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A comprehensive review on nutraceutical potential, clinical studies and application of beetroot as a source of bioactive compound

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Abstract

Beetroot (*Beta vulgaris*), a renowned root, recognized for its vibrant colour, nutritional value, and culinary versatility. It is rich source of diversified phytochemicals such as betalains, betacyanins, phenolic and flavonoid compounds, enhancing the health value. The enchanting colouration of beetroot is attributed to a unique nitrogen containing pigment betalain. This red and yellow tyrosine-derived pigments garnered significant attention in medicinal word owing to its potential nutraceutical value. Betalains, comprises betacyanins and betaxanthins, are well recognised for their potent antioxidant and anti-inflammatory activities. Phenolic compounds and flavonoids further enhance beetroot's health benefits through their antioxidant, anti-carcinogenic, and cardioprotective properties. This review focuses on variety of biologically active compounds of beetroot, their health promoting effect, mechanism of action and clinical studies The review also highlights on the diverse applications of beetroot in food products. Due to its appealing color and health benefits, beetroot is increasingly utilized in the formulation of functional foods, beverages, and nutraceuticals. The incorporation of beetroot powder, juice, and extracts into various food matrices not only enhances nutritional value but also improves the sensory attributes and shelf life of the products. Future research directions include optimizing processing methods to retain bioactive compounds and expanding the range of beetroot-enriched food products to meet consumer demands for healthier dietary options.

Keyword: Bioactive, Betalains, Beetroot, health benefits, nutraceutical, clinical studies

Introduction

Beetroot (*Beta vulgaris* L.) is a herbaceous biennial plant from the Chenopodiaceae family (Chauhan *et al.*, 2020). It is rich in various valuable compounds such as betaine, glycine, folates, saponins, betacyanin, carotenoids, betalains, flavonoids and polyphenols (Vali *et al.*, 2007). This plant is not only recognized for its colour but also for several medicinal properties such as antifungal, antidepressant, carminative, antibacterial, diuretic, anti-inflammatory, antioxidant (Yadav *et al.*, 2016). Additionally, it also possesses antibacterial and antiviral properties (Reddy *et al.*, 2005). Beetroot's antioxidant properties, derived from the nitrogen pigment betalain, promote consumer health and wellness. Beetroot Additionally, it has been shown to suppress the growth of human carcinoma cells. Beetroot, with a pH of 7.5-8, is an alkaline meal rich in vitamins C, B1, B2, niacin, B6, and B12, while its leaves are high in vitamin A. The nutraceutical potency of beetroot is coiling around betalains and other phytochemicals responsible for reducing risk of some diseases. These major bioactive compounds of beetroots are illustrated under subheadings (Fig 1.)

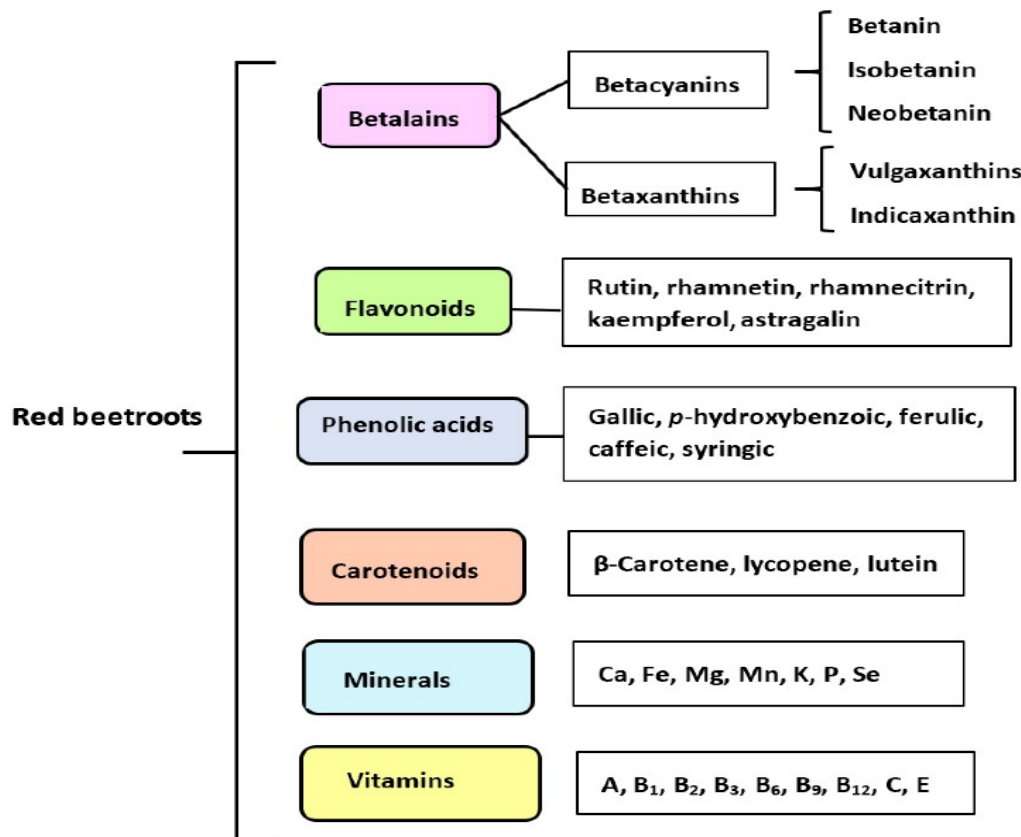


Fig1: Main bioactive compounds present in red beetroots (Sentkowska *et al.*, 2023)

Betalain as major bioactive compound

Betalains, a class of red and yellow pigments composed of aromatic indole derivatives containing nitrogen present in beetroot are soluble in water and may be found in plants such as bougainvillea, Caryophyllales, beets, and fungus like fly agaric (Otálora *et al.*, 2019). Betalains are vacuolar nitrogen containing pigments having a core structure (protonated 1,2,4,7,7-pentasubstituted 1,7-diazaheptamethin system) known as betalamic acid [4-(2-oxoethylidene)-1,2,3,4-tetrahydropyridine-2,6-dicarboxylic acid] (Fig 2).

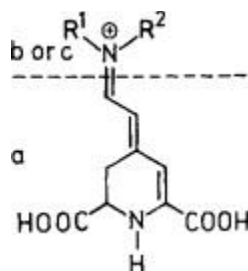


Fig 2: Structure of Betalains (Bohm and Rink, 1988)

Betalains in red beetroot brings it to the top ten vegetables with the highest antioxidant benefits. Betalains exhibit a red color and maintain stability within a pH range of 3 to 7. They are categorized into two primary groups based on their color: beta-cyanins (75-95%) which have a reddish-violet hue, and betaxanthins accounting for (5-25%) which possess a yellow-orange hue (Delgado-Vargas *et al.*, 2000). Chemically, betacyanins consist of cyclo-3,4-dihydroxyphenylalanine and betalamic acid (Milton *et al.*, 2021). The utilization of betalains as natural colorants is becoming increasingly popular in the food sectors due to their non-poisonous, non-toxic, anti-allergic, antioxidant, and anti-cancer properties, which offer significant health benefits (Gengatharan *et al.*, 2015). Effect of betalain and other bioactive compounds on human health are enlisted in table 1.

Carotenoid, a potent antioxidant of beetroot is considered to be crucial for the management and prevention of various diseases. It protects DNA, against free radical damage by scavenging oxygen radicals generated by chloroplasts during photosynthesis (Punia *et al.*, 2023). Beetroot derived carotenoids demonstrated as anti-mutagenic agent, responsible for attenuating several malignancies. Beta-carotene and lutein, two carotenoids of red beets, modulates the immune function and act as powerful antioxidants enhancing functional efficacy of it (Sardana *et al.*, 2018).

Oxalic acid, as a metal ion chelator, stimulates the production of nephroliths and is thus poses a health risk, particularly in persons prone to renal disease (Webb *et al.*, 2008). Consuming red beetroot is beneficial for health due to its bioactive compounds, including betalains, phenolic acids, flavonoids, and antioxidants, which are easily digested and accessible by humans (Vinson *et al.*, 1998).

Table 1: Betalain and other bioactive of Beetroot with their health benefits

Bioactive compounds	Health-Enhancing Properties	References
Betalain	• Anti-diabetic, hypotensive, hepatoprotective benefits, and wound healing • Decreased oxidative hemolysis in red blood cells • Significant anti-inflammatory properties • Anticancer activity against HEp-2 and MCF-7 cells • Antihypertensive and antidepressant effects • Potent antioxidant, anticarcinogenic, and cardioprotective effects	Clifford <i>et al.</i> , 2015 Sulakhiya <i>et al.</i> , 2016 Lechner <i>et al.</i> , 2019
Betacyanin	• Inhibit the growth of human tumor cells • Help prevent bladder and ovarian cancer • Lower blood pressure	Neha <i>et al.</i> , 2018 Rahimi <i>et al.</i> , 2019
Betanin and Isobetanin	• Suppress Alzheimer's disease progression • Inhibit the growth of breast, colon, stomach, and lung cancer cells • Enhance resistance to lipid peroxidation	Lechner <i>et al.</i> , 2019 Calvi <i>et al.</i> , 2023
Phenolic and Flavonoid compounds	• Prevent cancer and reduce cardiovascular diseases with potent antioxidant activity • Inhibit the synthesis of microbial cell membranes	Akan <i>et al.</i> , 2021
Nitrates	• Antihypertensive Effects • Ergogenic Aid to Improve Exercise Performance	Domínguez <i>et al.</i> , 2017 Laskibar <i>et al.</i> , 2021

Betalains as anticancer agent

Cancer, a complicated disease characterized by uncontrolled cell proliferation and infiltration into surrounding cells and organs. Environmental stress, food adulteration, microbial infection, smoking, heavy metal exposure, ionizing radiation, and hereditary factors all contribute to the cumulative accumulation of genetic mutations that cause cancer. Approximately 5-10% of malignancies are caused by genetic abnormalities acquired from parents (Alfawal *et al.*, 2022). Excessive synthesis of reactive oxygen and nitrogen species (RONS) can cause oxidative stress and long-term cell disruption, potentially leading to cancer. Many antioxidant food sources have been tested for their capacity to reduce oxidative stress and scavenge RONS (Lobo *et al.*, 2010).

According to research, beetroot may work as a chemopreventive drug, preventing the growth of multi-organ cancers while demonstrating lower toxicity. Kapadia *et al.*, 1996 began researching beetroot's anticancer efficacy (Kapadia, 1996). Betanin in beetroot can prevent skin and lung cancers in ICR mice treated with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). The decrease rate in lung cancers was very high, reaching 60%. It was the first study to use an animal model to investigate beetroot's anticancer potential. As study progressed, further data emerged to support this claim. He used DMBA, NOR-1, TPA, N-nitrosodiethylamine (DEN), and phenobarbital to create three tumor models in mice. During the experiment, they discovered that the betanin in beetroot dramatically inhibited the growth of tumors generated by chemical carcinogens, suggesting that beetroot was a powerful cancer chemopreventive agent. Furthermore, betanin and betaine isolated from beetroots have been shown to inhibit the proliferation of hepatocellular cells (Lee *et al.*, 2014).

Betacyanins, when combined with vitexin-2-O-xyloside, had a synergistic impact on reducing the growth of human urinary bladder cancer cells (T24), but not on normal human skin keratocytes. When taken concurrently, both drugs boosted pro-apoptotic BAX protein levels while decreasing expression of prosurvival components BIRC5 (survivin) and CTNNB1 (β -catenin) (Scarpa *et al.*, 2016). In another study, doxorubicin plus red beetroot extract demonstrated synergistic cytotoxicity against human pancreatic, prostate, and breast cancer cell lines. Because betanin and doxorubicin have comparable configurations and chemical structures, one probable mechanism is DNA intercalation (Kapadia *et al.*, 2013).

Another research discovered that betanin-enriched beetroot extract caused apoptosis in breast cancer cells. Betanin/isobetanin-enriched concentrate derived from red beetroots suppressed cancer cell multiplication and caused their death, but had minimal effects on normal cells. The

concentrate appears to reduce aggregated cancer cell growth (3D cell culture) by inhibiting cell cycle progression, reducing the amount of G1 cells, increasing S phase, and downregulating cyclin A2 and cyclin B1 levels in breast cancer cells (Nowacki *et al.*, 2015).

Oral administration of red beetroot food color, in the form of commercial dye E162, prevented N-nitrosomethylbenzylamine (NMBA)-induced tumor growth in the rat esophagus. The number of NMBA-induced esophageal papillomas was reduced by about half in rats given the meal color compared to controls. Furthermore, beetroot treatment decreased inflammation and angiogenesis while increasing apoptosis. Chemoprevention with red beetroot appears to entail a decrease in cell proliferation, angiogenesis, inflammation, and apoptosis. Because red beetroot contains betanins, these benefits may be achieved via suppression of oxygen radical-induced signal transduction (Lechner *et al.*, 2010).

In another investigation, oral betanin treatment in Institute of Cancer Research (ICR) mice reduced PMA-induced promotion of mouse skin cancers and glycerol-induced promotion of lung tumors as compared to controls. Betanin, in the form of beetroot extract, dramatically reduced tumor multiplicity and burden in two rat lung tumor models. Following immunohistochemistry analysis, betanin was found to decrease angiogenesis and promote apoptosis in treated mice and human cancer cells in vitro (Zhang *et al.*, 2013).

Table 2: Cancer-preventing effect of betalains

S. No	Compound	Experimental model	Dose and treatment period	Cancer protective actions	References
1.	Betanin (red beet extract diluted with dextrin; CAS-Nr.: 7659-95-2)	- Comet test using HT-29 cells - Nrf2 dual luciferase reporter gene assay in Huh7 cells - Huh7 cells express the antioxidant enzyme heme oxygenase-1 (HO-1). - GSH levels in Huh7 cells.	15 μM betanin (14 h) 1-15 μM betanin (24 h) 25 μM betanin (24 h) 1-15 μM betanin (24 h)	- Prevents DNA damage - Activates Nrf2 at 15 μM betanin. - Increased HO-1 protein concentration - Promotes cellular GSH.	Esatbeyoglu <i>et al.</i> , 2014

2.	Betanin (ABCR GmbH & Co. KG)	- In vitro DNA damage mediated by H₂O₂ in isolated human neutrophils and Caco-2 intestinal cells - Caspase3 activity	200 µM (24 h) 200 µM (24 h; neutrophils) 20-200 µM (24 h; Caco-2 cells)	- Betanin lowers DNA damage induced by H₂O₂ by approximately 14%. - Improves caspase-3 activity in neutrophils and Caco-2 cells. - Promotes apoptotic death of cells.	Zielińska-Przyjemka <i>et al.</i> , 2016
3.	Beetroot-carrot juice	Case study of a patient suffering from B-cell chronic lymphocytic leukemia (B-CLL)	330 mL of fresh raw beetroot-carrot juice orally (beetroot 200 g + carrots 250 g) 2 h before breakfast 6 times/week for 1.5 month 330 mL of fresh beetroot-carrot juice 6 times per week, in combination with the chlorambucil protocol 8 mg/day for 37 days	- Reduces leukocyte and lymphocyte count. - Lowered uric acid levels, higher appetite, and improved fatigue. - Reduces leukocyte, lymphocyte, and reticulocyte numbers. - Improves kidney function. - Improves blood urea, serum creatinine, and uric acid levels while decreasing LDH levels.	Shakib <i>et al.</i> , 2015
4.	Betanin extract (betanin)	VC and B(a)P-induced lung tumors in mice	5 and 100 µg/mL betanin in drinking	Reduces tumor multiplicity and load.	Zhang <i>et al.</i> , 2013

	(TCI America)		water for 20 weeks	• Activates caspase-3 • Inhibits angiogenesis	
5.	Beetroot extract (betanin) (TCI America, Portland, OR, USA)	• Mouse skin and lung carcinogenesis model	• 0.0025% (w/v) betanin in drinking water for 20 weeks	• Suppresses TPA-induced growth of mice skin cancers. • Decreases 60% of lung cancers.	Kapadia <i>et al.</i> , 1996
6.	Betavulgarin isolated from beetroot	• Breast cancer cells (MDA-MB-231 and MCF-7)	MDA-MB-231 ($\geq 100 \mu\text{M}$) and MCF-7 cells were treated at $\geq 50 \mu\text{M}$ for 24 hours	• Inhibits proliferation, migration, colony formation, and mammosphere development. • Reduces CD44+/CD24- subpopulation size and self-renewal-related gene expression, including c-Myc, Nanog, and Oct4. • Restricts the Stat3/Sox2 signaling pathway and causes breast cancer stem cell death.	Liu <i>et al.</i> , 2020
7.	Beetroot extract (diluted with dextrin)	• Trypan blue cell viability assay in human pancreatic (PaCa), breast (MCF-7) and prostate cancer (PC-3) cell lines	0.29-290 $\mu\text{g/mL}$ after 72 hours.	• When combined with doxorubicin, it has synergistic antiproliferative actions • Reduces IC50 values by over 40%.	Kapadia <i>et al.</i> , 2013

8.	Betacyanins purified from beetroot	Sulforhodamine B (SRB) apoptosis marker test in bladder cancer cells (T24) and normal human skin keratinocytes (NCTC 2544).	50 µg/mL at 24, 48 and 72 h	- Betacyanins and vitexin-2-O-xyloside limit the multiplication of tumor cells, but not normal ones. - Activates apoptotic markers in tumor cells.	Scarpa <i>et al.</i> , 2016
9.	Betanin and betaine isolated from beetroot extracts	MTT assay in HepG2 cells	0-200 µg/mL 800 µg/mL	- Reduces cell growth in a dose-dependent manner. - Hinders HepG2 cells by 25%.	Lee <i>et al.</i> , 2014
10.	Beetroot juice (79.3 mg/100 mL betaxanthins and 159.6 mg/100 mL betacyanins	Phase I and phase II enzymes, DNA damage and NDEA-induced liver injury model in rats	8 mL/kg/day for 28 days.	- Decreases biomarkers of liver impairment, such as ALT, SDH, GGT, and bilirubin. - Reduces DNA damage caused by the NDEA therapy.	Krajka-Kuźniak <i>et al.</i> , 2012

Application of Betalains as Nutritional Enhancers in Diverse Food Products

Beetroot-based products has long been prominent in the nutraceutical and therapeutic food industries. The use of them as functional compounds in various food items is becoming more widespread mainly attributed to nutraceutical potential of betalain. Beetroot, which is high in nutrients and bioactive substances, has long been utilized in food preparations due to its bright red color. Beetroot may be processed into a variety of types, including pulp, juice, powder, pomace, peels, and extracts, which are then employed for making functional food products enhanced with

various beetroot fractions. The utilization of beetroot in various food products were enlisted in table 2.

Table 3: Beetroot as betalain source in different food products

Sr. no	Food product	Form of beetroot	Findings	References
1.	Biscuits	Utilization of beetroot powder up to 15%	Records indicate that biscuits containing 5% beetroot powder exhibit superior physical and sensory properties.	Srivastava <i>et al.</i> , 2016
2.	Bread	Addition of beetroot powder upto 70%	Bread formulated with 10% beetroot was overall acceptable and demonstrated a high nutritional content.	Cui <i>et al.</i> , 2022
3.	Jelly	500mL of beetroot juice was utilized to develop jellies	Good quality jelly was formed	Chaudhari <i>et al.</i> , 2015
4.	Noodles	Addition of upto 40% of beetroot pulp	Noodles with 30% beetroot pulp demonstrated superior nutritional content, cooking performance, and overall acceptability.	Chhikara <i>et al.</i> , 2019
5.	Candy	Beetroot pomace utilization up to 10%	The phytochemical characteristics of candies were enhanced.	Kumar <i>et al.</i> , 2018
6.	Jam	Beetroot pulp incorporation up to 60%	Beetroot pulp can also be effectively utilized to produce a low-sugar extra veggie jam.	Perumpuli <i>et al.</i> , 2018
7.	Yogurt	Addition of beetroot juice upto 50ml	Adding 5-10 mL of beetroot juice to yogurt enhanced its physicochemical, color, and sensory properties, increased vitamin C and mineral content, and ensured safety	Dhineshkumar <i>et al.</i> , 2016
8.	Muffins	Utilizing beetroot extract as a natural colorant.	Beetroot can be used as an alternative to hazardous synthetic colorants, and it possesses high antioxidant levels.	Shetty, 2020
9.	Dairy free drinks	Fresh beetroot	The probiotic drink showed high antioxidant, total phenol, and flavonoid content, suggesting it as a suitable alternative for those with lactose intolerance or dairy allergies.	Panghal <i>et al.</i> , 2017
10.	Sattu beverage	Addition of Beetroot juice upto 20%	Addition of 5% beetroot juice showed highly acceptable in sattv beverage also enhanced antioxidant activity,	Sharma <i>et al.</i> , 2020

			phenolic, flavonoid, betacyanin, and betaxanthin content	
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Conclusion

The comprehensive review of betalains found in beetroot unveils a plethora of potential health benefits and applications in food products. Betalains, the vibrant pigments responsible for beetroot's striking color, exhibit promising antioxidant, anticancer, and bioactive properties. These compounds not only contribute to the visual appeal of food products but also offer potential health-promoting effects, including cardiovascular protection, metabolic support, and possibly cancer prevention. Furthermore, the versatility of betalains extends beyond their nutritional benefits to their application as natural colorants in various food products. However, despite the growing body of research supporting the health benefits and applications of betalains, there remain gaps in understanding their full therapeutic potential and mechanisms of action. Future studies should focus on elucidating these mechanisms, optimizing extraction techniques, and exploring novel applications in functional foods and nutraceuticals. Overall, the exploration of beetroot's bioactive components, particularly betalains, holds promise for both the food industry and human health, offering opportunities to enhance the nutritional value and sensory appeal of food products while promoting wellness and disease prevention.

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