients on controlled exercise and diet plans. Since *Brassica juncea* significantly prevented a rise in creatinine levels, it will delay the development of diabetic nephropathy (Grover et al., 2003). However, in this later mentioned study, the seeds were incorporated in animal food and its effects in an alloxan induced rat diabetes model were apparent. Depending on the seed powder content of the animals' food (5 to 15%), anti-hyperglycemic, anti-hyperlipidemic and other beneficial effects of the seeds against diverse metabolic disorders associated pathologies have also been observed in earlier reports from the same research group (Khan et al., 1995a; 1995b; 1996a; 1996b; 1997; Grover et al., 2001; 2002; 2003; Yadav et al., 2004).

Feeding a 10% *Brassica juncea* diet to rats for 60 days had no adverse effect on food intake and various hematological parameters (Khan et al., 1995a). Taken together, these reports could indicate that the water soluble components of *Brassica juncea* seed are involved in its beneficial effects against insulin deficiency, and that fairly high doses of its bio-active components are well tolerated by experimental animals. The broad spectrum of beneficial effects of the seeds observed in these studies warrant further exploration of *Brassica juncea* seeds as a potential source for obtaining pharmacologically standardized phyto-therapeutics that are potentially useful for combating diabesity, which is the rising century epidemic of the 21st century caused by, or associated with, metabolic disorders (Farag and Gaballa, 2011). Theoretically, the germinated seeds could as well be the better alternatives for such purposes (Cavallos-Casals and Cisneros-Zevallos, 2010). To date little information on the medicinal phytochemistry and pharmacology of such seeds has been reported.

Recently, it has been reported that the seeds of mustard (*Brassica juncea*) fed to rats at doses equal to normal human intake did not cause any adverse effects on the food efficiency ratio (FER), red blood cell count (RBC), white blood cells (WBC), total count, differential counts or on the levels of blood constituents, like serum electrolytes, blood urea, haemoglobin, total serum protein, albumin-globulin ratio, fibrin level, glycosylated haemoglobin and the activity of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase in serum. No histopathological changes were observed in the livers of rats administered mustard meal (Khan et al., 1995a). Another study showed the effects of graded levels of high-glucosinolate mustard (*Brassica juncea*) meal (MM) as substitute for soya-bean meal (SBM) in broiler rabbit diets. Forty rabbits were randomly allocated to one of four experimental diets containing MM 0, 80, 160 and 245 g/kg. MM-incorporated diets had higher digestible and higher metabolisable energy (ME) content. Caecum weight reduced linearly with increasing MM levels in diet. Blood haemoglobin, packed cell volumes and lymphocytes were higher in the 245 MM diet; whereas white blood cell counts reduced linearly. Serum aspartate aminotransferase increased linearly while alanine aminotransferase and alkaline phosphatase activity, protein, erythrocytes sedimentation rate and red blood cell counts were not affected by MM. Serum Cu, Na and K content increased linearly with increasing MM levels. It is concluded that MM can replace up to 66% SBM protein in rabbit feeding; whereas complete replacement of SBM with MM reduced feed intake (Tripathi et al., 2008). Taken together, these reports suggest that body weight reduction caused by mustard feeding is not associated with the major pathologies. Since reducing body weight is the major goal of preventive measures against diabesity, mustard meal could as well be a promising lead for such purposes.

Available preclinical information suggests that *Brassica juncea* leaves are better suited than its seeds for further exploration as a source for a remedy against diabesity. Reported pharmacological activity profiles of leaf extracts are quite analogous, but not identical, to those reported for the seeds of the plant (Kim et al., 2003; Rahmatullah et al., 2010; Valavala et al., 2011) and a few active constituents potentially involved in their beneficial effects against metabolic disorders have been more definitively identified and better characterized (Kim et al., 2002; Yokozawa et al. 2002, 2003). It seems that at least some active components of the leaves involved in modulating metabolic functions are water soluble, and that they could also be effective alternatives for combating diabesity associated hyperglycemia as well as hyperlipidemia. Since the

leaves are almost devoid of fats, and contain numerous vitamins, minerals and other micronutrients, they seem to be an especially recommendable vegetable for health care purposes. For designing and developing pharmacologically standardized phyto-pharmaceuticals from this vegetable, due attention has to be paid to agricultural practices yielding pharmaceutically appropriate quality of the vegetable. This is not only because its bioactive constituent spectrum can vary considerably according to the growing and harvesting conditions present, but also due to the fact that *Brassica juncea* leaves are a well known accumulator of toxic heavy metals (Mancini and Bruno, 2010). In any case, a Japanese group has reported (Yoshimasa and Yoko, 2001) that similar to other Brassicaceae vegetable, *Brassica juncea* leaves also reduces serum cholesterol levels in human. During more recent years several in vitro studies have revealed diverse bio-activities of Isorhamnetin, i.e. quantitatively the major flavonol of *Brassica juncea* leaves, indicating potential benefits against metabolic disorders (Lee et al., 2009; Sanchez et al., 2007). Taken together, numerous reports now available on this flavonol constituent of the plant strongly suggest that it could play an important role in the pharmacological activity profiles of *Brassica juncea* leaf extracts. It must be mentioned though that like in the case of herbal remedies, diverse other structural and functional classes of bio-active molecules are involved in their therapeutically interesting bio-activities. Sinapic acid and phytosterols are some such other constituents of the plant with demonstrated beneficial effects against metabolic disorders and associated hyperglycemia and hyperlipidemia in animal models or epidemiological studies (Gupta et al., 2011; Noh et al., 2009; Patch et al., 2006; Shyni and Kanchan, 2011).

Psychopharmacology
Obesity, and/or inappropriate food choices and sedentary behavior, are now well recognized to be the main causative factors leading to diabesity, cancer and numerous other health conditions. Moreover, it has now become apparent also that proper regulation of both eating and sedentary behaviors is crucial for combating all obesity associated medical conditions. However, despite consistent concentrated efforts for decades, as yet no safe, effective, and universally acceptable anti-obesity agent or other preventive or therapeutic measures could yet be identified. Therefore, extensive efforts are now being made in several laboratories to identify animal models and therapeutic leads for combating obesity associated co-morbidities. Currently, fructose or high fat diet fed animals are considered to be appropriate pharmacological models better suited for identifying potential therapies for such conditions. Although the beneficial effects of *Brassica juncea* seed against diverse metabolic disorders in these two animal models have been demonstrated (Khan et al., 1996b; 1997; Yadav et al. 2004), as yet no reports describing the effects of *Brassica juncea* seeds or leaves on eating and sedentary behaviors in obese animals have appeared. Interestingly, in more recent years, a few reports indicating central nervous system (CNS) function modulating effects of some known secondary metabolites of the plants have started appearing. Some of them dealt with sinapic and other phenolic acids (Yoon et al., 2007) known for a long period to be abundantly present in *Brassica juncea*. One of these reports (Yoon et al., 2007) reveals that low oral doses (4 mg/kg) of pure synapic acid possess strong anxiolytic activities in animal models and that this effect is due to its modulating effects on neuronal GABA-gated chloride channel functions. A recent review (Sharma, 2001) summarizes numerous bioactivities, including CNS function modulating effects, of these and numerous other phenolic acid present in *Brassica juncea* and in diverse types of extracts obtainable from its different parts.

Since many such acid are also well known human metabolites of flavonolic molecules present in *Brassica juncea*, they could indeed be involved in the observed psychopharmacological and other effects of its flavonol containing extracts and their sub-fractions.

Anxiety, depression and cognitive dysfunctions are the major psychopathological conditions commonly associated with diabesity and almost all other chronic diseases, and available psychotherapeutics often does not meet the therapeutic demands of patients with such disorders. Therefore, phyto-pharmaceuticals are often used by such patients, and the clinical efficacies of only a few of them have also been demonstrated in properly controlled clinical trials. Diverse types of extracts from *Hypericum perforatum* are now often used for such purposes. Efforts to more rationally define their pharmacological activity profiles and active constituents led not only to the identification of its potential beneficial effects against diabesity associated depression and other psychopathologies (Husain et al., 2011; Kumar et al., 2011), but also to the detection of antidepressants like the therapeutic potential of the flavonol Isorhamnetin (Chatterjee et al., 2004). Since Isorhamnetin has been reported to be the major antidiabetic flavonol of *Brassica juncea* leaves (Kim et al., 2002), efforts to clarify its potential involvement in the pharmacological activity profile of its methanol extract are now being made in our laboratories. Observations made to date reveal that the tested extract possess anxiolytic and other therapeutically interesting CNS function modulating activities in comorbid CNS disorders associated with diabetic rodents. Although as yet no definitive statements on active principles of the test extract can be made, it certainly seems to be a promising starting point for further therapeutic ventures for combating mental health problems associated with the still expanding epidemic of diabesity.

However, the ultimate goal of our efforts is to identify and develop animal models suitable for evaluating potential modulating effects of agents on CNS functions controlling eating and sedentary behaviors. These are the two well recognized causative factors for diabesity, and until now, little effort has been made to identify experimental models useful for identifying novel therapeutic interventions. This is mainly due to a lack of consensus, or existing misconceptions, on a brain function-based classification system of mental health conditions (Miller, 2010) and a lack of precise knowledge on the pharmacological targets and processes involved in control of obesity related behaviors. Moreover, until recently, most modern therapeutic sciences have continued to neglect the possibility that peripheral functions could play important role in controlling almost all cognitive brain functions. It is now becoming increasingly apparent though, that many, if not all, such functions are controlled and modulated by complex interactions between the gut-brain axis and intestinal microbiota as well. That such is especially the case for brain functions involved in controlling energy intake and expenditure dictating the body mass index, i.e. the currently widely used diagnostic criteria for diabesity, is now well established. Since several therapeutically interesting pharmacological activities of structurally diverse phyto-chemical constituents of *Brassica juncea* and other vegetables and plant products on gut functions and microbiota are now known, efforts to properly define their behavioral activity profiles in animal models could eventually lead to more reasonable pharmacological strategies and models urgently needed for identifying therapeutic leads against diabesity. Available information on some such phytochemical identified from *Brassica juncea* are summarized later.

Anti-oxidant/peroxynitrite scavenging activity

Antioxidants are scavengers of reactive oxygen radicals that attack polyunsaturated fatty acids in cell membranes, giving rise to lipid peroxidation. In general, they inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions, and can be phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogenous compounds (alkaloids, chlorophyll derivatives, amino acids and amines) or carotenoids as well as ascorbic acid (Velioglu et al., 1998; Larson, 1988). Peroxynitrite ($ONOO^-$) is a potent mediator of inflammatory processes and atherogenesis with strong oxidizing properties towards biological molecules (Podrez et al., 1999). As a member of reactive species, $ONOO^-$ has been implicated in several major chronic diseases such as Alzheimer's disease, rheumatoid arthritis, cancer, and atherosclerosis (Beckman and Koppenol, 1996). The antioxidant properties of sinapic acid isolated from *Brassica juncea* and other plants have been investigated by several methods and different experimental strategies. A spectrophotometric study revealed that sinapic acid suppressed the formation of $ONOO^-$ mediated tyrosine nitration through an electron donation mechanism (Zou et al., 2002). In these studies, sinapic acid was also found to be a dose dependant nitration inhibitor of bovine serum albumin and low-density lipoprotein (LDL). It also decreased the LDL peroxidation induced by $ONOO^-$ derived from the peroxynitrite donor 3-morpholinosydnonimine hydrochloride (SIN-1). Observations made in this study suggests that sinapic acid has an efficient $ONOO^-$ scavenging ability and may well be a potent $ONOO^-$ oxidant scavenger for the protection of the cellular defense activity against the $ONOO^-$ involved diseases.

Antioxidants like the activities of *Brassica juncea* leaf extracts have also been studied using four types of *Brassica juncea* preparations (CH_2Cl_2, EtOAc, BuOH and H_2O fractions obtained from leaves). In one of these studies (Kim et al., 2003), the *in vitro* spin trapping assay was used, whereupon 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) served as the spin trap reagent. The tested EtOAc and BuOH fractions showed strong antioxidant activities. In this study, the BuOH fraction was also tested *in vivo* using streptozotocin (STZ) induced diabetic rats as the experimental model. Ten consecutive daily doses (50 to 200 mg/kg/day) of the BuOH fraction revealed dose dependent superoxide (O_2^-) scavenging activities, and reduced serum levels of nitrite/nitrate, glucose, glycosylated hemoglobin and thiobarbituric acid (TBA) reactive substances. The conclusion of this study was that BuOH fraction of mustard leaf controls glucose metabolism and reduces lipid peroxidation as well as the levels of oxygen radicals, ameliorating the damage caused by oxidative stress in diabetes (Kim et al., 2003). Another simultaneous report from the same group (Yokozawa et al., 2003), using same models reveals though, that the EtOAc fraction was the most active ones in all models used in the study. Observations reported in these two reports could be useful for designing *Brassica juncea* leaf extracts concentrated in its active constituents involved in its antidiabetic activities.

Several flavonol constituents of *Brassica juncea* leaves have also been identified as potent scavengers of free radicals and peroxynitrite (Choi et al., 2002; Jung et al., 2009; Yokozawa et al., 2002) and the usefulness of its ethanol extract for preventing lipid peroxidation in ground pork has also recently been suggested (Lee et al., 2010). Isorhamnetin 3,7-di-O-β-D-glucopyranoside (Isorhamnetin diglucoside), is one of the flavonol constituents of the leaves that has also been shown to posses beneficial effects against hyperglycemia in STZ induced diabetic rats (Yokozawa et al., 2002). This report demonstrates also that Isorhamnetin diglucoside is metabolized by intestinal bacteria to the flavonol Isorhamnetin, and strongly suggest that the parent flavonol, and not its naturally occurring

glycoside, is the active principle involved in perceived anti-diabetic efficacy of leaf extracts in animal models. In this study, ten oral daily doses (10 and 20 mg/kg/day) of the diglucoside effectively reduced blood glucose and glycosilated hemoglobin levels. After intraperitoneal administration, Isorhamnetin diglucoside did not show these activities. These observations add further evidence to the conviction that unlike many other naturally occurring flavonols, Isorhamnetin is metabolically a more stable one.

However, the question whether antioxidant activities of Isorhamnetin and other phenolics of the plant are solely responsible for their observed effects have also been questioned (Stevenson and Lowe, 2009). It has recently been shown, indeed, that some observed effects of Isorhamnetin are due to its direct binding to kinases MEK1 and PI3-K, and that Akt suppression is involved in its mode of action (Kim et al., 2011). Involvement of nuclear signaling mechanisms in diverse other naturally occurring, so called, phyto-antioxidants (also known to be present in *Brassica juncea*) are now also well recognized (Gupta et al., 2010). A more recent critical review on the antioxidant activities of flavonoids clearly points out the existing controversies on their modes of actions (Mladenk et al., 2010). Thus, although the anti-hyperglycemic and anti-hyperlipidemic effects of *Brassica juncea* seeds have also been attributed to its anti-oxidative properties (Khan et al., 1997), such interpretations still remain speculative only. Moreover, it cannot be ignored that phytosterols and other constituents with no antioxidant activities are also encountered in the plant, and the cholesterol lowering activities of phytosterols are now also known (Gupta et al., 2011; Fassbender et al., 2008).

Bioactivities of isolated components

Numerous known chemical classes of *Brassica juncea* constituents are common secondary metabolites in other plants, whereupon their many identified health affecting compounds are identical to those produced by other plants of the Brassicaceae family (Jahangir et al., 2009). As a matter of fact, no chemotaxonomic marker specific for this plant has yet been identified. However, relative concentrations of some such plant secondary plant metabolites in this plant are higher. Glucosinolates and poly unsaturated fatty acids are examples of some such constituents with well recognized cancer preventing and other medicinal benefits. Several reports describing such efficacies of products containing them have appeared during recent years, and some such pharmacologically tested molecules and products were derived from different parts of the *Brassica juncea* only (Karakida et al., 2007; Khan et al. 1996a, 1996b, 1997; Kumar et al., 2009; Manesh and Kuttan, 2003; Joardar et al., 2007; Thejass and Kuttan, 2007). Critical discussion on the available voluminous information on such bioactive secondary metabolites of the plant is beyond the scope of this overview. It must be mentioned though that the therapeutic benefits of unsaturated fatty acids consumed with mustard oils or seeds depend on the relative amounts of individual fatty acids present in them. It is well established that all unsaturated fatty acids are not equally relevant for health care purposes and that some of them might have adverse effects as well (Berquin et al., 2008; Gleissman et al., 2010). Oils and other products obtained from *Brassica juncea* are enriched in erucic acid and the little available information on its bioactivities are not sufficient for predicting either its potential therapeutic value or its adverse effect potential (Crowther et al., 1995; Ferri and Chance, 2005). Analogous are the cases also for almost all known bioactive constituents of any medicinal plant, and little effort has until now been made to properly understand and define the potential therapeutic implications of the biological interactions between diverse bioactive constituents of a given type of extract obtained from a given plant. To our judgment, more rational predictions of preventive and/or therapeutic usefulness of *Brassica juncea* preparations can be made only by paying due attention to the existence of structurally and functionally diverse bioactive molecules in the plant itself. Efforts necessary for such purposes must pay due attention to the fact that *Brassica juncea* not only produced therapeutically useful fatty acids and other molecules, but also several other such molecules, like volatile oils and extractable oils, with known adverse effect potentials on human health.

Along with oils, phytosterols and flavonoids, sinapic acid and its conjugates are some nontoxic bioactive phyto-chemicals abundantly encountered not only in *Brassica juncea*, but also in a number of diverse edibles and other plants. Numerous components of volatile and fixed oils of mustard seeds as well as of *Brassica juncea* leaf extracts are conjugates of such phenolic acids, and flavonoids could also be the metabolized to phenolic acids after oral intake (Jäger and Saaby, 2011; D`Archivio et al., 2010). In any case, some reports on CNS function modulating and neuro-protective activities of sinapic acid in animal models have appeared (Karakida et al. 2007; Kim et al., 2010). Potential involvements of phenolic acids in general, as well as of other constituents of *Brassica juncea*, in such and other effects have often been implicated (Jahangir et al., 2009). This review can be recommended for obtaining an overview on diverse potential health benefits of *Brassica juncea* as well.

Antitumor activity

It has been reported that mustard seeds and curry leaf inhibited colon tumourogenesis and they also decreased plasma cholesterol concentration. Both of these may have a higher water-holding capacity, and/or may be dilutable and absorb any carcinogens or promoters contained within the intestinal lumen. Both these spices may reduce the absorption of bile acids in the ileum and thereby enterohepatic circulation of bile acids may be affected. The higher levels of bile acids in the feces may be due to the absorption of less water soluble bile acids by these spices. Thus, the enterohepatic pool is initially reduced and may be renewed by the increased synthesis of bile acids from cholesterol, thereby reducing the body cholesterol. These studies suggest that feeding the spices mustard and curry leaf reduced the incidence of colon tumor in rats induced by 1, 2-dimethyl hydrazine. Thus, the inclusion of these spices in a daily diet plays a significant role in the protection of the colon against chemical carcinogenesis (Khan et al., 1996b). It has been reported also that mustard essential oil containing allyl isothiocyanate (AITC) significantly reduced ascite secretion and tumor cell proliferation by about 80% and inhibited vascular endothelial growth factor expression in tumor-bearing mice in vivo. It also reduced vessel sprouting and exhibited potent antiangiogenic activities in the chorioallantoic membranes and corneas of the tested rats. AITC arrested the growth of EAT cells by inducing apoptosis and effectively arrested cell cycle progression at the G1 phase. The results clearly suggest that AITC inhibits tumor growth by both antiangiogenic and proapoptotic mechanisms (Kumar et al., 2009). Two naturally occurring isothiocyanates, Allyl isothiocyanate (AITC) and phenyl isothiocyanate (PITC) were investigated for their antioxidant and anti-tumor properties. Both AITC and PITC showed antioxidant and tumor reducing activities when administered intraperitoneally at a dosage of 25μg/dose/animal for 5 consecutive days (Manesh and Kuttan, 2003). AITC and PITC significantly inhibited tumour-specific angiogenesis which can be attributed to their downregulatory actions of NO and TNF-α (Thejass and Kuttan, 2007).

Antimicrobial activity

Allyl isothiocyanate (AIT) is derived from the glucosinolate sinigrin found in plants of the family Brassicaceae and is responsible for the characteristic pungency found in horseradish and mustard pastes (Cejpek et al., 2000). It is a well-recognized antimicrobial agent against a variety of organisms, including food borne pathogens such as *Escherichia coli* O157: H7 H7 (Luciano and Holley, 2009). The antibacterial action of AIT was increased at lower pH values. This fact can be related to the higher stability of AIT in more acidic environments. In addition, the degradation products of AIT in water were ineffective against E. coli O157: H7 growth. Therefore, the aqueous decomposition of AIT and basic conditions will limit its antimicrobial activity. Furthermore, allyl isothiocyanate inhibited the catalysis of both thioredoxin reductase and acetate kinase, which are responsible for important metabolic reactions in bacteria. Thus, it can be concluded that AIT and other isothiocyanates have multi-targeted antimicrobial activities, since they caused enzymatic inhibition and membrane damage (Lin et al., 2000). Volatile compounds from *Brassica juncea* were fungicidal to all the plant pathogenic fungi. Allyl isothiocyanate is also responsible for the fungicidal activities of *Brassica juncea* (Mayton et al., 1996). An antifungal juncin was isolated from the seeds of the *Brassica juncea* var. integrifolia. The protein exhibited antifungal activities toward the phytopathogens *Fusarium oxysporum*, *Helminthosporium maydis*, and *Mycosphaerella arachidicola* (Ye and Ng, 2009). Another study discovered a plant chitinase (*Brassica juncea* BjCHI1) found in *Brassica juncea*. BjCHI1 showed dual roles as a defence protein against both pathogenic bacteria, including R. solanacearum, as well as fungal phytopathogens, *Conophytum truncatum, Colletotrichum acutatum, Botryti cinerea*, and *Ascochyta rabiei* (Guan et al., 2008).

Allergenicity

The allergenicity to *Brassica juncea* seed has been reported by in vivo and in vitro methods in Indian atopic cases. To assess sensitization, a skin prick test was carried out with an antigen extract (1:10 w/v) of *Brassica juncea* Total IgE and *Brassica juncea* specific IgE was estimated by an enzyme-linked immunosorbent assay. To determine the allergenically important protein, immunoblot was carried out. The results showed sensitization against the seeds of *Brassica juncea* does exist in the Indian population. Braj IE, a major protein isolated from oriental-mustard (*Brassica juncea*) seeds has been shown to be allergenic (Monsalve et al., 1993). Another protein named napin from *Brassica juncea* is also allergenic in nature. Allergenicity as well as the resistance to trypsin, limits the utilization of napins in food. One of the features of allergens is that the protein must have properties that protect its structure against degradation in the gastrointestinal tract (Jyothi et al., 2007).

Defense against insects

Glucosinolates are feeding deterrents and are toxic to nonadapted herbivores (Blau et al., 1978) but are feeding and oviposition stimulants for crucifer (Brassicaceae) specialists (David and Gardiner, 1966; Nault and Styer, 1972). Isothiocyanates, the most common hydrolytic products of glucosinolates, are more volatile and more toxic than glucosinolates. Isothiocyanates are toxic to generalist insects (McCloskey and Isman, 1993), and crucifer specialist insects (Wadleigh and Yu, 1988). The effect of myrosinase activity and glucosinolate profiles of *Brassica juncea* on feeding behavior, feeding damage, and larval growth of different insect species i.e. the crucifer specialist, Plutella xylostella and the generalist, *Spodoptera eridania*, have been studied. The study showed the proportions of time feeding and areas damaged by *Plutella xylostella* were lower on lines with high myrosinase activity than on lines with low myrosinase activity. In contrast, the proportion of time feeding and area damaged by *Spodoptera eridania* were not related to myrosinase activity, but were lower on cotyledons of lines with high glucosinolate concentrations than on lines with low glucosinolate concentrations. Relative growth rates (RGR) of both insect species were lower on lines with high glucosinolate concentrations, but were not related to myrosinase activity in the lines. The toxicity study that used artificial diets, indicated that allyl isothiocyanate, but not allyl glucosinolate, was lethally toxic to neonate *Plutella xylostella*; whereas isothiocyanate and the glucosinolate were lethally toxic to neonate *Spodoptera eridania*. Results of the study suggested that myrosinase activity might be more important for plant defense against specialist insects that have adapted to intact glucosinolates, but less important for defense against generalists, which are susceptible to the intact glucosinolates. The different responses of *Plutella xylostella* and *Spodoptera eridania* suggest that glucosinolates may have originated as defences against generalist herbivores or other exploiters (Li et al., 2000).

Safety aspects

Mustard meal is the product that remains following the oil extraction of mustard (*Brassica juncea*) seeds. In spite of its well balanced amino-acid composition and high protein content, the use of mustard meal in animal feed is limited because it contains compounds such as glucosinolates that reduce its nutritive value and make it unpalatable as well as goitrogenic. Glucosinolates, whose degradation products thiocyanate, isothiocyante and nitriles suppress the thyroid uptake of iodine, which may result in lowered levels of the thyroid hormones T3 (tri-iodothyronine) and T4 (thyroxin) (Barrett et al., 1997), which can induce metabolic disorders. These problems are associated with glucosinolate degradation into toxic compounds either by the myrosinase enzyme present in the mustard meal or the enzyme present in rumen bacterial microflora (Nugon-Baudon et al., 1990). A more recent study investigated the influence of diets containing HCl treated mustard meal, copper and iodine supplemented untreated mustard meal as well as untreated mustard meal as replacements for soybean meal in diets of growing calves, on nutrient utilization, blood parameters, liver enzymes, thyroid hormones and calf growth. Results of the study reveal that the plasma thyroid hormone levels (T3 and T4) were reduced after untreated mustard meals, which indicated an iodine deficiency in calves fed the diet with this meal. Plasma T3 concentrations were found to be positively correlated with the growth rates of calves. Mustard meal, if treated with HCl or supplemented with copper and iodine, will result in better overall calf performance than if fed untreated and similar to that of soybean meal-based diets (Tripathi et al., 2001). Another study tested the nutritional performance and thyroid hormone status of adult goats fed graded levels of iodine when their diet contained a goitrogenic mustard (*Brassica juncea*) cake-based supplement. The results of the study indicated that the apparent digestibility of nutrients and N metabolism of goats were not influenced by supplementary iodine levels tested when fed a mustard cake diet. However, it had a positive influence on live weight gain and the thyroid status of the goats (Pattanaik et al., 2001).

Recently, it has been reported that the topical application of mustard oil (allyl isothiocyanate) to the skin or injection into joints induces hyperalgesia, allodynia (Haas et al., 1992) and neuroinflammation (Cairns et al., 2002). However, when

applied to the oral or nasal mucosa, mustard oil evokes a desensitizing pattern of irritation (Khan et al., 1996a). It was also reported in another study that the topical application of mustard oil to a mouse ear produces acute skin inflammation differently from capsaicin. Mediators derived from mast cells, such as histamine and 5-hydroxytryptamine, appear to be minor factors in the response to mustard oil. In addition, the tachykinin NK receptor is involved principally during the first 5 min of the inflammatory response to mustard oil (Inoue et al., 1997). A further study compared the extent of plasma-protein extravasation and oedema induced by mustard oil application to the temporomandibular joint (TMJ) region with that induced by glutamate. The application of mustard oil resulted in plasma-protein extravasation into the TMJ tissue and oedema of the TMJ region. In contrast, glutamate did not cause a plasma-protein extravasation or oedema (Fiorentino et al., 1999). Yet another study investigated the responses of neurons in the superficial laminae of trigeminal subnucleus caudalis (Vc) to noxious thermal (53°C) and chemical (pentanoic acid; 200 mM) stimuli prior to and following lingual mustard oil application. A low concentration of mustard oil (0.125%) applied at a constant flow (0.5 ml/min; 15 min) initially excited Vc neurons and was followed by partial desensitization. Responses to noxious heat were unchanged following mustard oil. A high concentration of mustard oil (1.25%) initially excited Vc neurons and was followed quickly (within 20 s) by nearly complete desensitization. The study suggests that the effect of mustard oil on subsequent lingual nociceptive responses is concentration dependent, transient, and modality specific (Simons et al., 2004). It is also reported that application of the mustard oil to single molar tooth pulp, causes significantly increased cutaneous mechanoreceptive field (RF) size and responses of nociceptive neurons in both ventroposterior medial nucleus (VPM) and posterior nuclear group (PO). These changes in the RF and response properties of thalamocortical neurons to noxious stimuli likely contribute to the behavioural consequences of peripheral inflammation manifesting as pain referral, hyperalgesia and allodynia (Zhang et al., 2006).

Concluding remarks and future perspectives

Available preclinical information on *Brassica juncea* not only adds considerable experimental evidence justifying widespread Ayurvedic uses of mustard seeds and oils, but also suggests that its leaf could as well be better exploited for health care purposes according to the holistic principles of Ayurveda. Along with other recommendations, proper choices of edibles for specific health care purposes are highly recommended by almost all Ayurvedic practitioners. Modern therapeutic researchers are now also consistently recommending fruits and vegetables in general and Brassicaceae vegetables in particular, for helping patients suffering from, or prone to, diabesity and related mental health conditions. However, as yet little concentrated effort has been made to design and develop a pharmacologically well standardized phyto-pharmaceutical or nutraceuticals, especially suited for such purposes. None of the numerous available, and widely used, nutritional supplements (containing vitamins, minerals, phytochemical and other products) were specially designed and have not yet been pharmacologically evaluated, for such purposes. Ideally, the therapeutic potentials of such products must not only be predictable from their bio-activity profiles, but also must be safe, sustainable, and affordable to the vast majority of population in the underdeveloped and developing countries as well. However, in reality, this ideal goal is at present neither scientifically achievable nor economically feasible. This is not only due to lack of precise scientific know-how, but also due to complex socioeconomic problems involved in the etiology, pathogenesis and progression of diabesity and its network (Williams and Fruhbeck, 2009). In view of the situation, efforts to better understand the therapeutic potentials *Brassica juncea* seeds and leaves could as well lead to more rational, or appropriate, medicinal uses of this edible plant. These efforts should eventually be useful for generating the knowledge necessary for more rationally designing other medicinally useful products not only from this plant, but also from others containing similar bio-active phyto-chemicals.

Several medicinal phytochemistry based pharmacological strategies are now available for such purposes, Most of them are analogous to the so called "Reverse pharmacology" strategy (Patwardhan and Mashelkar, 2009) which is now being widely recommended in India for discovering drug leads from Ayurvedic and other traditionally known medicinal plants. This approach is useful only when medicinal benefits of a given plant are better established either by observational or by more objective clinical studies. Since this criterion is not yet fulfilled for *Brassica juncea* (except for the oil obtained from its seeds; potential health benefits of which are indicated by an epidemiological study), other approaches have to be used for this plant. All strategies suitable for the purpose not only necessitate close collaboration between medicinal phyto-chemists, pharmacologists, and other researchers pertaining to diverse other sub-disciplines of modern medicine, but also involve large investments of time and expensive modern technologies. Since *Brassica juncea* derived product patent rights can easily be commercially misused, or bypassed, modern pharmaceutical industries will most probably not be interested in research investments on this plant that can easily be grown and harvested by many. On the other hand, diabesity is already a major health problem of the underdeveloped and developing countries, and as yet no very reasonable, practicable and cost effective measures for properly combating the epidemic in such countries are yet available. Such a situation demands the urgent necessity of economically feasible strategies and models potentially useful for identifying edible vegetables and fruits potentially useful for meeting the health care demands of these countries by the use of available and economically more affordable experimental techniques.

With the assumption that *Brassica juncea* leaf could as well be an anti-diabesity vegetable, attempts are now being made in our laboratories to better understand its health care benefits according to the holistic principles of Ayurveda which suggest that health can be maintained only by proper adjustments of the balance between the bodily functions and those of mind and soul. These efforts led to the identification of a provisionally standardized extract from commercially available leaves of the plant which was well tolerated by experimental animals as well. After repeated daily oral doses it not only effectively counteracted hyperglycemia and hyperlipidemia in diabetic rats, but also revealed a broad spectrum of psychopharmacological activity profiles in such animals only (Manuscript under preparation). These observations not only add further experimental evidence to our conviction that widespread popularities of many herbal remedies are due to their subtle and as yet not properly defined effects on gut-brain axis, but also strongly suggest its potential usefulness for combating the diabesity epidemics. Since diverse classes of bio-active phytochemical of Brassicaceae plants are also encountered in other edible and medicinal plants, convenient pharmacological models and tools capable of identifying their therapeutically interesting bioactivities could as well be used also for better, or more rational, pharmacological standardization of numerous medicinal plants.

It must be mentioned also that *Brassica juncea* seeds and numerous other products obtainable from the plant also contain different concentrations of the bioactive phytochemical present in leaf extracts pharmacologically screened in our and other laboratories during recent years. Consequently many commercial byproducts, or wastes, of *Brassica juncea* could as well have medicinal values other than those known for veterinary purposes. They could even be economically more affordable starting materials for obtaining medicinal benefits, other than involving diabesity. The holistic strategies based on the post modern concepts of poly-pharmacology could as well be a more rational and economically more feasible ones for making more appropriate uses of traditional knowledge and better commercial as well medicinal exploitation of medicinal plants. Thus, *Brassica juncea* seems to be another example of edible Ayurvedic plants which could not only be better explored for medicinal purposes, but also for identifying unconventional pharmacological models for helping patients with diverse health problems, including those involving brain pathologies of the modern decades.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest in the present study.

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