Nutraceuticals with high antioxidant properties based on fruits and medicinal plants

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<td>Submission</td>
<td>Recently, consuming natural food supplements derived from natural resources has become widespread in many developed societies due to the lack of side effects, low cost, and high antioxidant properties to prevent and treat many diseases. The research and formulation aimed to design a safe and effective medicinal and edible healthy natural nutraceutical with high antioxidant properties and rich mineral elements based on fruits and medicinal plants. Antioxidant, Protein, and inorganic contents of fruits and natural herbal plants were determined to evaluate the contents of active constituents of the meal in milligrams. The results were then used to formulate an edible herbal supplement as a solid oral system. The raw protein content of each ingredient was evaluated through the Kjeldahl method. The contents of the nutrient elements were measured in mg/kg (D.W) by ICP-Mass. All components were mixed thoroughly in some proper percentages, milled, and then dried by heating for 48 hours. The Flowability of the powder was subsequently evaluated using the work index and Hausner ratio. The work index of the sample was 34.3, and because the Flowability was not optimal, aerosol was added to 10 grams of a mixture of 1% by weight of the material. Finally, the working index reached 23, which is desirable. Furthermore, the ingredients were volumetrically encapsulated in OO-sized capsules and subjected to quality control tests. Performing organoleptic assays for controlling the physical and chemical properties of the formulation, the active ingredient was determined in terms of the results of Kjeldahl and inductively coupled plasma Mass spectrometry (ICP-Mass). The results of all control tests, including the weight deviation test, opening time test, and active ingredient dosage test, were conducted for the developed formulation according to credible pharmacopeia. According to the allowable daily dosages, the developed capsule is a proper herbal supplement with very high antioxidants.</td>
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Introduction

People worldwide are enthusiastically looking for new substances that can improve biological function or make them healthier without fearing synthetic chemical side effects\(^1\)\(^-\)\(^3\). These products are called differently: vitamins, dietary supplements, functional foods, “Nutraceuticals,” phytochemicals, bio-chemopreventants, and branded food\(^4\)\(^-\)\(^6\). These terms vary in meaning from country to country, as do the regulations for these substances. Nutraceuticals are biologically active ingredients that can be present in functional or separate food and have a documented, and, at the same time, beneficial effect on human health through metabolic processes\(^7\). Nutraceuticals in the USA, as food additives, also have other terms: therapeutic foods, phytochemicals, preventive substances, pharmaceutical foods (pharma-food), and functional foods\(^2\)\(^-\)\(^3\). The Act on Food Safety and Nutrition lists only three categories: dietary supplements, food for special nutritional uses, and dietary food for special medical purposes.

Nutraceuticals have a specific definition other than those in other categories of food products, such as food, dietary supplements, herbal products, functional foods, and enriched foods. Nutraceuticals include individual nutrients and biologically active phytochemicals, supplements, functional foods, and herbal products\(^7\). Consumer’s desire for food products with health benefits continues to increase\(^8\)\(^-\)\(^11\). Furthermore, people want food with desired health benefits rather than taking medicine separately\(^12\)\(^-\)\(^13\).

In this case, “Nutraceuticals,” often called ‘phytochemicals’ are natural bioactive, chemical compounds with health-promoting, disease-preventing, or medicinal properties\(^5\). The ascribed health benefits of nutraceuticals are countless. This product is claimed not only to reduce the risk of cancer and heart disease but also to prevent or treat hypertension, high cholesterol, excessive weight, osteoporosis, diabetes, arthritis, and macular degeneration, boosting the immunity system probably against Covid-19 (leading to irreversible blindness), cataracts, menopausal symptoms, insomnia, diminished memory and concentration, digestive upsets, constipation, and headaches\(^3\). Nutraceuticals are marketed as a single substance or as a combination of preparations. Herbs can be grouped into Nutraceuticals and have been used as food and medicine for centuries. Plants often contain substantial amounts of antioxidants, including tocopherols (vitamin E), carotenoids, ascorbic acid, flavonoids, and tannins\(^14\)\(^-\)\(^16\), and we suggest that antioxidant action may be an important property of plant medicines associated with diabetes\(^2\)\(^,\)\(^17\)\(^,\)\(^18\).

Since the beginning of human civilization, herbs have been an integral part of society, valued for their culinary and medicinal properties. Herbs have played a significant role in maintaining human health, improving the quality of human life, and serving us with valuable components of seasoning, beverages, cosmetics, dyes, and medicines\(^19\)\(^-\)\(^21\). Both pharmaceutical and nutraceutical compounds might be used to cure or prevent diseases, but only pharmaceutical compounds have governmental sanction\(^2\).

Generally, nutraceuticals should only contain doses lower than those contained in medicines and demonstrate a higher level of safety of use (especially long-term) than typical medicines. Hence, in most cases, importance is compared to the role of vitamins. These substances, however, are not classified as essential ingredients, because their synthesis in a healthy body is usually sufficient for proper functioning. However, in some situations, there is a justified or even indicated dietary supplementation in these ingredients.

The aim of designing the novel formulation was to provide the supplement with high antioxidant properties as well as rich in mineral and trace elements the 8 fruits and natural herbal plants with high antioxidant properties, such as Lonicera caerulea L. (abbreviated as, L.c.), also known as blue honeysuckle or Haskap, and other types of elderberries (the native red and blue elderberries from British Columbia, Canada ingredients were chosen. One of the main ingredients in the novel formula of our nutraceutical is Haskap. Lonicera caerulea L.
(abbreviated as L.c.), also known as blue honeysuckle or Haskap is an emerging fruit\textsuperscript{22,23} with known health benefits\textsuperscript{24-26}. Haskap is native to northern regions of North America, Europe, and Asia, and it has recently gained popularity as a commercial crop in North America\textsuperscript{27,28}. Haskap production is increasing in North America due to the potential health benefits of eating the fruit, and the slightly tart flavor of its blueberries, which are eaten fresh or used in various food products\textsuperscript{28}. Honeysuckle berry is declared one of the richest sources of vitamin C among all the berries. The content can differ depending on factors such as climate, cultivation conditions, genotype, stage of ripeness, and harvesting time\textsuperscript{29-31}.

A study reported that ascorbic acid values of certain honeysuckle berry cultivars can even exceed other fruit generally considered as rich in vitamin C. Oranges contain 31 mg/100 g of ascorbic acid, red currants from 35 to 90 mg/100 g, black elderberries around 30 mg/100 g and raspberries between 16 to 32 mg/100 g\textsuperscript{32}. It has been revealed that the consumption of vitamin C reduces reactive oxygen species levels and triglyceride content, indicating anti-inflammatory and anti-atherosclerotic properties\textsuperscript{33-35}.

As a nutraceuticals category, antioxidants form an ingredient class that is becoming increasingly popular among consumers. From time-tested ingredients such as vitamins C and E to newer ingredients like lutein and lycopene, antioxidants are not a fad. As this paradigm shift from treatment to prevention unfolds, antioxidants represent one of the most recognized and beneficial ingredients among consumers. Consumer interest in antioxidants remains high. Consumption of products containing high antioxidants positively counteracts cancer tumors and inflammatory diseases\textsuperscript{26,36-39}. The main goal of current research and formulation was to design a safe and effective medicinal and edible healthy natural nutraceutical with high antioxidant properties and rich mineral elements based on fruits and medicinal plants in the form of a solid system that can be available for all people around the world.

**Method**

**Using Novel Low-cost Enriched Material Strategy**

In the current study, 8 different fruits and herbal plants in the novel nutraceutical’s ingredients samples, including haskap (\textit{Lonicera caerulea} L.), as well as 3 types of elderberries (the native red and blue elderberries) which were growing wild in the Okanagan, or other parts in north Ontario, Canada selected. The Okanagan Valley is well known for its array of orchards and commercially grown fruit, offering locals and tourists a chance to pick fresh produce from the field\textsuperscript{40}. The Okanagan Valley, sometimes the Okanagan Country, is a region in the Canadian province of British Columbia.

**DPPH Method for Antioxidant Capacity**

**Preparation of DPPH solution**

DPPH solution was composed by taking 7.89 mg of 1,1-Diphenyl-2-picrylhydrazyl (DPPH, purchased from Sigma-Aldrich) weighed on a chemical balance, dissolving with 100 ml 99.5% ethanol (Merck), and finally allowed to germinate at a controlled temperature (25 °C) kept in dark for 2 hr. Fresh DPPH stock solution (200 micromoles) was prepared on each analysis day\textsuperscript{41}.

Haskap berries, redberries, blueberries, blackberries, and other freeze-dry powders were produced in-house. Accurately, 1.00 gm of fruit freeze-dry powder was weighed into 250 ml round bottom flask, and 100 ml methanol was added, refluxed in the water bath at 80 ± 5°C for 30 minutes, cooled to room temperature, and transferred to a 100 ml volumetric flask.

To determine the antioxidant capacity by DPPH, 25 μl of the original methanol extract of each ingredient was poured into a test tube, and 2000 μl of DPPH solution. The resultant solution was shaken in a dark room at room temperature for 30 min, and the absorption at a wavelength of 517 nm was read using an absorbance of each sample was measured at 517 nm with the UV-1800 Shimadzu Spectrophotometer (Shimadzu Cooperation, Kyoto, Japan).
Methanol was the blank used in this assay. The results obtained for the DPPH standard curve were plotted as DPPH concentration vs. absorbance at 517 nm\(^41\).

**Pharmaceutical Tests on the Solid form of final Nutraceutical Product**

**Tests for quality control of Natural products in the Form of Oral Solid Systems**

Tests performed in a raw materials laboratory include titrations, loss on drying, Karl Fischer moisture determination, heavy metals limit tests, and Microbial tests.

**Tests to formulate an edible new herbal supplement by R&D**

All quality control tests on capsules were conducted using pharmacopoeial methods\(^1,2\). The physical parameters of pharmaceutical tablets controlled by IPQC tests are temperature, pressure, moisture content, time, weight, particle size, hardness, loss on drying, disintegration time, color, compactness, integrity, etc. On the whole, Chemical composition tests of ingredients, Flowability of The powder Test, Dissolution Controlling, Water Penetration Controlling, Osmotically Controlled Release Systems, and Organoleptic testing of pharmaceutical products (organoleptic assays for controlling the physical and chemical properties of the formulation), were determined to be sure of the target for quality of formulation stage. In-process quality control (IPQC) tests were strongly considered. IPQC aims to produce a perfect finished product by preventing or eliminating errors at every production stage.

The raw protein content of each ingredient was evaluated through the Kjeldahl method, and samples were then heated in an oven at 75 ± 5 ℃ to be dried and reach constant weight. The contents of the nutrient elements iron (Fe), calcium (Ca), potassium (K), magnesium (Mg), zinc (Zn), copper (Cu), Phosphorus (P), and manganese (Mn) were measured in mg/kg (D.W) by ICP-Mass\(^41-45\). All ingredients were mixed thoroughly in some proper percentages, milled, and then dried in an oven at 65 ± 5 ℃ for 48 hours. The Flowability of the powder was subsequently evaluated using the work index and Hausner ratio. The work index of the sample was 34.3, and because the flowability was not optimal, aerosol was added to 10 grams of a mixture of 1% by weight of the material. Finally, the working index reached 23, which is desirable. Once finished with the required evaluations, the ingredients were volumetrically encapsulated in OO-sized capsules (in Fig 1) and subjected to quality control tests. OO-sized capsules were selected as this size has a light ingredient volume; the capsule can handle 430 mg of filling. It can handle 665 mg with a medium volume and 950 mg with a heavy volume. Performing organoleptic assays for controlling the physical and chemical properties of the formulation, the active ingredient was determined in terms of the contents of protein, and the seven elements: Fe, Ca, K, Mg, Zn, Cu, P, and Mn were determined in final formulations as per the results of Kjeldahl and inductively coupled plasma optical emission spectrometry (ICP-Mass).

![Fig 1. Final encapsulating the mixtures of natural ingredients](image-url)
**Results and Discussion**

**Healthy properties of nutraceuticals against health problems (Case Studies Perception of Our Nutraceutical Product)**

The difference between drugs and nutraceuticals is debated among scientists and health authorities. It is generally accepted that nutraceuticals should only contain doses lower than those contained in medicines and demonstrate a higher level of safety of use (especially long-term) than typical medicines.

The expectation of nutraceuticals after the Covid-19 pandemic or a huge number of breast cancer patients leads us to new formulations that should be less expensive, available, and, on the other hand, environmentally green novel formulations. Now, focusing on some fatal diseases such as cancer or Covid-19 is the priority of most countries, even in Canada. The investigation accomplished in a population-based cross-sectional study initiated in the 8 central zones in a Province to compare the effect of diet, and lifestyle on the incidence of Breast Cancer (stages I to III Breast Cancer usually includes surgery; Stage III is divided into subcategories known as IIA, IIIB, and IIIC) in a total study population of 150 Breast Cancer patients participated along with 37 women in the control group at the same time, aged 20 to 69 years, with incident invasive Breast Cancer identified.

We studied urinary Cd levels in 150 women with Breast Cancer (diagnosis age 20 to 69 years). Cd levels trend aggressively with different risk profiles from later-onset disease “stage III”. According to the results, we recommend women older than 40 years, particularly with family Breast Cancer history, to largely avail themselves of a plant-based diet (Nutraceuticals), along with adequate heavy metal ameliorating trace elements e.g. selenium (Nutraceuticals), to drink anti-inflammatory herbal tea (Nutraceuticals) in daily diet, and the most important factor utilizing Nutraceutical formulated by Natural fruits and Herbal plants which is highly enriched by essential mineral trace elements, antioxidants and phytochemicals.

The purpose of this conversation, we'll focus on the nutraceuticals that may prevent cancer and also reduce corona and flu per recent studies published, along with proven biologically active phytochemicals, including phenolic compounds, polyphenols, anthocyanins, flavanones, isoflavones, ellagitannins, ellagic acid, resveratrol, proanthocyanidins, procyanidins, flavan-3-ols, mineral and trace elements such as Selenium, Calcium and Zinc.

**Novel Formulation of Nutraceutical Product**

Our product has the novelty in providing not only Multivitamin-Multimineral supplements but also a great antioxidant which is formulated 100% based on a Mixture of endemic or native fruits and herbal plants [Berries, fruits, and herbal plants in our Formulation with high antioxidant values] (see Fig 2).

**Nutraceutical and supplements market**

The nutraceutical market is currently developing very dynamically. At present, the segment of functional food and nutraceuticals belongs to the most dynamically developing branches of the food industry. The annual sales volume is not in millions but in billions of dollars. Nutraceuticals are biologically active ingredients that can be found in functional foods or separately and have a significant beneficial effect on health through their participation in metabolic processes various scientific organizations, and government agencies, have increased the popularity of nutraceuticals and functional foods in the public sector. Raising awareness of public health, an aging population, escalating healthcare costs, the latest advances in research and technology, changes in government regulations and accountability, global market expansion, nice media, and numerous scientific pieces of evidence contribute to the popularity of so-called “functional food”. Recognition of the functional diversity of food and nutraceutical composition will allow this industry to provide consumers with many new products that can be developed into specialized markets.
The DSHEA [Dietary Supplement Health and Education Act] governs dietary supplements in the United States. The DSHEA (1994), adopted to manage the human nutraceuticals market, does not allow the Food and Drug Administration FDA to consider a new product as a “medicine” or “food additive” if it is covered by the definition of “dietary supplement”, which includes, among other things, all possible dietary ingredients as well as concentrates, ingredients, extracts or metabolites of these ingredients. With the adoption of the DSHEA Act, the definition of nutraceuticals was extended to include vitamins, minerals, herbs and other botanicals, amino acids, and all dietary sub-stances for human use to supplement the diet by increasing total dietary intake to comply with regulations.

**Advantages of the Formulated Novel Product**

The Nutraceutical market is developing dynamically, Our Formulated nutraceuticals possess multiple therapeutic properties. Nutraceuticals belong to the most dynamically developing branches of the food industry. The annual sales volume is not in millions but in billions of dollars. Awareness in public health, an aging population, escalating healthcare costs, the latest advances in research and technology, changes in government regulations and accountability, global market expansion, and media. All components and processes are 100% natural without any side effects (based on scientific studies), Improving psychophysical efficiency.

This is an area of new, recently discovered significance, crucial for the environment, economy and health all over the world, The invented nutraceutical formulation is based on high contents of nutrients which reducing the risk of cancer, boosting immunity systems, Utilizing natural endemic or native ingredients and turning them to the beneficial food supplement which preventing osteoporosis due to the source of natural calcium, magnesium, Iron,Require very simple and low-cost Technology, Focusing in food security, Nowadays, nutraceuticals have received considerable interest due to potential nutritional, safety and therapeutic effects, We
can formulate many nutraceuticals in the forms of tablets, capsules, or powders. All of the nutraceuticals formulated in the form of insoluble dietary fiber (IDF), which includes celluloses, some hemicelluloses and lignins which are fermented to a limited extent in the colon. The number of Formulations of Nutraceutical based on their focuses on medicinal properties could be vary between 20 up to 100 products; regarding to the dried fruits, percentages of herbs. The preservative in our product is natural, too, despite many preservatives that are often added to prevent the growth of bacteria and mold in the gelatin solution during storage. Common preservatives include potassium sorbate and methyl, ethyl, and propyl hydroxybenzoate, while the natural herb we added to the mixture will preserve the product. The current product can be offered in soft gelatin capsules as a 100% green/ Natural product.

Conclusion

There is an increasing awareness of new antioxidants that can be used for specific and targeted health benefits, such as anti-aging and cardiovascular and immune health. Berries have also hit the radar screen in the nutraceutical arena for its antioxidant activity, and people know they historically used not only as food, but also as medicine. The above studies highlight of this novel formulated product reveal high antioxidant property, as well as enriched in mineral elements and high nutritional value. Based on the above-mentioned studies, it can be concluded that due to the purported health benefits of the new product, which has been developed with special active ingredients. The results of all control tests, including the weight deviation test, opening time test, and active ingredient dosage test, were conducted for the developed formulation according to credible pharmacopeia. According to the latest analyses and the allowable daily dosages of different elements, the developed capsule is a proper herbal supplement with very high antioxidant properties for compensating deficiencies in protein, iron, and other necessary elements for the human body. The content of this methodology is for informational purposes only. The content is not a substitute for professional medical advice, diagnosis, or treatment. All authors confirm that when possible (after recording the formulation as a patent), the supplement will be tested by a trusted third party, such as USP, Consumer Lab, or NSF.

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**Author contributions**

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by [Parisa Ziarati]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.