The Pharma Innovation Journal 2023; 12(7): 01-05

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 01-05

www.thepharmajournal.com
Received: 03-04-2023
Accepted: 02-05-2023

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Extraction of pharmacologically beneficial peptides from highly nutritious broccoli and its applications: A review

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Abstract
In recent years, plant-derived bioactive peptides are significant due to their potential to prevent and treat various diseases and disorders. These peptides exhibit anti-inflammatory, anti-cancer, anti-ageing, anti-acne, anti-microbial, antioxidant, and other properties which make them suitable constituents of natural medications. Broccoli (*Brassica oleracea*) is rich in phytonutrients such as glucosinolates, sulforaphane, polyphenols, carotenoids, flavonoids, vitamin K, vitamin C, selenium, iron, potassium and calcium respectively. The studies conducted on it found that they also contain a considerable amount of protein, which is utilized as an alternative to synthetic chemicals in food, pharmaceutical and cosmetic industries. The peptides with ACE-inhibitory and anticancer properties are extracted from Broccoli through gastrointestinal digestive enzyme hydrolysis and identified using UPLC-MS/MS. Broccoli waste also contains an abundance of bioactive compounds and nutrients. Therefore, utilization of its waste can reduce post-harvested losses and aid in maintaining a sustainable food chain system. Therefore, this review paper focuses on the nutraceutical and pharmaceutical properties of bioactive compounds and applications of Broccoli.

Keywords: Broccoli, bioactive compounds, peptides, applications

Introduction
Brassica oleracea is the most significant crop from the Brassicaceae family included in the human diet. The Italica variety of broccoli is one of the most popular vegetables in Western cuisine. They are referred to as "superfoods" since they are abundant in biologically active compounds with well-researched health benefits (Orlando *et al*., 2022) [25]. The phytochemicals in the Brassicaceae family are divided into a number of macronutrients (high in proteins, dietary fibre and low in carbs) and micronutrients (vitamins, minerals and amino acids) (Favela-González *et al*., 2020) [10]. The principal bioactive compounds are glucosinolates, vitamin C, sterols, chlorophyll, flavonoids, carotenoids, alkaloids, phytosterols, phenolic compounds, and slightly amount of minerals (Vega-Galvez *et al*., 2023) [35]. These bioactive compounds have certain potential, including anticancer, antioxidant, antibacterial, anti-inflammatory and antihypertensive effects. In addition, Broccoli's advantageous composition also enables it to treat diabetes, hypercholesterolemia, cardiovascular illnesses, and photosensitivity disorders (Gudiño *et al*., 2022) [14].
Currently, there is growing awareness regarding plant-derived bioactive peptides (BPs) for their use in nutraceutical, cosmeceutical, and medicinal applications (Nicolas-Espinosa *et al*., 2022) [21] because of less toxicity and minimum adverse effects than several chemotherapeutics (Udenigwe *et al*., 2021) [34]. A bioactive peptide liberated by the hydrolysis of parent proteins basically has 2-20 amino acid fragments and can display varied biological effects based on its amino acid content, chemical structure and length (Olivares-Galván *et al*., 2020) [23]. Numerous plants have been studied to have bioactive peptides such as mustard, soybeans, chickpea, spinach, tomato, bottle gourd, mango, peach and others have been found to exhibit antioxidant, anti-viral, antithrombotic, hemolytic, antihypertensive activities (Nicolas-Espinosa *et al*., 2022; Olivares-Galván *et al*., 2020) [21, 23]. However, a few studies on broccoli protein and hydrolysates reported the presence of a peptide (Tyr-Pro-Lys) with ACE inhibitory peptide potential (Dang *et al*., 2019) [7].
Moreover, the stem and the leaves of broccoli are regarded as waste since they are rotting and scraped components of the vegetable. Numerous nutrients and bioactive substances, including phytochemicals (glycosylates, phenolics, carotenoids, and flavonoids), are present in these

wastes (Pereira *et al*., 2022) [28]. These nutrients and nutraceuticals, which have a wide range of uses in the food supplement, pharmaceutical and cosmetic sectors, are abundant in broccoli waste (Socas-Rodríguez *et al*., 2021) [33]. The maceration method was used to extract the phytochemicals from the leaf and stalk, and the extract from the stalk exhibited greater anti-inflammatory and antioxidant effects at a concentration of 500 g/mL. It also demonstrated effective anti-microbial activity at 300 g/ml, indicating its potential as a source of significant biologically active compounds with anti-ageing and anti-acne activity (Ramesh *et al*., 2023). Therefore, discovering new applications for broccoli may result in the food, cosmetic, and pharmaceutical industries, which can contribute to a more sustainable food system (Galanakis *et al*., 2020) [13].

Nutritional value of broccoli

The Brassicaceae family, which includes cruciferous vegetables with astringent tastes, particularly broccoli (*Brassica oleracea* L. var. Italica) is a nutrient-dense vegetable that offers a variety of necessary vitamins, minerals, and other health-promoting components (Kumar *et al*., 2022) [17]. It is particularly a good source of fibre, which helps promote digestive health and lower the risk of certain ailments, vitamin K is essential for bone health and blood clotting, folate is necessary for cell formation and proliferation, and vitamin C supports the immune system and skin health due to its antioxidants property (Nagraj *et al*., 2020) [20]. Other antioxidants such as beta-carotene and quercetin are also present in broccoli. These substances aid in defending cells against damage that is caused by free radicals, which contribute to ageing and diseases (Yadav *et al*., 2016). The seedlings of broccoli are abundant in sulforaphane, a phytochemical that belongs to the class of isothiocyanate and its precursor known as glucosinolates. These are beneficial substances which have been associated with anti-cancer properties and can reduce inflammation (Li *et al*., 2022) [19]. In addition, broccoli is an excellent source of various important minerals such as iron, potassium and calcium. Iron is essential for oxygen transport in the body, potassium helps to control blood pressure and fluid balance and calcium is essential for bone health (Davison *et al*., 2017) [8].

According to the available evidence, broccoli sprouts and microgreens serve a significant role in human health and lower the chance of developing chronic ailments. Various *in vivo* and *in vitro* investigations have shown the chemical composition and a variety of biological properties of broccoli microgreens and sprouts, including anticarcinogenic, antioxidant, antibacterial, antidiabetic and anti-inflammatory properties. Recent years have seen extensive research, especially into the anticancer and antioxidant potential (Le *et al*., 2020) [18]. Overall, broccoli is a very nutritious vegetable that, when included in a balanced diet, can offer a number of significant health benefits (Ferguson *et al*., 2012) [11].

Biologically Active Compounds of Broccoli

Bioactive molecules are extra-nutritional constituents of food, mostly found in grains, fruits and vegetables that have the ability to regulate metabolic functions and have a positive impact on the human body (Sindhu *et al*., 2021) [32]. Broccoli (*Brassica oleracea* var. italica) health benefits are primarily due to bioactive secondary plant metabolites, rendering it a functional food. Glucosinolates (GSLs), sulforaphane and polyphenols (PPs) are three of those compounds that are of particular interest (Andini *et al*., 2020) [1]. In addition, selenium (Se) can accumulate in broccoli, and Se metabolites have been linked to a lower risk of cancer. The most common strategy used for preventing human selenium deficiency is agronomic biofortification, which involves supplementing plant-based products enriched with selenium to provide an adequate supply (Bouranis *et al*., 2023) [5].

Bioactive components are extracted from herbs, vegetables, and fruits using a variety of techniques, including modern methods such as pressurized hot water extraction, pulsed electric extraction, or microwave-assisted extraction (Panja *et al*., 2018) [27]. Both traditional and advanced methods were used to explore the isolation, identification, and characterization of the compounds from broccoli seedlings of the species Brassica. Among these techniques, high-performance liquid chromatography (HPLC) linked to electrospray ionization (ESI), ultraviolet-visible (UV-vis), or mass spectrometry (MS) detectors with C18 analytical columns were mostly used to analyze broccoli samples (Kumar *et al*., 2017) [16]. The hydrolysis byproducts of glucosinolates, and isothiocyanates, were also studied using gas chromatography and mass spectrometry (GC-MS), spectrophotometric (UV-vis), which is the easiest method to estimate the total phenolic and flavonoid contents (Le *et al*., 2020) [18]. Therefore, the use of these vegetable extracts can cause a variety of positive effects of high biological value in the treatment of diseases due to their bioactive properties (anti-inflammatory, anti-obesity, anti-anaemic, anti-cancer, antimicrobial, antioxidant, cardio-protective, immunomodulator, gastroprotective, and hepatoprotective) (Favela-González *et al*., 2020) [10]. A study suggested the use of the Vacuum drying (VD) method can preserve the bioactive compounds of dried broccoli to manufacture powders with high biological potential. The effects of temperature (at the five levels of 50, 60, 70, 80, and 90 °C) and constant vacuum pressure (10 kPa) were assessed to accomplish these objectives (Ramírez-Pulido *et al*., 2021) [31]. The findings indicate that drying time reduced as the temperature increased. The Midilli and Kucuk models are best suited to drying kinetics, whereas the statistical tests revealed that the Brunauer-Emmett-Teller (BET) model fits effectively to sorption isotherms. VD significantly influences a number of proximal composition parameters. Vacuum drying at higher temperatures (80 and 90 °C) enhanced broccoli powder's antioxidant capacity (Vega-Galvez *et al*., 2023) [35]. However, at 50 °C, greater antibacterial and neuroprotective effects were seen, most likely as a result of the production of isothiocyanate (ITC) (Oliveira *et al*., 2016) [24]. Overall, the results of this study show that VD is a potential method for the production of broccoli powder extracts that may be employed as natural neuroprotective agents or as a preservative (Vega-Galvez *et al*., 2023) [35]. Thus, several researches showed that phenolics, glucosinolates, selenium, and related compounds constitute the main class of phytochemicals explored in broccoli that can provide a wide range of health benefits and play a vital role in the pharmaceutical as well as nutraceutical industries (Oleszek *et al*., 2023) [22].

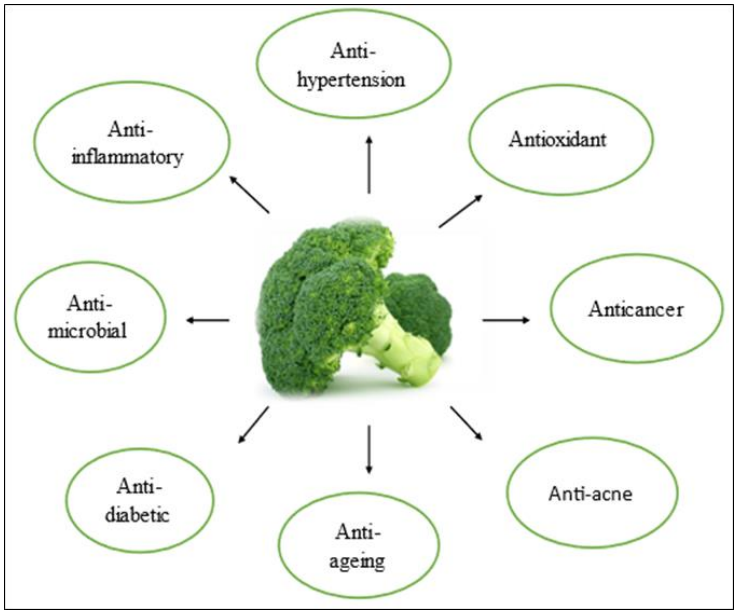

Fig 1: Pharmaceutical properties of bioactive compounds of Broccoli

Extraction of bioactive peptides from broccoli

According to (Zhang *et al*., 2022) [37], KSVLLKF (KF-7) ACE-inhibitory peptides produced from natural sources are gaining remarkable interest due to the negative effects of artificial ACE inhibitors such as captopril used to treat hypertension. The purpose of this research is to isolate and characterize new peptides from protein hydrolysates of broccoli while also testing the *in vitro* ACE inhibitory efficiency. The enzymatically hydrolyzed fraction BHs-IV-I-IV of broccoli was produced using gel chromatography, centrifugation, ultrafiltration, and high-performance liquid chromatography (HPLC). This fraction has 100% ACE inhibitory activity. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was employed to identify the new ACE inhibitory peptide KSVLLKF (KF-7) from fraction BHs-IV-I-IV. The least concentration of KF-7 in the protein hydrolysates of broccoli is shown by the low ACE inhibition rate of 0.05 mg of KF-7 and the low ACE inhibition rate of 12.48 mg of pepsin hydrolysate of broccoli crude extract. These findings indicated that KF-7 may be used in functional foods and pharmaceuticals to treat hypertension, and protein from broccoli may be a source of such peptides.

Three Angiotensin-converting enzyme inhibitory peptides-TFQGPPHGIQVER, LVLPGELAK, and IPPAYTK isolated, purified, and identified from broccoli protein hydrolysates in the current investigation to assess their hypotensive and angiotensin-converting enzyme (ACE) inhibitory activity *in vitro* impact *in vivo*. The relative inhibitory IC50 values were 23.5, 184.0 and 3.4 M. The effect of gastrointestinal digestion on ACE inhibitory activity was also examined, the findings revealed that the peptide LVLPGELAK had nearly twice as much ACE inhibitory action after simulating digestion than before. The two new peptides with significant ACE inhibitory activity, LAK and LVLPGE, were identified followed by digestion with IC50 values of 48.0 and 13.5 M, respectively. The hypotensive impact of peptides was evaluated by oral administration of the peptides to spontaneously hypertensive rats (SHRs) (Dang *et al*., 2019) [7].

Proteomics was used to analyze peptide combinations from the broccoli stem total proteins and membrane proteins. These peptides were used to treat wound healing which was examined after 24 and 48 hours of therapy application and HaCaT cells in order to assess cytotoxicity in a concentration range between 20 and 0.15625 mg of protein/mL. In addition, keratinocyte proteome and gene expression data were evaluated. These findings indicate that depending on the protein source, distinct peptide combinations derived from broccoli halt cell proliferation and inhibited the carcinogenic, uncontrolled expansion of the cells (Nicolas-Espinosa *et al*., 2022) [21].

The findings of these studies support the beginning of new lines of investigation into the employment of Brassica peptides in pharmaceutical or cosmetics products.

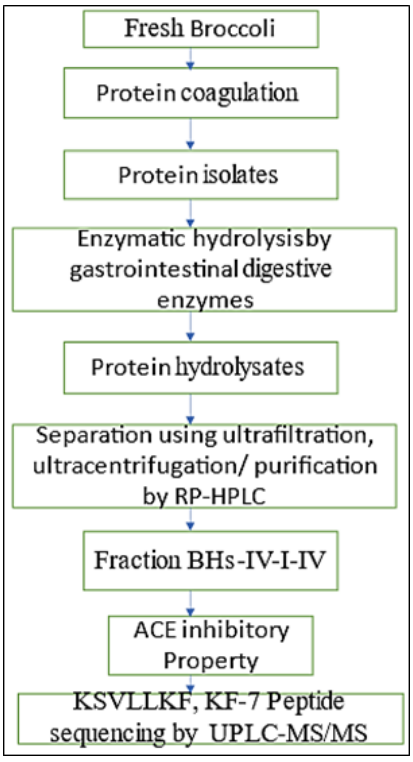

Fig 2: Flow diagram for the extraction of Bioactive peptides from Broccoli stem

Application of bioactive peptides derived from broccoli

Broccoli is a cruciferous vegetable that is high in vitamins, minerals and phytochemicals. Peptides obtained from Broccoli are physiologically active substances. These peptides are being employed more often in the pharmaceutical, cosmetic and food sectors due to their numerous health advantages (Baenas *et al*., 2014) [3].

Broccoli peptides have been discovered to have antibacterial, antioxidant, and anti-inflammatory activities which makes them suitable ingredients in the field of pharmaceuticals (Owis *et al*. 2015) [26]. They may aid in the prevention and treatment of a number of ailments and disorders and have been proven to have potential anti-cancer qualities (Podhorecka *et al*. 2017) [29]. In fact, some research has indicated that broccoli peptides may help lower the chance of developing cancer, Alzheimer's disease, and cardiovascular disease (Barnard *et al*. 2014) [4]. Furthermore, Broccoli peptides are employed in skin care products in the cosmetics industry because they can hydrate the skin better, reduce inflammation and shield it from harm to the environment (Frantz *et al*., 2023) [12]. They could also aid in enhancing skin tone and texture while decreasing the visibility of wrinkles and fine lines (Wunsch *et al*., 2014) [36].

Broccoli peptides are functional components in many different food items used in the food industry. They are frequently employed as a natural preservative due to their antibacterial activity (Gyawali *et al*., 2014) [15]. They can be incorporated into food products to increase their nutritional value. The texture, flavour and shelf life of various food products can also be enhanced by these peptides of Broccoli (Axel *et al*., 2017) [2].

Overall, the use of broccoli peptides in the pharmaceutical, cosmetic, and food sectors is still relatively new, and a study is currently being done to fully understand their potential (El *et al*., 2012) [9]. Early research, however, points to the possibility that these substances might have several potential uses in a variety of sectors and provide major health and nutritional advantages (Chaudhry *et al*., 2008) [6].

Conclusion

Nutrient-rich food is in high demand currently due to its potential health outcomes. The importance of Brassica is rising because of its nutritional benefits and pharmacological properties. Bioactive peptides extracted from various plants and animal sources are gaining considerable importance because these peptides not only fulfil the demand for protein but can also aid in the preparation of medicines due to their pharmaceutical properties which include anti-oxidant, anti-inflammatory, anti-hypertensive, and others. Scientists are focusing more on plant-derived bioactive peptides than animals and finding new sources of plants which are rich sources of bioactive peptides. This is because peptides obtained from plants are safe, cost-effective, and can easily produce on a large scale. The studies conducted on *Brassica oleracea* reported peptides with bioactive potential. Additionally, plants from the Brassicaceae family include several useful metabolites that are also directly connected to a variety of recognized biological functions, prompting extensive research into their phytochemical composition. Numerous medicinal characteristics are confirmed by research to be effective in treating chronic ailments including osteoporosis, cancer, hypertension, type 2 diabetes, and obesity as well as cardiovascular ailments. The waste of broccoli also contains the same quantity of phytonutrients. Hence, its utilization can reduce the global problem of waste production.

Data availability: Not applicable.

Data deposition: Not applicable.

Conflict of Interest: Not applicable.

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