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MICROGREENS AS A FUNCTIONAL COMPONENT OF THE HUMAN DIET: A REVIEW

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ABSTRACT

The alarming growth of chronic diseases is a major problem in Europe, and current data suggest an even greater burden in the future. Although genetic predispositions remain important determinants in the development of certain disorders, the appropriate diet can considerably minimize the risk of many diseases. Many plant species do, in fact, have health-promoting properties due to their high levels of physiologically active chemicals. Glucosinolates, vitamins, tocopherols, saponins, tannins, and other polyphenols have been shown to have beneficial impacts on human health, including cancer, diabetes, cardiovascular, kidney, and Alzheimer's disease prevention. A new plant category known as microgreens has evolved as a rich source of vitamins, minerals, carotenoids, and physiologically active chemicals against these backdrops. Microgreens could have 10 to 100 times more effective phytochemical concentrations than adult equivalents. Furthermore, they are environmentally cultured in regulated environments with no dirt, harmful residues, or excessive water use. However, existing limitations include uncertain methods of action, varying bioaccessibility, and a paucity of published clinical research. Indeed, microgreens could be a viable new food source for people who are interested in consuming healthy diets.

Keywords: microgreens, phytochemicals, cancer, inflammatory

INTRODUCTION

Chronic diseases are becoming more common in Europe, and they are one of the primary causes of mortality and morbidity. Chronic diseases like heart attack, cancer, diabetes, osteoarthritis, Alzheimer's, and Parkinson's were a few decades ago thought to be primarily a concern of the elderly population. In the European Union (EU), life expectancy has increased dramatically in recent years, yet senescence is accompanied by at least two chronic disabilities. In addition, we know that middle-aged and young people are strongly affected by chronic conditions nowadays. This reflects a large extent of the cumulative effect of more difficult living or working conditions and greater exposure to various risk factors earlier in life. The EU approach to addressing the challenge of chronic diseases involves an integrated response focusing on prevention across sectors, combined with efforts to strengthen the health system to improve the management of chronic conditions. It is a public call and an important issue for policy-makers and researchers across the world. Many chronic diseases and conditions are linked to a genetic predisposition of individuals. Additionally, lifestyle choices such as smoking, alcohol drinking, fitness, or sexual behavior are significant factors determining the development of chronic disabilities (**Busse *et al.*, 2019**). Human evolution and new advances are mirrored by the mutual injection of various information from medicine, biochemistry, and nutrition. The lack of vitamins and minerals in the human diet, as well as drastic changes in overall health, sparked a surge in interest in food and nutrition. The current evidence from experimental research largely supports the health-protective effects of eating a diet high in plant-derived foods. Brassicaceae, Fabaceae, Solanaceae, Apiaceae, Amaranthaceae, and other vegetable families are deemed important due to evidence of their health-promoting properties linked to physiologically active chemicals found in the edible parts of the plants (**Choe *et al.*, 2018; Bella *et al.*, 2020; Ghoora *et al.*, 2020**). The Brassicaceae family consists of more than those 3 000 species and includes 350 genera such as *Sinapis*, *Camelina*, or *Brassica* which are worldwide economically important. In addition, *Lepidium*, broccoli, and cauliflower are rich in glucosinolates, and more in detail, in glucoraphanin, a molecule that is transformed into sulforaphane with anticarcinogenic effects (**Raiola *et al.*, 2018; Le *et al.*, 2020).** *Fabaceae* family is one of the largest groups consist around 19 000 plant species. Fenugreek, clover, and soybeans have a high number of bioactive compounds such as caffeic acid, alpha-tocopherol, trigonelline, and kaempferol with strong antitumor and anti-inflammatory properties (**Salehi *et al.*, 2019**). *Solanaceae* is a group of trees, shrubs, and herbs including more than 2 000 species. Datura, capsicum, or Withania are known for possessing a diverse range of alkaloids whose toxicity to humans and animals ranges from mildly irritating to fatal in small quantities. However, antioxidant or anticholinergics benefits are well known (**Lima *et al.*, 2021**). Many of the benefits of mature plant ingestion in humans are well-known, but the continued increase of land field chemigation can raise various concerns. In this context, agricultural innovations that can reduce the impact of more external influences are highly welcomed. Microgreens are currently grown using hydroponic techniques, which are the most popular. We speculate that when proof of their biological impacts increases, these plants may become an interesting new food source for the improvement of human health (**Fraga *et al.*, 2019; Majid *et al.*, 2021).** This review is dedicated to gathering current experimental findings on the monitoring of chemical and nutrient compositions, biological efficacies, and health benefits of various microgreens regarding overall consequences on human health.

MICROGREENS

Microgreens have grown in popularity in recent years, and their popularity is growing due to a global increase in public awareness of healthy eating. Microgreens are young immature greens that are between the sprout and baby greens stages of growth. They're not as big as baby greens, and they're harvested later than sprouts. Microgreens are produced from the seeds of herbs, vegetables, and grains. It is the seedlings of edible plants with established roots and its first true leaves, the cotyledons, are fully developed. Additionally, microgreens consist of a central stem and a pair of young true leaves that start to emerge (**Kyriacou *et al.*, 2016; Choe *et al.*, 2018**). **Di Gioia et al. (2017)** declare that not all tender young plant greens are considered microgreens. The size of them is approximately 1 to 3 inches tall, and they are harvested on average, between 7 - 14 to 21 days post-planting (species-dependent). Baby greens and sprouts, in addition to microgreens, are recognized, and the primary differences are provided in (Table 1).

Table 1 Cultured and morphological differences: sprouts, baby greens, microgreens

GROWTH PHASE	HARVEST TIME (DAYS)	SIZE (INCHES)
sprouts	from 2 to 6	from 0.8 to 1
baby greens	from 15 to 40	from 2 to 4
microgreens	from 7 to 21	from 1 to 3

The composition of sprouts and microgreens is also a significant difference. Microgreens are harvested without the roots, while a sprout includes seed, root, and stem. An abundant supply of neutral clean water or slightly acidic water is required for microgreens production. If seeds are immersed overnight at a constant temperature and under low light conditions, germination may be improved. The sprouts should be exposed to light after 2 or 3 days and watered continuously by the spreader until real leaves emerge (**Turner *et al.*, 2020**). Shrinking of agricultural field, increasing pesticides application in the environment, radical population growth and needs to ensure the food security continually support indoor farming of microgreens. The attractivity of the microgreens also comes from their short production cycle, acceptable cultivation conditions (e.g., less water usage), and loose or soilless germination media as well as their strong abilities to produce multiple cycles of microgreens compared to mature vegetables. Controlled conditions such as climate, humidity, lighting, and irrigations with minimizing external environmental growth factors are other attributes, that markedly popularise the microgreen market (**Goodman and Minner, 2019; Riggio *et al.*, 2019**). Despite much positive information regarding indoor farming, microgreens bring several challenges to current days. The primary limitation is postharvest quality degradation and reduced shelf life. Due to their early senescence, microgreens are difficult to store. A high surface area to volume ratio, rapid postharvest degradation transpiration, a high respiration rate, and early tissue damage all have a negative impact on their quality (**Renna *et al.*, 2017**). These miniature greens are a special category of plants for their vibrant colors, freshness, flavor, aromas, cultivation conditions, and nutritional value. Owing to the enormous content of bioactive molecules they could be characterized as disease-preventing and health-promoting plants. Previous data suggest that microgreens are richer in polyphenols, glucosinolates, vitamins, minerals, or carotenoids. Saturated or unsaturated fatty acids are also more abundant compared to adult plants. For radish microgreens, it was detected a high content of glucosinolates, which are crucial in human cancer prevention. Likewise, broccoli microgreens exhibit a robust antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, or anti-obesity potential compared to their corresponding adult plant (**Xiao *et al.*, 2019; Ghoora *et al.*, 2021**).

PHYTO-COMPOSITION OF MICROGREENS

Although a wide range of microgreens is currently being cultivated, scientific data about their direct potential to affect human physiological functions are not widely available. Nowadays, there are many herbs and vegetables grown as microgreens. Commonly cultivated taxonomic families include *Brassicaceae* (broccoli, mizuna, cabbages, radishes), *Fabaceae* (fenugreek, sweet pea, alfalfa), *Apiaceae (*carrot, parsley, celery) *Asteraceae, Amaranthaceae*, and many others. It is also well documented that the chemical composition of microgreens differs considerably from that of mature plants. There is strong evidence about a huge abundance of bioactive compounds, including carotenoids, vitamins or minerals, glucosinolates, and polyphenols (**Turner *et al.*, 2020; Marchioni *et al.*, 2021**).

Vitamins

Phylloquinone known as vitamin K1 is highly presented in *Amaranthaceae*, *Brassicaceae,* and *Lamiaceae* families. The level of phylloquinone concentrations fluctuated between 0.6 to 4.1 μg/g fresh weight. Among the species, red garnet amaranth had the highest concentration (4.1 μg/g fresh weight), followed by green basil (3.2 μg/g), red cabbage (2.8 μg/g fresh weight), and magenta spinach (0.9 μg/g fresh weight). Compared to mature amaranth and cabbage, phylloquinone has been detected at a lower level (0.41 to 1 fresh weight.14 μg/g fresh weight) respectively. Detailed analyses also suggested that a total of 18 out of the 25 commercially grown microgreens contain a greater amount of phylloquinone compared to their adult form (**Xia *et al.*, 2012; 2019**). Besides phylloquinone's role in blood coagulation and maintaining healthy bone tissue through the prevention of vascular calcification, it has been established, that phylloquinone has also immunosuppressive and anti-cancer effects (**Halder *et al.*, 2019**). Vitamin C (total ascorbic acid) is considered as the sum of free ascorbic acid and dehydroascorbic acid, which are highly bounded in *Malvaceae*, *Brassicaceae,* or *Cucurbitaceae* families. **Ghoora *et al.* (2020)** declare, that ascorbic acid was the most abundant at all evaluated microgreens (e.g., fenugreek, radish, roselle, etc.) with concentrations ranging from 41.6 to almost 140 mg/hundred grams. Roselle microgreens from the *Malvaceae* family have the highest value of ascorbic acids followed by basil and radish. In the previous study, **Xiao *et al.* (2019)** confirmed a higher level of total ascorbic acid in cauliflower and broccoli microgreens. Compared to accumulated data of the National Nutrient Database for Standard Reference Legacy Release (**Haytowitz *et al.*, 2018**), the total ascorbic acid concentration of red cabbage, broccoli, and amaranth microgreens is 6 times greater than its mature counterpart. Ascorbic acid is a potent antioxidant, and it is also essential for a variety of human biological functions generally. In addition, it has a significant effect on collagen synthesis and immune system regulations. Severe deficiency in vitamin C could result in scurvy, and increased risks of many noncommunicable disseise such as cancers, arthritis, or Alzheimer´s disease (**Abeysuriya *et al.*, 2020**). The most active form of vitamin E is α-tocopherol. Together with other tocotrienols (β, γ, and δ) belongs to the vitamin E family. On the other hand, γ-tocopherol is the most frequent in plants (**Sadiq *et al.*, 2019**). Especially, *Brassicaceae* and *Apiaceae* families of microgreens are considered the rich source of them. **Xiao *et al.* *(*2019)** confirmed green daikon radish has the greatest γ and α-tocopherols levels (mg/100 g fresh weight). In addition, pepper cress and cilantro also showed high levels of both vitamin E isoforms. According to a previous study, α-tocopherol was the most abundant in radish or sunflower (58.6 and 48.7 mg/100 g fresh weight), while mustard and spinach contain over 15-fold respectively 8-fold higher α-tocopherol content, compared to mature counterparts (**Ghoora *et al.*, 2020).** Due to the unique antioxidant properties of tocopherols, current studies suggest an essential role in maintaining human health. They can protect against oxidative damages in many tissues and play an important role in muscle or immune functions. At the same time, tocopherol supplementation could be the prevention of many aging-related diseases such as cardiovascular or Alzheimer's disease (**Thompson *et al.*, 2019**).

Minerals

Some previous studies suggest that microgreens are excellent sources of minerals. Some of them are important but the majority are considered essential nutrients. In general, the concentrations of macro-minerals (e.g., potassium, magnesium, calcium, phosphorus) and trace minerals (e.g., manganese, zinc, sodium, and copper) is greater in 90% of microgreens cultivars compared to mature plant (**De la Fuente *et al.*, 2019; Zhang *et al.*, 2021**). The results of the recent study suggest that a valuable source with the highest concentrations of Ca, K, and Na could be the Fennel microgreen. Spinach microgreens had a significantly richest content of Mg, while roselle had a maximum source of P, Zn, and Se (**Ghoora *et al.*, 2020**). **Pinto *et al.* (2015)** previously showed that lettuce microgreen had significantly higher concentrations of most minerals such as Ca, Fe, Zn, Mg, or Mn compared to mature counterparts. **Weber (2017)** declare that mustard microgreen contains a higher concentration of Ca and Mg but, at the same time, the other two mustard genotypes have a lower level in both. It could be affected by varietal differences and the composition of growth substrate or culture conditions. A lack of experimental data on the essential mineral content was published so far. Therefore, a concrete conclusion is not fully created.

Polyphenols

As we discussed previously, the nutritive and functional value of microgreens rest in their rich vitamin, minerals, sugar, and also carotenoid content. However, the most important molecules essential for human health are phytochemicals that include phenolic compounds subdivided into classes such as flavonoids, phenolic acids, tannins, stilbenes, or lignans. As relatively unknown plant materials with limited scientific knowledge, the overall phenolic content, antioxidant potential, and nutritional profile of microgreens have yet been scarcely examined (**Bella *et al.*, 2020**). Currently, common commercial microgreens families such as *Brassicaceae*, *Apiaceae*, *Lamiaceae*, or *Malvaceae* exhibited enormous antioxidant activity with a wide variation ranging from 303.3 mmol/kg in jute to 878.3 mmol/kg in cress. Overall, the highest antioxidant capacity was confirmed in species of the *Brassicaceae* followed by the *Lamiaceae* family. If we look at the evidence in detail, coriander had the highest level of total polyphenol content (5920 mg/g dry weight) followed by green basil and tatsoi (3506 and 2645 mg/g dry weight). Compared to the data from mature plants (database on polyphenol content in foods) coriander has reached a level of only around 2260 mg/g in dry weight (**Kyriacou *et al.*, 2019**). If we look in deep, kaempferol, quercetin, isorhamnetin, and hydroxy-cinnamic acids with derivatives are the most potent health-promoting phytoconstituents commonly found in mature brassicaceous vegetables. Experimental data showed that the level of isorhamnetin and quercetin-3-O-glucoside is 80 - respectively 3-fold lower in mature pakchoi than in microgreen counterparts. Similarly, comparing the level of caffeic and ferulic acid were significantly lower values detected in mature plants. More than a 100-fold higher level of quercetin-3-O-glucoside was measured in cress, while the content of ferulic acid has been estimated at the same level, respectively. According to current evidence, microgreens have more diverse polyphenol profiles, strong antioxidant potential, and could be favored in the human diet due to their bioavailability (**Li *et al.*, 2018; Kyriacou *et al.*, 2019**).

Glucosinolates

The last group of phytochemicals strongly abundant in microgreens is glucosinolates. Glucosinolates are essential to plant secondary metabolites recognized as nitrogen-sulfur derivatives (β-D-thioglucoside-n-hydroxysulfates). The major group of glucosinolates is aliphatic derivates (derived from valine, isoleucine, etc.) followed by aromatic (derived from phenylalanine) and indole (derived from tryptophan) derivatives (**Le *et al.*, 2020**). **Huang *et al.* (2016)** compared the level of glucosinolates in microgreen red cabbage and mature plant. The results revealed a 2-fold higher concentration in microgreens (17.1 μmol/g) compared to 8.3 μmol/g in mature counterparts. Similar reports demonstrated that microgreens contain more functional substances including glucosinolates (**Gan *et al.*, 2017; Mir *et al.*, 2017**). Evidence about glucosinolates and their biological effects is currently recognized bactericidal, nematocidal, and fungicidal properties. In addition, antioxidant, anti-diabetic, and anti-inflammatory activities are well documented. Recent research also explores their modulatory potential to cardiovascular, lung, and cancer diseases (**Maina *et al.*, 2020**).

HEALTH-BENEFICIAL EFFECTS OF MICROGREENS

As we have already outlined in the previous sections, microgreens are becoming recognized as a novel source of physiologically active substances, and their popularity is skyrocketing due to a variety of nutritional and chemical factors. Current research has been expanding gradually while the pilot study confirmed effectiveness in the reduction of blood glucose, weight control, and cardiovascular diseases prevention. The high bioaccessibility of phytochemicals after digestion can provide the antimicrobial, anti-inflammatory, antioxidant, or anti-diabetic potential of microgreens (**De La Fuente *et al.*, 2020; Le *et al.*, 2020**). Overleaf, the rich content of ascorbic acid, carotenoids, and isothiocyanates are responsible for a strong anti-proliferative effect. Additionally, **Huang *et al.* (2016)** describe inhibition of cholesterol and triglycerides synthesis, together with regulation of plasma and liver lipid metabolism.

Anti-cancer potential of microgreens

Malignant tumors are the second leading cause of death globally and there is a prediction of increasing incidence during the next few years. Therefore, there is an urgent requirement for cost-effective cancer prevention through the boost of plant bioactive phytochemicals intake (**Koh *et al.*, 2020**). Microgreens, with a variety of polyphenols, vitamins, carotenoids, and minerals, are considered promising in cancer prevention and have the regulated ability to affect specific metabolic processes and mechanisms within cancer cells. Overleaf, clear evidence was first reported only recently (**De La Fuente *et al.*, 2020**). Those authors reported the anti-proliferative effect of the bio-accessible fractions extracted from four *Brassicaceae* microgreens (broccoli, radish, etc.) on human colorectal adenocarcinoma cells. Cell cycle arrest in G2 / M, immediate reactive oxygen species generation, and apoptotic cell death were reported. It is well recognized that the exact mechanism underlying cancer development is unknown, however, inflammation plays a crucial part in carcinogenesis. This pathway is related to the Kelch-like ECH-associated protein (Keap) -nuclear factor erythroid 2-related factor 2 (Nrf2), AhR (aryl hydrocarbon receptor), and nuclear factor Kappa B (NF-kB). Simultaneously, all of them could be affected by diet-derived compounds (indole-3-carbinol, diindolylmethane, glucoraphanin, or oxazolidines) involved in microgreens (**Choe *et al.*, 2018).** Especially, the *Brassicaceae* family is a significant source of glucosinolates metabolites. Their effect starts with the activation of Phase I and II in xenobiotic-metabolizing enzymes that could be effective in cancer protection. They can activate specific enzymes involved in cancer metabolism and allow cells to start defending against potential carcinogens (**Singh *et al.*, 2019**). In general, glucosinolates are not functional phytochemicals, but their hydrolysis products have a strong potential to affect molecular and signaling pathways in organisms. It detailed, glucosinolates could be hydrolyzed by the enzyme myrosinase into several functional substances such as isothiocyanates, oxazolidines, or epithionitriles. Notably, isothiocyanates have been proven to present excellent anti-carcinogenic potential. Enzymatic conversion of them is responsible for sulforaphane, iberin, and erucin production in broccoli microgreens. It was confirmed that all of them could significantly reduce the risk of colon and bladder malignant growth (**Baenas *et al.*, 2017; Le *et al.*, 2020**). In current days, breast and prostate cancer are common diseases, and malignant growth is promoted by the hormone-mediated route. It suggests that inhibiting or regulating estrogen and androgen receptor binding activity could have a significant impact on the development of hormone-dependent cancers. A previous study confirmed that plant-derived phytochemicals such as indoles or flavonoids, which are primarily represented in microgreens, act an effective role in prostate and breast protection at earlier stages. Indole-3-carbinol significantly repressed estrogen receptor alpha (ER-α) signaling in MCF-7 breast cancer cells. In addition, downregulated expression of the estrogen-responsive genes, suppression of trefoil factor 1 (TEF1), and cathepsin-D, followed by upregulation of the tumor suppressor gene was also reported (**Baenas *et al.*, 2017; Tomas *et al.*, 2021**).

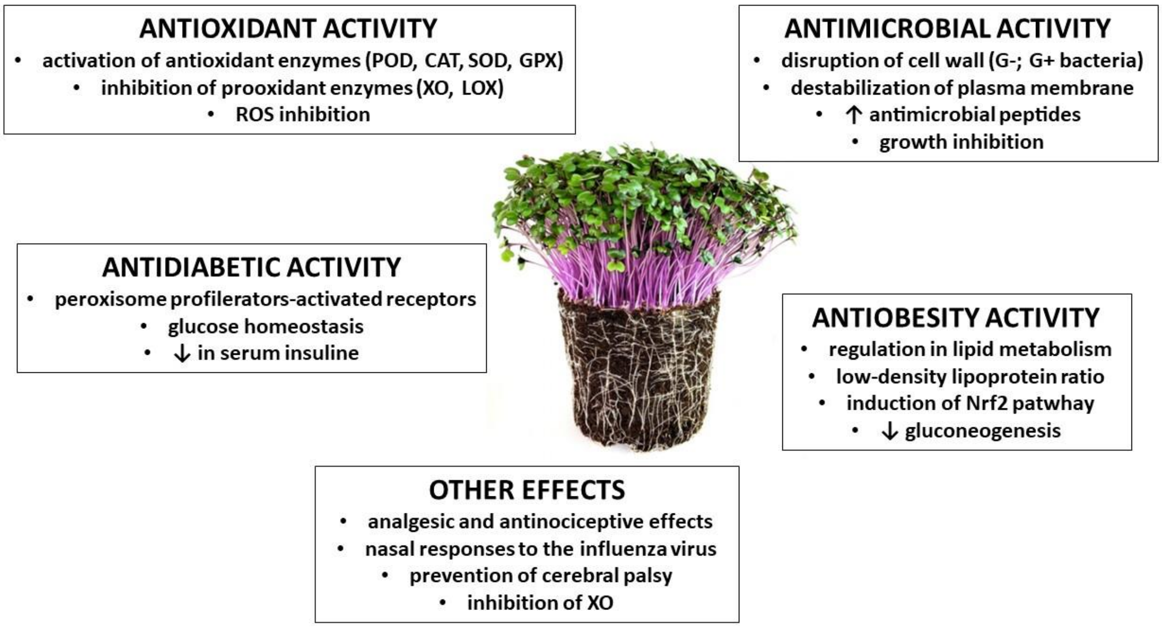

Figure 1 Another biological activity of microgreens. Abbreviations: ROS – reactive oxygen species, XO – xanthine oxidase, LOX – lipoxygenase, POD – peroxidase, CAT – catalase, SOD – superoxide dismutase, GPX – glutathione peroxidase, G- - Gram-negative bacteria G+ -Gram-positive bacteria, Nrf2 - nuclear factor erythroid 2-related factor 2

Anti-inflammatory potential of microgreens

In general, inflammation plays a critical role in the progression of several diseases, including cancer, cardiovascular diseases, and obesity. Higher quantities of phytochemicals, which are prevalent in microgreens, are thought to regulate the immune system and prevent the diseases and health issues stated above. It is well known that the NF-kB pathway has a major role in many inflammatory stimuli. NF-kB induces transcription of pro-inflammatory genes and lunches the synthesis of pro-inflammatory cytokines, concretely tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin 6 or 8 (IL-6, IL-8). A high level of polyphenols and glucosinolates presented in microgreens could inhibit phosphorylation or ubiquitination of kinases, essential in the NF-kB signaling pathway. Overleaf, glucosinolates may potentially inhibit catabolism of nuclear factor kappa light polypeptide gene enhance in B-cells inhibitor alpha and subsequently interfere with NF-kB (**Lopez-Chillon *et al.*, 2019; Subedi *et al.*, 2019**). An important enzyme involved in inflammation is cyclooxygenase-2 (COX-2). Its upregulation can lead to the destabilization of an inflammatory process through prostaglandin production. However, various polyphenols (kaempferol, quercetin, etc.,) presented in microgreens could suppress COX-2 activity (**Subedi *et al.*, 2019**). Previous reports suggest, that AhR also plays an important role in the regulation of the immune system. It has been shown to affect the transcription of interleukin-17 (IL-17). AhR also participates in the differentiation of FoxP3-IL-10- producing type 1 regulatory T-cells induced by interleukin-27 (IL-27). Microgreens have a high concentration of AhR ligands in their phytochemical composition. This suggests that indole-3-carbinol, as well as other polyphenols and glucosinolates, can influence AhR-mediated immunological responses and T-cell regulation (**Wheeler *et al.*, 2017, Subedi *et al.*, 2019**). In addition, **Huang *et al.* (2016)** confirmed, that the consumption of red cabbage microgreen inhibits TNF-α due to the ability to reduce liver lipids, an excess of which has been known to induce inflammatory responses. **Marotti *et al.* (2021)**

found that Glycyrrhize glabra L., also known as licorice, has anti-inflammatory properties that are mediated through suppression of the proinflammatory cascade. **Subedi *et al.* (2019)** revealed evidence of immune system modification through ingestion of broccoli microgreens. Sulforaphane-enriched broccoli inhibited the NF-kB signaling pathway as well as inflammatory proteins such as TNF-α, IL-1β, and prostaglandin E2 (PGE2). Microgreens have a variety of health-promoting properties in addition to anti-carcinogenic and anti-inflammatory properties, as shown in Figure 1.

CONCLUSION

Microgreens are still a relatively new subject, thus research into their nutritional content and evaluation of health-promoting characteristics is at the beginning. Microgreens have a more powerful overall profile (amounts of carotenoids, glucosinolates, polyphenols, and vitamins) than their mature counterparts, and thus there is a presumption of more significant health advantages, including the ability to prevent the development of a wide range of chronic diseases. To this date, anti-cancer, anti-inflammatory, anti-diabetic, anti-fungal, anti-microbial, anti-diabetic, and antioxidant properties have been identified. Current evaluations highlight a range of constraints in the microgreens research area overleaf. External factors, for instance, can have an impact on nutritional and phytochemical compositions. Therefore, there is essential to comply with the proper cultivation, harvesting, and processing approach of microgreens. Other critical points, which could be addressed are: identifying the mechanism of cellular action, determining bioaccessibility, and validation of health-promoting effect in human studies. Indeed, microgreens could be a promising new food source for people who are interested in consuming healthy diets. As a result, more research is required to properly comprehend the importance of microgreens in human health.

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