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Broccoli; The Green Beauty: A Review

A. I. Owis
Department of Pharmacognosy, Beni-Suef University, Beni-Suef,Egypt Telephone: +202-01202500017

Abstract

Context: Plants are nature's blessing to mankind to make malady free sound life, and assume an essential part to protect our wellbeing. Broccoli - *Brassica oleracea* L.var. *italica* Plenk (Brassicaceae) - is considered as a nutritional powerhouse. The present review comprises the phytochemical and therapeutic potential of broccoli.

Objective: This aim of this review to collect results obtained from various studies in order to spot more light towards the surprising green world of broccoli. In addition to, a number of recommendations that will help to secure a more sound „proof-of-concept" to complete the whole picture providing significant information could be used as a dietary guideline that encourage broccoli consumption for the management of various diseases.

Methods: This review has been compiled using references from major databases such as Chemical Abstracts, ScienceDirect, SciFinder, PubMed, Henriette's Herbal Homepage and Google scholars Databases.

Results: An extensive survey of literature revealed that broccoli is a good source of health promoting compounds such as glucosinolates, flavonoids, hydroxycinnamic acids and vitamins. Moreover, broccoli is the kind of nutrient that has so many wonderful applications including gastroprotective, antimicrobial, antioxidant, anticancer, hepatoprotective, cardioprotective, anti-obesity, anti-diabetic, anti-inflammatory and immunomodulatory activities.

Conclusion: There are still missing areas need further in-depth investigation such as effect of broccoli on central nervous system.

Keywords: biological activities, *Brassica oleracea,* Brassicaceae, phytochemistry.

INTRODUCTION

Plants have been the primary source of medicines for early drug discovery. In developing countries, due to economic factors, nearly 80% of the population still depends on plant extracts as a source of medicine. Due to increasing risk of chronic illness worldwide, World Health Organization (WHO) is encouraging developing countries to use traditional herbal medicines for the treatment of various chronic ailments [1]. American Cancer Society, (1984) and National Research Council, (1982) have been advised to include more cruciferous vegetables, such as cabbage, broccoli, brussel sprouts, kohlrabi, and cauliflower, in their diets. Today, physicians are making the same recommendation. Broccoli has gained attention because of the struggles on patenting genotypes with high concentrations of glucosinolates showing positive effects in cancer treatment [2]. Broccoli - *Brassica oleracea* L. var. *italica* Plenk - belongs to family Brassicaceae. The word *"Brassica*" means to cut off the head. Broccoli is an Italian word from the Latin *brachium,* meaning an arm or branch. The term sprouting as used in sprouting broccoli refers to the branching habit of this type, the young edible inflorescences often being referred to as sprouts. The sprouting broccolis are thought to have originated from the eastern Mediterranean then introduced into Italy. A remarkable diversity of broccoli-like vegetables has been developed in Italy. According to (FAO statistics, 2012), China is the top world producer of broccoli (9,596,000 tons) [3]. The flowers of broccoli are borne on a faceted floral shoot so that the inflorescence terminates the axis of the plant. The inflorescence, which has been described as a corymb, a corymbose panicle or a modified racemose panicle, consists of functional floral buds, perfect flowers, stem and bracts. At the time of harvesting, the inflorescence is a growing, faceted axis bearing a large number of immature, stalked flowers, floral buds and varied bracts which are smaller and simpler in form than the vegetative leaves. The bracts are absent from the terminal portion of the inflorescence [4, 5]. The aim of the present study was to develop a database for the amazing green world of broccoli that can be used to encourage daily dietary intake through investigating diet–disease relationships. We highlight the phytochemical content, discuss its relation with the pharmacological activities, evaluate toxicity, summarize nutritional value and appreciate the influence of cooking method on phytochemical content to complete the whole picture providing significant information could be used as a dietary guideline that encourage broccoli consumption for the management of various diseases. In addition to, provide notes on studies on broccoli tissue cultures and its by-products. This review provides a number of recommendations that will help to define a more sound „proof- of-concept" for the complete benefit of broccoli.

METHODS

This review has been compiled using references from major databases such as Chemical Abstracts, ScienceDirect, SciFinder, PubMed, Henriette's Herbal Homepage and Google scholars Databases.

PHYTOCHEMISTRY

Broccoli inflorescence is a good source of health promoting compounds since it contains glucosinolates, flavonoids, hydroxycinnamic acids and other minor compounds [6]. Glucosinolates are produced almost exclusively in Brassica plants, where they are thought to play a role in microbe and insect defence. When plant cells are disrupted (e.g. during cutting, chewing, cooking and freezing), glucosinolates are hydrolyzed by a β-thioglucosidase enzyme (myrosinase) to various bioactive breakdown products (isothiocyanates, nitriles, thiocyanates, epithicyanates, epithionitriles and oxazolidines). Various glucosinolates were identified in broccoli sprouts by means of liquid chromatography–mass spectrometry and liquid chromatography/tandem mass

spectrometry such as glucoiberin, glucocheirolin,glucoraphanin, progoitrin, sinigrin, glucoalysin, gluconapin, glucoiberverin, 4-hydroxyglucobrassicin, glucoerucin, glucobrassicin, 4-methoxyglucobrassicin, gluconapoleiferin and neoglucobrassicin (Fig. 1) [7] and by GC/MS analysis such as disulphide dimethyl, trisulphide dimethyl, 1, 2, 4-trithiolane 3,5-dimethyl, ethenyl isothiocyanate, allyl isothiocyanate, 4-methyl thiobutyl isothiocyanate and 2-phenylethyl isothiocyanate [8]. In addition to, isolation of uridine, uridine 9-acetate, obtucarbamate, *N*-(4- hydroxy phenyl) acetamide, and *p*-hydroxy benzoic acid [9, 10], a new glucosinolate Cinnamoyl derivative [6'-*O*-trans-(4″-hydroxy cinnamoyl)-4-(methylsulphinyl) butyl glucosinolate] [11]. Including sulforaphane quantification; 5-methylsulfinylpentylnitrile was found in the greatest concentration in the chloroform extract (475.7 mg/kg of fresh sprouts), followed by sulforaphane (222.6 mg/kg) and 4-methylsulfinylbutylnitrile (63.0 mg/kg) [12]. Broccoli has been reported to be one of the main flavonol sources in diet. The major inflorescences flavonols were quercetin-3-*O*-sophorotrioside-7-*O*-glucoside; quercetin-3-*O*-sophoroside-7-*O*-glucoside; quercetin-3-*O*-sophorotrioside-7-*O*-sophoroside; kaempferol-3- *O*-sophorotrioside-7-*O*-glucoside; kaempferol-3-*O*-sophorotrioside-7-*O*-sophoroside; kaempferol-3-*O*-sophoroside-7-*O*-glucoside; isorhamnetin-3-*O*-sophorotrioside-7-*O*- glucoside; kaempferol-3-*O*-sophoroside-7-*O*-sophoroside; isorhamnetin-3-*O*-sophorotrioside- 7-*O*-sophoroside; quercetin-3,7-di-*O*-glucoside; quercetin-3-*O*-glucoside-7-*O*-sophoroside; kaempferol-3,7-di-*O*-glucoside; kaempferol-3-*O*-glucoside-7-*O*-sophoroside; isorhamnetin-3,7-di-*O*-glucoside; isorhamnetin-3-*O*-glucoside-7-*O*-sophoroside; quercetin-3-*O*- sophoroside; kaempferol-3-*O*-sophoroside; quercetin-3-*O*-glucoside; kaempferol-3-*O*-glucoside. All of the previous flavonoids were found acylated with hydroxycinnamic acids (*p*-coumaric, caffeic, ferulic and sinapic). So that broccoli contains large complex combinations of hydroxycinnamic acids with highly glycosylated flavonoids [6].Concerning sterols, the dominant sterol, both in florets and stems, were β-sitosterol and brassicasterol [13]. Indeed, broccoli is considered a storehouse of various phytochemicals.

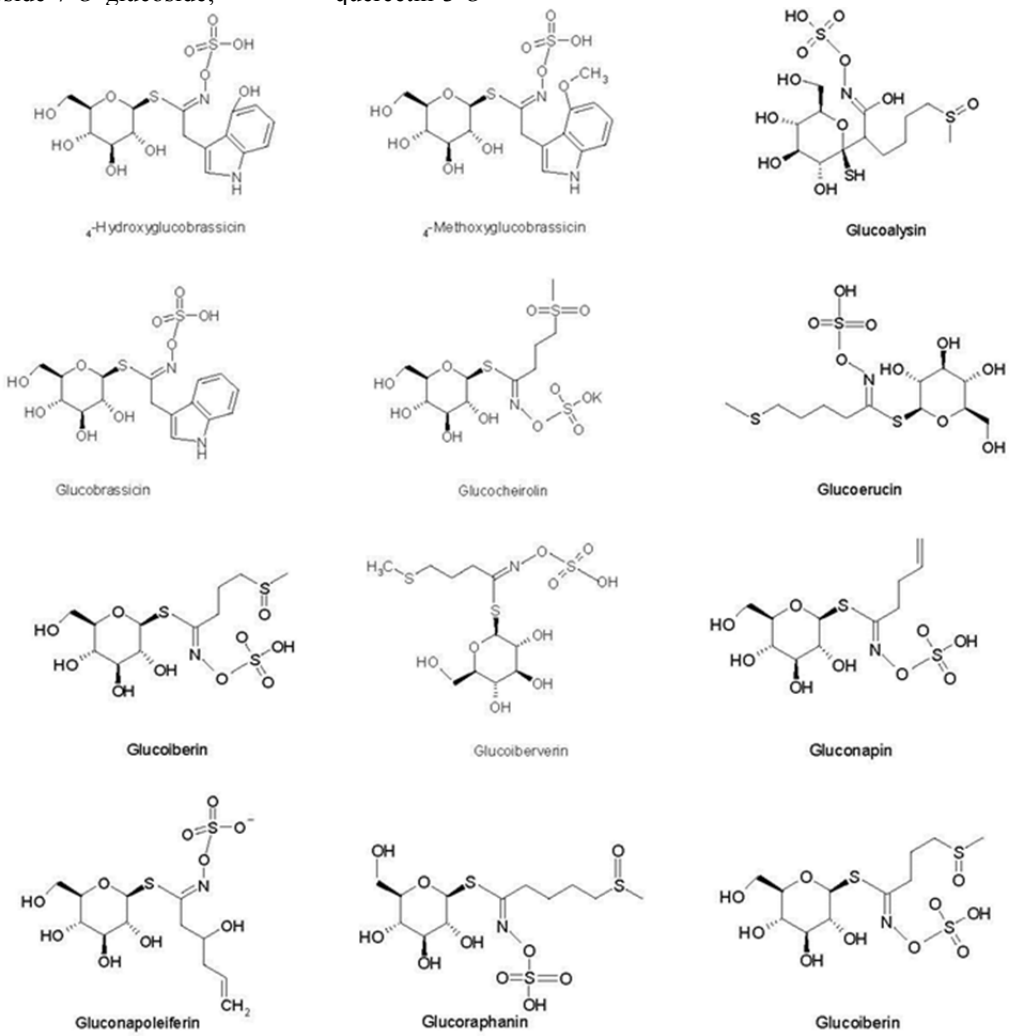

Fig. 1: Structures of some glucosinolates present in Broccoli

BIOLOGICAL ACTIVITIES

Dietary use of broccoli has encouraged scientists to test for a wide range of biological activities including gastroprotective, antimicrobial, antioxidant, anticancer, hepatoprotective, cardioprotective, anti-obesity, anti-diabetic, anti-inflammatory and immunomodulatory activities. Results from these studies are discussed below.

Gastroprotective Activity

Gastric infection with *Helicobacter pylori* is a cosmopolitan problem, and is especially common in developing regions where there is also a high prevalence of gastric cancer. Sulforaphane has dual actions as potent bacteriostatic agent against 3 reference strains and 45 clinical isolates of *H. pylori* and blocking formation of benzo[*a*]pyrene-evoked fore stomach tumors in mice thus offer hope that these mechanisms might function synergistically to provide diet-based protection against gastric cancer in humans [14]. In 2004, Galan and co-workers published a preliminary report on the temporally associated eradication of *H.pylori* infection in three of nine patients with oral consumption of broccoli sprouts and suggested the need of further studies [15]. In 2009, Yanaka and his group proved that daily intake of sulforaphane-rich broccoli sprouts for 2 months reduces *H. pylori* colonization in mice and improves the sequelae of infection in infected mice and in forty-eight *Helicobacter pylori*–infected patients [16]. Moon et al., (2010) noted that the greatest inhibition zones against *H. pylori* (>5 cm) were by the chloroform extract of broccoli sprouts, followed by the hexane extract (5.03 cm), the ethyl acetate extract (4.90 cm), the butanol extract (3.10 cm), and the crude methanol extract (2.80 cm), whereas the residual water fraction did not showany inhibition zone. Broccoli sprouts can be an excellent food source for medicinal substances [12].

Antimicrobial Activity

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [17]. Benko-Iseppon et al., (2010) investigated the presence of antimicrobial peptides in broccoli leaves [18]. Ethyl acetate and chloroform extracts of broccoli florets were found to be effective against *B. cereus* and *B. subtilis,* respectively. Ethyl acetate and ethanol extracts were highly active against *E. coli*. Additionally, ethyl acetate and chloroform extracts showed high activity against *Candida albicans* [9]. Biologically synthesised Ag nanoparticles from aqueous extract of broccoli florets were found to be effective in controlling growth of human pathogens viz. *Klebsiella Pneumonia, Staph. Saprophyticus* and *E. coli* [19]. Minimum inhibitory concentration (MIC)values of 10 - 320 μg mL^{-1} were recorded against most of six food borne bacteria; *B. cereus*,*B. subtilis*, *Staph. aureus*, *E. coli*, *S. typhi.* and *Shigella flexneri* with acetone and methanol as the potential extracts. *B. subtilis* (15.4 mm) and *B. cereus* (16.3 mm) were found to be the most sensitive organisms among the pathogens tested [20]. Broccoli stems extract showed antimicrobial activity against *Listeria monocytogenes*, which MIC was 102.4 mg/mL. Analysis by CG-MS allowed the identification of organic acids, as ascorbic and malic acids, and phenolic compounds, as sinapinic, ferulic and caffeic acids [21].

Anti-oxidant Activity

Oral broccoli consumption is related to an overall improved antioxidant status [22]. *In vivo* models using chickens fed low antioxidant diet with 10% apple/broccoli mixture showed a remarkable increase in erythrocyte stability toward H_2O_2, decrease in carbonyls in insoluble muscle proteins and slowdown lipid oxidation in heat-treated liver samples [23]. The antioxidant capacity of hydrophilic extracts of broccoli using the oxygen radical absorbance capacity (ORAC) assay ranged from 65.8 to 121.6 μmol trolox equivalents (TE)/g of tissue, and the capacity of lipophilic extracts ranged from 3.9 to 17.5 μmol TE/g [24]. After broccoli consumption in twenty-seven young healthy smokers (250 g/day) for 10 days, the level of oxidised DNA lesions decreased by 41%, the resistance to H_2O_2-induced DNA strand breaks increased by 23% and a higher protection was observed in subjects with glutathione S-transferase (GST) M1-null genotype [22]. *In vitro* models clearly suggest that, broccoli is a natural source for antioxidants, which could serve as a nutraceutical with potential applications in reducing the level of oxidative stress and related health benefits. Inhibition of superoxide scavenging by aqueous and ethanolic extracts showed an IC_{50} of 0.93 and mg/mL, respectively. Metal ion chelation showed an IC_{50} of 0.35 mg/mL of both extracts and was equipotent to positive control, ethylenediamine tetra-acetic acid. The ethanolic extract exhibited higher antioxidant activity in DPPH radical and superoxide anion scavenging than that of aqueous extract [25]. Furthermore, 3 day old broccoli seedlings showed the highest antioxidative activity than mature plant when tested for antioxidative activity using DPPH radical method. Consuming small quantities of broccoli sprouts has the same protective effect as consuming large amounts of mature plants [26]. Caroling et al., (2013) assigned the reducing property of biologically synthesised Ag nanoparticles from aqueous extract of broccoli florets due to the presence of antioxidant viz. ascorbic acid, polyphenols which is confirmed by quantitative assay and scavenging effect of free radicals proved by DPPH scavenging activity [19].

Anticancer Activity

During broccoli preparation, chewing, and digestion, the glucosinolates are broken down to form biologically active compounds such as indoles, nitriles, thiocyanates, and isothiocyanates. Indoles and isothiocyanates have been found to inhibit the development of cancer in several organs in rats and mice, including the bladder [27], breast [28], liver [29], lung [30], prostate [31, 32], renal [33], crown-gall [26], oral mucosa [34], colon [35] and skin [36]. National Cancer Institute (NCI) studies in animals and experiments with cells grown in the laboratory have identified several potential ways in which these compounds may help prevent cancer:

- They help protect cells from DNA damage.
- They help inactivate carcinogens.
- They have antiviral and antibacterial effects.

- They have anti-inflammatory effects.
- They induce cell death (apoptosis).
- They inhibit tumor blood vessel formation and tumor cell migration

NCI also mentioned that a concentrated form of sulforaphane found in broccoli has been shown to reduce the number of acute lymphoblastic leukemia cells in the lab setting and have both preventive and therapeutic properties in solid tumors. Studies have shown that people who eat a diet rich in broccoli have a lower risk of some cancers Our idea is that "Broccoli is unmatched in its anti-cancer effect".

Hepatoprotective Activity

Broccoli extract at the doses of 150 and 300 mg/kg produced significant hepatoprotection by decreasing the activity of serum enzymes, bilirubin, while it significantly increased the levels of NP-SH and decreased MDA of liver tissue. Histopathological observations of the liver also showed the protective effect of broccoli in CCl 4 -induced hepatic injury in rats. The obtained results suggest that broccoli possesses hepatoprotective capacity and may have potential therapeutic value in the treatment of some liver disorders probably by its antioxidative effects on hepatocytes, due to flavonoids and sulfurated compounds [10, 37].

Cardioprotective Activity

Current epidemiological predictions show that the world is heading for a vascular typhoon of cardiovascular disease burden. It is estimated that by 2020, cardiovascular diseases will be the largest cause of disability and mortality in developing countries [1]. Consumption of broccoli sprouts rich in sulforphane was found to decrease oxidative stress in spontaneously hypertensive stroke-prone rats (SHR) and thus, improves blood pressure as well as decreases inflammation. It was found through twelve patients-clinical study that consumption of fresh broccoli sprouts [100 gm/day] for a week leads to reduction in LDL, total cholesterol and increase in HDL cholesterol. Another related prospective study of 34,492 postmenopausal women in Iowa showed that broccoli was strongly associated with reduced risk of coronary heart disease [1]. Recent study demonstrated that broccoli consumption can prevent the reduction of mRNA level and protein level due to ischemic reperfusion injury which causes cardiomyocyte death. Moreover, another study demonstrated that broccoli treatment can improve cardiac function, reduce myocardial infarction and cardiomyocyte apoptosis after ischemic reperfusion injury [1].Broccoli exerts cardioprotective effects through various mechanisms such as: (a) inhibition of phase I enzymes and DNA adducts; (b) induction of phase II antioxidant detoxifying enzyme;(c) antioxidant function; (d)induction of cell cycle arrest; (e) inhibition of angiogenesis; and anti-inflammatory properties and thus considered as a potential functional food [1].

Anti-obesity Activity

The incidence of obesity is rising worldwide at an alarming rate and is becoming a major public health concern with incalculable social and economic costs. Dietary phytochemicals appear to be able to target different stages of the adipocyte (fat cell) lifecycle [38]. The body weight gain and mesenteric adipose tissue weight were increased by high fat diet in rats, but gradually decreased to the corresponding level of normal diet group after administration of ethanol and aqueous extract of broccoli sprouts [39, 40]. In another study, chloroform extract, combined ethyl acetate and ethanol and the crude extract of broccoli florets showed significant loss in the body weight of female rats at 5% and 1%. When the obtained results were compared with water extract of green tea (117 g total loss in body weight), chloroform extract showed higher total loss in body weight (180 g) [8].

Anti-diabetic Activity

Consumption of antioxidants existing in broccoli leaves contributes to decrease damages to cells and, specially, accelerates restoration of pancreatic cells and subsequently increases insulin and decreases blood glucose. That was proved through treatment of streptozotocin induced diabetic rats with 100 mg/kg and 200 mg/kg body weight broccoli sprouts aqueous extract which leads to significant decrease in blood glucose and liver glycogen at ^{14}th and ^{21}st day [40 - 42]. Broccoli sprouts may improve IR in type 2 diabetic patients. It was proved through eighty-one patients-clinical study that consumption of fresh broccoli sprouts [10 g/day] for 4 week leads to significant decrease in serum insulin concentration and homoeostasis model assessment of IR index [43].

Anti-inflammatory Activity

The effects of 10-day broccoli (250 g/day) intake on markers of inflammations in young male smokers were studied. Circulating levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 6 receptor (IL-6sR) and adiponectin were measured. Broccoli intake has no significant effect on TNF-α, IL-6, IL-6sR or adiponectin while plasma CRP decreased by 48%. An inverse correlation between lycopene, TNF-α and IL-6sR was observed at baseline [44]. In another study, the ethyl acetate fraction of broccoli florets inhibited nitric oxide release from lipopolysaccharide-stimulated RAW 264.7 cells in a dose-dependent manner and inhibited IκB-α degradation and nuclear factor-κB activation in lipopolysaccharide-stimulated RAW 264.7 cells [45]. Moreover, sulforaphane might be a new therapeutic agent for rheumatoid arthritis. Sulforaphane inhibits unstimulated and interleukin-1β (IL-1β)-induced proliferation of rheumatoid arthritis synovial fibroblasts (RASFs); the expression of matrix metalloproteinases (MMP-1, MMP-3), and cyclooxygenase COX-2 mRNA and protein; and the prostaglandin E2 (PGE2) production induced by IL-1β. Sulforaphane also inhibits the phosphorylation of ERK-1/2, p-38, and JNK and activation of NF-kB by IL-1β [46]. In conclusion, broccoli is considered as potent antiinflammatory.

Immunomodulatory Activity

Intraperitoneal administration of Sulforaphane (500 μg/dose/animal/day) in BALB/c mice was found to enhance the total WBC count (12,950 cells/mm^3) on 9th day, the phagocytic activity of peritoneal macrophages, significantly reduced the elevated level of TNF-*α* production by LPS stimulated macrophages, increase bone marrow cellularity

(23×10^6 cells/femur) and number of α-esterase positive cells (1346.66/4000 cells). Treatment with Sulforaphane along with the antigen, sheep red blood cells (SRBC), produced an enhancement in the circulating antibody titre and the number of plaque forming cells (PFC) in the spleen (315.83 PFC/10^6 spleen cells) on the 6^{th} day. These results indicate the immunomodulatory activity of Sulforaphane [47]. Intestinal immune functions are dependent on dietary aryl hydrocarbon receptor (AhR) ligands. Indole-3-carbinol is an AhR ligand found in broccoli sprouts. After oral consumption, indole-3-carbinol is converted in the presence of gastric acid to high-affinity ligands such as indolo[3,2-b] carbazole or 6- formylindolo[3,2-b]carbazole [48].

Toxicity Study

Broccoli is considered one of the main goitrogenic foods consumed (27·7 %) [49]. This idea conflicts with Shaipro et al., 2006 results who conducted a placebo-controlled, double-blind, randomized clinical study of sprout extracts containing either glucosinolates (principally glucoraphanin, the precursor of sulforaphane) or isothiocyanates (principally sulforaphane) on healthy volunteers to evaluate their safety and tolerance in human subjects using thirty-two types of hematology or chemistry tests in addition to indicators of liver (transaminases) and thyroid [thyroid-stimulating hormone, total triiodothyronine (T3), and free thyroxine (T4)] function. No significant or consistent subjective or objective abnormal events (toxicities) associated with any of the sprout extract ingestions were observed [50]. Moreover, when an LD50 test was performed on broccoli florets crude extract, no toxicity was shown up to 10 g/kg body weight [8, 10].

NUTRITIONAL VALUE

Broccoli is considered one of 20 most frequently consumed raw vegetables [51]. According to USDA, 2014 (Table 1), broccoli is a rich source of carbohydrates, potassium, vitamin K, vitamin C, vitamin A, vitamin E, potassium and folate. It is a very good source of dietary fiber, protein, calcium, phosphorus, magnesium and sodium. Dietary vitamins A, C, and E are important in an optimal diet, due to their antioxidant and free radical scavenging activities, which play important roles in human nutrition [52]. In addition, the prevention of carcinogenic nitrosamine formation in the stomach is another protective mechanism for vitamin C. Broccoli is also concentrated in phytonutrients especially in one particular phytonutrient category-glucosinolates- broccoli is simply outstanding. The isothiocyanates (ITCs) made from broccoli's glucosinolates are the key to broccoli's cancer-preventive benefits [53]. Broccoli is recommended in case of xerophthalmia resulting from vitamin A deficiency [54], infantile scurvy resulting from vitamin C deficiency [55] and anemia resulting from folate deficiency [56]. The following amino acids were identified by ion exchange chromatography in raw broccoli florets: glutamine, proline, asparagine, valine, arginine, isoleucine, threonine, leucine, phenylalanine, aspartic acid, lysine, alanine, tyrosine, *S*-methylcysteine, histidine, ornithine, glutamic acid, γ-aminobutyric acid, glycine and serine [57]. A clinical study on fourteen volunteers, consumed 200 g broccoli once a day for seven days, leads to an increase in serum concentrations of lutein and γ-tocopherol [58].

The consumption of broccoli may alter the stannous dichloride toxicity. Broccoli extract may use as a new protective strategies against the toxic effect of $SnCl_2$ on patients who were taken Technetium-99m [59].

EFFECT OF COOKING METHOD

Processes such as blanching, cooking and cutting, affect the content of glucosinolates, sulforaphane, polyphenols and antioxidant activity in broccoli. Steam processed broccoli should have enhanced antioxidant (through phenols and flavonoids) and anticarcinogenic (through glucosinolates) properties. Cutting or chopping broccoli then cooking or blanching at temperatures lower than 100^oC is favorable for anticarcinogenic properties. These issues seem promising for the use of processed broccoli as a functional food, with improved health promoting properties [60]. Steam processed broccoli showed a higher antioxidant capability, due to the significantly increased extractability of phenols and flavonoids, increased the bioavailability of these compounds *in vivo*, thus improving the health-promoting properties [61 - 63]. A significant decrease in polyphenols was observed in conventional and microwave cooked compared to fresh broccoli [64]. Blanching and cooking at temperature ranging between 50^oC and 90^oC affect the content of glucosinolates in broccoli [65, 66], mostly attributed to their loss from vegetal tissue through leaching into the cooking water and being dependent on the cooking conditions. In addition to, glucosinolates can be hydrolyzed by the action of myrosinase present in the plant tissue [67], besides chopping and crushing which facilitates the release of myrosinase, thus stimulating the formation of active isothiocyanates, such as the anticarcinogenic sulforaphane and consequently, improving the potential of broccoli as a functional food [60, 68, 69].

BIOAVAILABILITY OF GLUCOSINOLATES

The final level of glucosinolates in the prepared vegetable, the absorption, metabolism and delivery of glucosinolate breakdown products to target tissues depends, to a large extent, upon the residual level of myrosinase activity. The relative concentrations of glucosinolates and myrosinase present in broccoli prepared for human consumption will vary in a complex way determined by aspects of the food production chain, all the way from the farm to the cooked food. The activation of myrosinase is brought about by the physical disruption of the plant tissue during harvesting, processing, food preparation and consumption. However chopping induces glucosinolate hydrolysis only at the cut surfaces [70]. Thus large intact leaves, or florets of broccoli, will undergo only minimal losses of glucosinolates up to the point of cooking. If such vegetables are eaten raw, both intact glucosinolates and active myrosinase are ingested simultaneously, which enables the breakdown of the glucosinolates to occur within the alimentary tract. Some of the ingested glucosinolates were also broken down in the colon, but plant myrosinase

appeared to be the dominant factor. The bacterial microflora of the human colon also expresses myrosinase activity, except in case of antibiotics administration. In the absence of myrosinase activity, some intact glucosinolates are thought to be absorbed from the human alimentary tract, but the biological significance of this is unknown. Further research is needed to define the main site of absorption within the upper gastrointestinal tract is not entirely clear, bioavailability of the glucosinolate breakdown products in greater detail, so that the balance of benefit and consumer preference can be properly defined [70].

Table (1): USDA National Nutrient Database for raw broccoli

Nutrient	Unit	1value per 100 g	1 cup chopped = 91.0g	1 bunch =608.0g	1 spear (about 5" long) = 31.0g	1 stalk =151.0g	0.5 cup, chopped or diced = 44.0g	1 NLEA serving =148.0g
Proximates								
Water	g	89.3	81.26	542.94	27.68	134.84	39.29	132.16
Energy	kcal	34	31	207	11	51	15	50
Protein	g	2.82	2.57	17.15	0.87	4.26	1.24	4.17
Total lipid (fat)	g	0.37	0.34	2.25	0.11	0.56	0.16	0.55
Carbohydrate, by difference	g	6.64	6.04	40.37	2.06	10.03	2.92	9.83
Fiber, total dietary	g	2.6	2.4	15.8	0.8	3.9	1.1	3.8
Sugars, total	g	1.7	1.55	10.34	0.53	2.57	0.75	2.52
Minerals								
Calcium, Ca	mg	47	43	286	15	71	21	70
Iron, Fe	mg	0.73	0.66	4.44	0.23	1.1	0.32	1.08
Magnesium, Mg	mg	21	19	128	7	32	9	31
Phosphorus, P	mg	66	60	401	20	100	29	98
Potassium, K	mg	316	288	1921	98	477	139	468
Sodium, Na	mg	33	30	201	10	50	15	49
Zinc, Zn	mg	0.41	0.37	2.49	0.13	0.62	0.18	0.61
Vitamins								
Vitamin C, totalascorbic acid	mg	89.2	81.2	542.3	27.7	134.7	39.2	132
Thiamin	mg	0.071	0.065	0.432	0.022	0.107	0.031	0.105
Riboflavin	mg	0.117	0.106	0.711	0.036	0.177	0.051	0.173
Niacin	mg	0.639	0.581	3.885	0.198	0.965	0.281	0.946
Vitamin B-6	mg	0.175	0.159	1.064	0.054	0.264	0.077	0.259
Folate, DFE	µg	63	57	383	20	95	28	93
Vitamin B-12	µg	0	0	0	0	0	0	0
Vitamin A, RAE	µg	31	28	188	10	47	14	46
Vitamin A, IU	IU	623	567	3788	193	941	274	922
Vitamin E (alpha- tocopherol)	mg	0.78	0.71	4.74	0.24	1.18	0.34	1.15
Vitamin D (D2 + D3)	µg	0	0	0	0	0	0	0
Vitamin D	IU	0	0	0	0	0	0	0
Vitamin K (phylloquinone)	µg	101.6	92.5	617.7	31.5	153.4	44.7	150.4
Lipids								
Fatty acids, total saturated	g	0.039	0.035	0.237	0.012	0.059	0.017	0.058
Fatty acids, total monounsaturated	g	0.011	0.01	0.067	0.003	0.017	0.005	0.016
Fatty acids, totalpolyunsaturated	g	0.038	0.035	0.231	0.012	0.057	0.017	0.056
Cholesterol	mg	0	0	0	0	0	0	0
Other								
Caffeine	mg	0	0	0	0	0	0	0

PLANT TISSUE CULTURE

Plant regeneration procedure using hypocotyl protoplasts and a culture medium with a high NAA: 2,4-D auxin ratio permits highly efficient formation of colonies that regenerate shoots at frequencies of 8–17% in 8–11 weeks [71]. 0.1 mg/l TDZ with 0.1 mg/l NAA is the recommended combination for adventitious shoot regeneration [72]. Foliar Methyl Jasmonate (MeJA) application 4 days prior to harvest of broccoli at commercial maturity resulted in enhanced total glucosinolate (GS) concentrations. Although a single application of 250 µmol L−1 MeJA maximized GS concentrations in broccoli florets, two days of consecutive treatments (4 and 3 days prior to harvest) of 250 µmol L−1 MeJA further enhanced neoglucobrassicin concentrations and floret extract quinone reductase (QR)- inducing activity [73].

BROCCOLI-DERIVED BY-PRODUCTS

The possibility of adding health-promoting value to inexpensive and unused agro waste material is worth the effort of recycling it to obtain bioactive components that could benefit the food and drug industry. The analysis of extracts of the broccoli by-products showed a higher capacity for scavenging DPPH· in leaves than in the stalks. Moreover, the total concentration of phenolic compounds in leaves was almost 10 times higher than in the stalks while the stalks were richer in glucosinolates than the leaves [74].

CONCLUSION AND RECOMMENDATION

Plants are nature′s gift to humankind. They are considered as the most important resources of human nutrition and medicines. Rapidly increasing knowledge on nutritional plants and therapy has brought in a revolution on them. Nutritional therapy has emerged as a new term and has quickly spread in last few years. Strong recommendations for consumption of natural plant food and the use of nutritional therapy have become progressively popular for health improvement, prophylaxis and treatment of various ailments.

Broccoli is a widely used plant as food in many countries. It is obvious from this review that a lot of studies using different techniques have been carried out, and they showed excellent outcomes in terms of their application in different domains especially in cancer treatment. But little phytochemical study so far has been carried out on terpenoids and sterols content of broccoli. Moreover, some biological studies are still missing and needs further in-depth investigation such as effect on the central nervous system. Biologically-guided fractionation is needed for a stronger „proof-of-concept" for therapeutic potential of broccoli. Some recommendations are summarized as follows:

1. Identification and authentication (voucher specimen and number) of broccoli is necessary, as there is considerable confusion between different species and varieties of *Brassica*.
2. Method of drying, preparation and storage of extracts need to be well-defined.
3. In a bioassay-guided fractionation, a scattered activity across the different fractions is mostly indicative for non-selectivity.
4. Test organisms either rats, mice, microbes …etc. should be well-characterized.
5. *In vitro* models are the „golden" standard especially if microorganisms can be cultivated in cell cultures since it resembles more the *in vivo* situation [75].
6. Total extracts and derived primary fractions exhibiting strong non-selective action in the panel of *in vitro* screens can only be properly evaluated in animal models.
7. Antimicrobial activity must be discriminated from nonspecific toxicity by parallel cytotoxicity evaluation on mammalian cell lines or integration into a panel of unrelated microbial screens.
8. The used dose can have a profound impact on the test results. Extended dose ranges with at least three doses are needed for establishing representative dose–response curves.
9. To correct for too many false-positives, stringent endpoint criteria must be adopted.
10. Inclusion of appropriate controls in each test replicate (blank-, treated- and reference controls) is necessary [75].
11. Each test should contain at least one reference drug to ascertain test performance and proper interpretation of the results.
12. Differences in composition of the growth medium and animal food can greatly affect the potency of a natural product.
13. Further bioavailability studies are needed.
14. Real critical dose assessment of plant extracts and pure compounds is necessary.
15. he phenomenon of „additive" or „synergistic" effects in extracts frequently causes loss-of-activity during bio-guided fractionation efforts. In that case, further studies should focus on the defined crude extract [75].

DECLARATION OF INTEREST

The author declares that there"s no conflict of interest.

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