



Research Article

Kanji Drink: The Tangy Carrot Beverage for Good Health

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Introduction

Kanji is an Indian ready-to-serve fermented drink, particularly popular in North India, especially during festivals like Holi. Fermented foods are a category of functional foods that offer health benefits beyond their basic nutritional value. The diverse Indian food culture provides a wealth of functional foods that can meet our nutritional requirements and offer additional health advantages. The fermentation process not only imparts a unique flavor to the food-stuff, but also provides benefits in terms of good gut health, primarily associated with the probiotic potential of Lactobacilli.

Fermented foods and beverages also contain certain compounds that can serve as prebiotics for beneficial host microorganisms (probiotics), such as various strains of Lactobacillus and Bifidobacterium [1]. Prebiotics help to promote the growth and activity of these probiotic microbes, leading to many health benefits, including improved digestive health, nutrient absorption, and potential immune system support. Probiotic lactic acid fermented foods have become popular around the world due to their promotive health effects. Thus, the identification and incorporation of prebiotics into fermented foods and beverages has become an area of interest for researchers and food producers looking to produce food products with potential health-promoting effects.

Kanji is made using a natural lactic acid fermentation process [2]. It is traditionally prepared with grated or diced Anthocyanin-rich **black carrots** (*Daucus carota* subsp. *sativus*), black mustard seeds (rye) and various condiments and spices for a unique tangy flavor. It has a cultural and traditional significance in some regions, where it is consumed during specific seasons to combat the effects of extreme heat and promote well-being. This unique beverage, having both prebiotic and probiotic components, has shown good diuretic effect, soothing effect on the digestive tract, and hepato-protective actions, testifying to its immense beneficial potential.

Recipe of the beverage

The ingredients used in making the fermented black carrot drink are only a few. These include, black carrots, Brassica nigra (called *raee* in Hindi) seeds, powdered black pepper (*Piper nigrum*), red chilli (*Capsicum annum*) powder, black salt (*kala namak*) and table salt (Figure 1). The Brassica nigra seeds are coarsely ground. The black carrots are peeled and cut into thin, long slices. These diced carrot pieces are immersed in water, the other ingredients are added and the mixture is stirred. The mixture is then placed in sun or sunlight for few days (3-5 days), preferably in a baked mud pot (*matka*), after which the beverage is ready for consumption.



Figure 1: The ingredients used in preparing Kanji beverage. All the ingredients are mixed together in a utensil and kept in sunlight for few days. Traditionally, clay pots are used.

Phytochemical Constituents and Therapeutic Benefits of Kanji

Phyto-compounds contained in black carrots and their beneficial effects

Carrots are rich in phytochemicals and nutrients, and provide the necessary minerals to support the growth of several bacterial strains. Phenolic compounds and antioxidants contained in carrot pulp promote anaerobic conditions and multiplication of probiotic microbes [3]. Nutritional analysis has indicated that Kanji can be a promising alternative approach to probiotic dairy products while serving as an effective plant-based vehicle for novel probiotic strains. According to the Food Safety and Standards Authority of India, it is safe to consume Kanji drinks. With 2% (w/v) sucrose, 1% (w/v) glucose, and 0.8% (w/v) fructose, carrot juice provides an excellent growth medium for probiotics by providing a good source of energy and carbon, mainly from glucose and sucrose. *L. rhamnosus* and *L. bulgaricus* showed a good growth rate in fermented carrot juice [4].

Being a multi-nutritional food source, black carrots are rich in minerals such as calcium, potassium, magnesium, phosphorus, sodium, zinc, and iron [5]. Black carrots are also a good source of

ascorbic acid (vitamin C), which is a vital nutrient for various bodily functions such as collagen synthesis, wound healing, and immune system support. Black carrots contain a higher concentration of flavonoids compared to orange carrots [6]. Furthermore, black carrots are rich in carotenoids, including lutein and beta-carotene [7]. Black carrots have a high content of anthocyanin pigments, a group of phenolic compounds that provide color and are known for their anti-inflammatory, antioxidant, and anti-cancer effects [7]. The major anthocyanins in these carrots are derived from the acylation of cyanidins. Other phenolic compounds including chlorogenic acid, caffeic acid, quercetin, and ferulic acid are also present in black carrots.

Being a cheap source of anthocyanins, which have gained popularity in the food industry as natural alternatives to food colorants, black carrots have received much attention [8,9]. The Anthocyanin pigments have been found to exhibit antioxidant, anti-cancer, anti-inflammatory, antimicrobial, anti-obesity, and anti-diabetic effects [10-12]. Thus, adding black carrot anthocyanins to food products as a coloring agent can promote better health [13,14]. Song et al., 2016 highlighted the antioxidant properties of black carrot sponge cakes. In their study, they observed an increase in total phenolic content and DPPH radical scavenging activity as the proportion of black carrot flour (BCF) was increased in

sponge cake. It was concluded that black carrot sponge cake meets consumer preferences while offering several health benefits [15].

Anthocyanins present in black carrots have been found useful in the prevention of metabolic syndrome through nine bio-mechanisms [16,17]. Poudyal et al. 2010 found that anthocyanin-rich black carrot juice was beneficial for glucose tolerance, liver function, plasma lipid profiles, abdominal obesity, and cardiac fibrosis with anti-inflammatory effects in a rat model [18]. Furthermore, anthocyanins from black carrots have shown good anti-cancerous effects [17,19]. Netzel et al found that Anthocyanin pigments from ethanolic extracts of black carrots exhibited substantial antiproliferative activity against cancer cells [20].

An experiment was conducted by Cho and Chung, 2019 where black carrot cookies were prepared with varying concentrations of black carrot powder (0%, 5%, 10%, 15%). The ingredients, including flour, baking powder, and black carrot powder, were mixed with eggs. In a separate bowl, butter and sugar were creamed and then added to the mixture. Finally, this dough was shaped into cookies and baked. Though there were no significant differences in texture, odor, and taste, however, the phenolic content was found to be increased as the concentration of black carrot powder increased, ranging from 24.0 to 103.5 mg GAE/100g. Furthermore, the addition of black carrot powder improved the cookies' DPPH radical scavenging ability, highlighting their antioxidant potential. It was recommended that 15% black carrot powder be added to the cookie formulation to meet consumer preferences [21].

The phenolic content present in black carrot roots is higher than in other colorful root vegetables [22]. Black carrots contain a substantial quantity of phenolic acids like hydroxycinnamates and caffeic acid [9]. These phenolic compounds aid in amelioration of inflammation by inhibiting inflammatory pathways [23]. The polyacetylene compounds present in black carrots can decrease Nitric oxide production (macrophage-derived NO) by 65%. Polyacetylenes content helps in enhancing the anti-inflammatory effects of black carrots [24]. The phenolic compounds in black carrots also prevent the risk of diabetes, and fermented black carrots are effective in diabetes management and prevention [25]. The phytochemicals found in black carrots have also been associated with reduced risk of cardiovascular diseases (CVDs).

Effect of fermentation in augmenting the therapeutic effects of black carrot

During the fermentation process of this unique beverage, microflora mainly Lactobacilli, play a crucial role in breaking down the sugars in the carrots and producing lactic acid. Fermentation not only imparts a unique flavor but also provides benefits in terms of good gut health and digestion, primarily associated with

the probiotic potential of Lactic Acid Bacilli (LAB). Sowami and Thorat in 2012 carried out a study to analyze the antimicrobial effect of bacteriocin derived from the Lactobacillus growing in fermented carrot kanji [26]. Bacteriocin shows potent bacteriocidal activity against *Staphylococcus aureus*, which is a major cause of food poisoning through toxin-mediated virulence [27]. This inhibitory activity suggests potential application of black carrot kanji as a probiotic. Singh et al. in 2021 compared the therapeutic potency of homemade Kanji and Marketed Probiotic Drink. The study results proved that traditional homemade Kanji possesses a higher probiotic, antioxidant, and antimicrobial profile as compared to the bottled probiotic drink [28].

A study by Park et al. in 2015 demonstrated that carrying out the fermentation process using *Lactiplantibacillus plantarum* or *Aspergillus oryzae* can substantially increase the total carotenoid content of black carrots while also altering the anthocyanin composition [29]. It may be noted that *Lactiplantibacillus plantarum* (formerly called *Lactobacillus plantarum*) is commonly found in many fermented food products as well as anaerobic plant matter. *L. plantarum* was first isolated from saliva. It is a hardy microbe and demonstrates the ability to temporarily persist in plants, and in the intestinal tract of insects and vertebrate animals. *Aspergillus oryzae*, is a filamentous fungus (a mold) used in East Asia, especially Japan, to saccharify rice, sweet potato, and barley in the making of alcoholic beverages. It is also used to ferment soybeans for making soy sauce and miso. The Park study confirmed that fermented carrot beverage has higher bioavailability and bio accessibility of important phyto-constituents, as compared to carrot juice.

In an experimental attempt to explore an alternative way to produce a functional bread type for healthy diet, black carrot fiber (BCF) was used to fortify bread. The quality and antioxidant properties of the Fiber Fortified Flat Bread were evaluated. It was found that there was a regular increase in phenolic content and anti-oxidant activity of both the outer and inner parts of the bread due to BCF level used. The process of making the fortified bread involved collecting black carrot pomace from blended carrots, drying it to 12% moisture, pounding it, and storing it at 4°C. Wheat flour was then mixed with carrot fiber to prepare dough, which was fermented and baked at 250°C for 5 minutes. The experiment's results showed that a black carrot fiber level of up to 2.5 percent is suitable for formulating black carrot fiber-fortified bread, considering its physical qualities [30]. Thus, adding black carrot fiber to flatbread was recommended for boosting its antioxidant content and physical appearance. This approach presents a potential method for producing a functional bread variety for a healthy lifestyle.

Phytochemicals contained in *B. nigra* seeds and their health benefits

Brassica nigra (black mustard) seeds, called *rae* in north India, contain a variety of phytochemicals, including alkaloids, saponins, tannins, and a range of polyphenols such as flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, and isoflavones) and non-flavonoids (phenolic acids, hydroxycinnamates, stilbenes, etc. [31]. The oil extracted from the seeds is rich in major fatty acids such as palmitic, stearic, oleic, linoleic, linolenic, eicosanoic, and erucic acids. The essential oil of *B. nigra* seeds contains components like di-(9-octadecenoyl)-glycerol, octadecadienoyl chloride, and hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. Additionally, glucosinolates such as Sinigrin and mustard oil glucosides are significant biomolecules in *B. nigra* seeds [32,33]. The above phytochemicals contained in black mustard seeds have a range of very useful health effects as given below (Figure 2).

Anticancer Potential of Brassica nigra Seed Extract

Brassica nigra seed extract has demonstrated significant anticancer activity, especially against human non-small cell lung cancer cell lines. The growth-inhibitory effects include reducing the viability and clonogenic survival of cancer cells in a concentration-dependent manner. The extract also induces apoptosis, evidenced by increased caspase-3 activity, and causes DNA double-strand breaks. The cell cycle analysis revealed that the extract arrests cancer cells in the S and G2/M phases. Moreover, it suppresses the migratory and invasive properties of the cancer cells, by modulating the expression of key proteins involved in these processes. In liver tissue, the presence of isothiocyanate in the seed extract plays a key role in anti-proliferative effects, suggesting protective benefits against the development or progression of liver cancer [34,35]. Furthermore, studies have shown that *B. nigra* extract, with sinigrin as a predominant glucosinolate, exhibits notable antiproliferative activity on both non-tumor and tumor cell lines [36]. The addition of myrosinase enhances these effects, correlated with the modulation of Mitogen-Activated Protein Kinases.

Antimicrobial effects of Brassica nigra Seed Extract

The aqueous extract of Brassica nigra seeds demonstrates notable antimicrobial activity. Utilizing the agar well diffusion method, Danlami et al. found that the extract effectively inhibited several harmful bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella para-typhi*, *Pseudomonas aeruginosa*, and

Staphylococcus aureus, with inhibition zones ranging from 7mm to 23mm [37].

Antimalarial Activity

Brassica nigra seed extract exhibited significant antimalarial properties against *Plasmodium berghei* infection in mice. Administering different doses of the extract (100, 200, and 400 mg/kg) showed notable chemosuppressive and chemoprophylactic activities, with higher doses enhancing survival time and weight gain in *Plasmodium* infected mice [38]. This indicates the presence of active compounds in the seeds that could be effective for malaria treatment and prevention. This unique effect could prove to be beneficial in tropical areas like north-west India, where mosquitoes start breeding with the onset of summers around Holi (in March), and proliferate all through the summers. Co-incidentally, the dark maroon coloured Kanji drink is prepared in north India at the time of Holi, since the drink is traditionally associated with this festival of colours.

Antiviral Properties against SARS-CoV-2

Guijarro-Real et al. 2021 found that the methanolic extract of Brassica nigra seeds inhibited the SARS-CoV-2 chymotrypsin-like protease (3CLPro), crucial for the virus's replication [39].

Protective Effects against Cadmium Chloride Toxicity on lungs

An experimental study on Brassica nigra seeds revealed their protective potential against the toxic effects of cadmium chloride (CdCl₂) in female albino rats [40]. Treatment with the aqueous extract of the seeds restored normal blood parameters and mitigated lung tissue damage induced by cadmium chloride, suggesting protective effects against oxidative stress and tissue damage caused by toxic substances.

Anticonvulsant Effects

The anticonvulsant effects of Brassica nigra seed extract were observed in a study using the kindling method in mice. Doses of 75, 150, and 300 mg/Kg significantly reduced the intensity, frequency, and duration of seizures. Additionally, the extract positively affected brain tissue biochemistry, indicating antioxidant properties and suggesting its potential as a treatment for grand mal seizures [41].

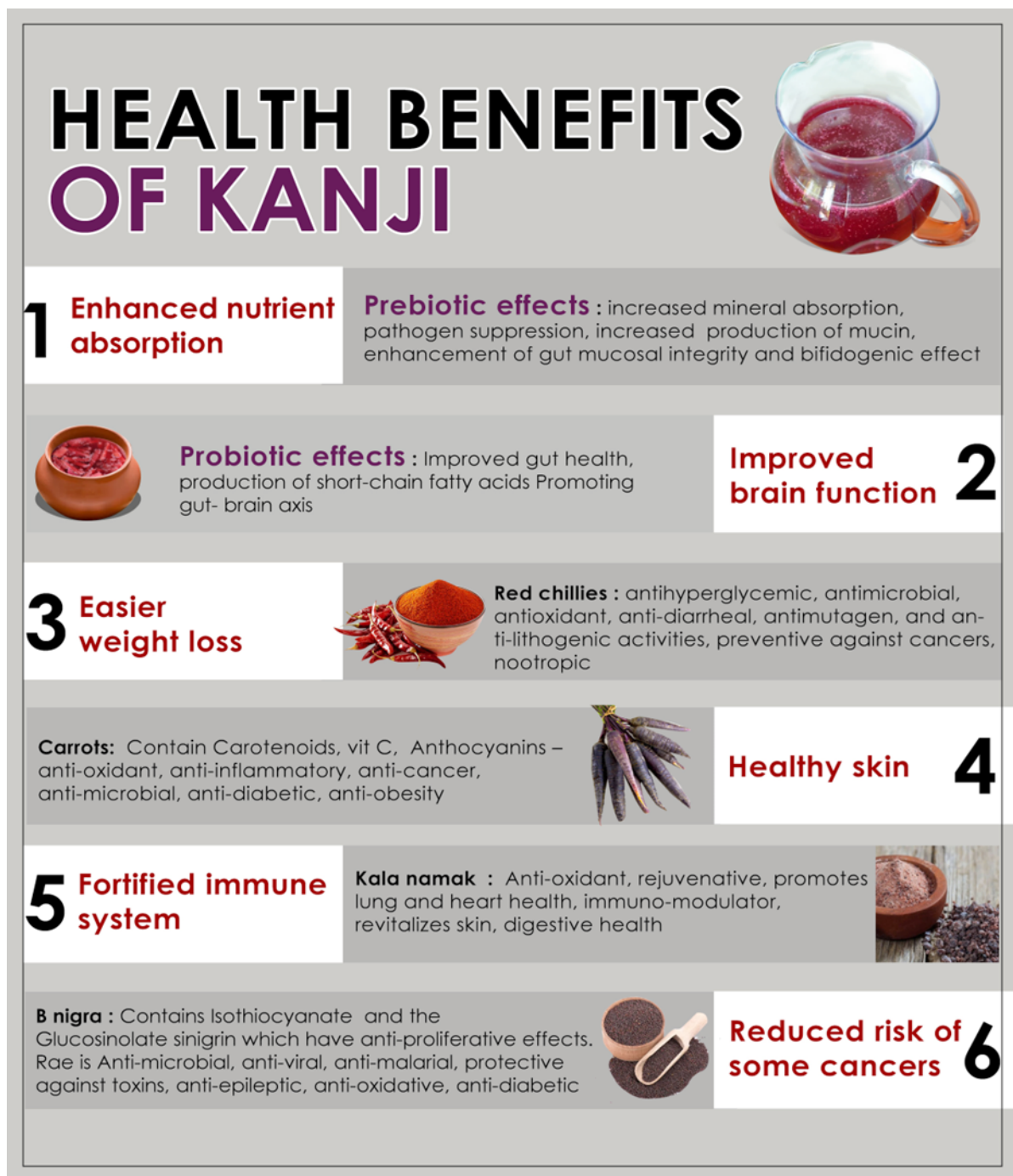


Figure 2: Shows a summary of the health advantages accruing from drinking the fermented beverage made from black carrots. Black carrots contain some very useful phytochemicals that are not present in red carrots. Brassica nigra is the other important component of Kanji, that helps in fermentation, and imparts a sour, tangy taste to the beverage.

Anti hyperglycemic Potential

Brassica nigra seed oil (BNSO) showed significant antihyperglycemic and antioxidant effects in streptozotocin-nicotinamide induced type 2 diabetic rats. Oral administration of BNSO reduced blood glucose levels, improved diabetes-related parameters, and exhibited strong antioxidant properties. This suggests BNSO's potential as a natural therapeutic agent for managing type 2 diabetes and its oxidative complications [42]. In a separate study, various extracts of Brassica nigra seeds were tested on streptozotocin-induced diabetic rats, with the aqueous extract proving most effective in controlling blood sugar levels. It significantly reduced fasting serum glucose levels and limited the increase in glycosylated hemoglobin (HbA1c) and serum lipids, indicating its potential as an antidiabetic treatment [43].

Kala Namak (Black herbal salt)

Black salt is another ingredient used in preparing Kanji. This type of salt is prepared by a special method in brick kilns, adding the three myrobalams to produce the final herbal salt. The phytochemicals contained in the myrobalams, which is known as Triphala in Ayurveda, are thus incorporated into the black salt, conferring several medicinal attributes to this herbal salt [44]. Triphala is a respected Ayurvedic powdered herbal mixture used for various health benefits. It is a combination of two words; 'Tri' meaning 'three' and 'Phala' meaning 'fruit', thus Triphala is a blend of three vital fruits native to the Indian subcontinent. Each of these fruits contributes unique therapeutic qualities, making Triphala a holistic remedy in Ayurveda [45]. Triphala includes Amalaki (*Embllica officinalis*), Bibhitaki (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*).

Embllica officinalis is a potent source of Vitamin C and antioxidants. It promotes immune function, revitalizes the skin, supports vision, and aids in overall rejuvenation [46]. *Terminalia chebula*, often referred to as the "king of medicines" in Tibetan tradition, is renowned for its purgative properties, promotes digestive health, and rejuvenates tissues [47]. *Terminalia bellerica* is known to be effective against diseases related to mucus and congestion. It acts as a natural astringent, detoxifying the body and aiding in the removal of excess water [48]. In addition to these, Babul (*Acacia nilotica*) bark is another key Ayurvedic ingredient used in the production of black salt [49]. Babul bark complements the other herbal components, and their combined presence in specific proportions exalts black salt from a mere seasoning to a wellness supplement rooted in Ayurvedic tradition. The herbal constituents work synergistically with the rock salt and may potentially combat various health conditions, from digestive issues to skin problems.

Red chillies

Red chillies (RC) contain several bioactive phytochemicals, such as capsaicinoids and carotenoid pigments. Chamikara et al., in 2015 reviewed the role of phytochemicals found in chilli pepper in decreasing the risk of several chronic diseases [50]. Phenolic compounds of red chilli like flavonoids, β -catenin, capsaicinoids, glycolipids, and carotenoids showed antihyperglycemic, antimicrobial, antioxidant, anti-diarrheal, antimutagen, and anti-lithogenic activities [51]. The carotenoid pigments like capsanthin, cryptocapsin, and capsorubin exhibited high free radical scavenging capacity [52]. Capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, and nordihydrocapsaicin are the crucial capsaicinoids found in chilli pepper [53]. **Capsaicin inhibits the growth of cancer cells** by activating the apoptosis pathway in human KB (human epithelial carcinoma) cancer cells and by mitochondrial-mediated caspase activation [54]. The protective effect of capsaicin helps reduce the risk of cancers through the inhibition of the NF- κ B pathway. Studies have demonstrated the inhibitory effects of capsaicin on cellular metabolism and autophagy [55,56]. Apoptosis-inducing potential of capsaicin makes it a strong prophylactic agent in the prevention of skin cancers [57]. It was also found to be effective in the prevention of prostate cancer through the down regulation of prostate-specific antigen (PSA), mitochondrial depolarization, and caspase-3 activation [58].

Chopan and Littenberg in 2017 evaluated the role of red chilli pepper on mortality in general, by conducting a population-based study. Study results show that adults who took red chilli peppers had a 13% reduced risk of death [59]. Chilli peppers can promote lipid catabolism in various tissues and organs [60]. The antimicrobial properties of chilli pepper can alter the gut microbiota, improving the **microbiome** [61]. Capsaicin has been proven to protect against heart disease through the regulation of coronary blood flow. Saleh and Teweldemedhin in 2018 explored the health and medical benefits of chilli pepper [62]. They concluded that the therapeutic properties of chilli pepper have a key role in the prevention of heart arrhythmias, cancer, stiff joints, rheumatism, bronchitis, chest colds, and arthritis [63]. Capsaicin can promote the depletion of substance P in the nerve endings, thus reducing pain and tenderness [64].

Bioactive compounds found in red chillies (RC) have been found to have several beneficial effects on **brain functioning** [65]. The carotenoids, lutein and β -carotene have demonstrated a promotive effect on memory [66]. Red chili extracts have also been found to be effective in suppressing major enzymes associated with Alzheimer's disease, such as butyrylcholinesterase,

acetylcholinesterase, and β -secretase [67]. Capsaicin exhibits high free radical scavenging capacity [52], and also helps regulate the mitochondrial enzymes which are beneficial in minimizing the risk of lung carcinoma [68,69]. This finding holds significance for degenerative brain disorders, since increase of Mitochondrial Lipid peroxidase (LPO) levels are associated with damage to brain cells [70,71].

Bukhari et al. in 2020 evaluated the effects of a diet incorporating red chilli powder on the liver function of rats in hypercholesterolemic conditions. A concentration-dependent reduction in LDL and cholesterol was observed when RC powder and standard diet were taken together, thus confirming the anti-atherogenic potential of red chilli powder [72]. Red chilli consumption has been linked to reduced blood glucose levels in rabbit models [73]. Chilli pepper has demonstrated the potential to inhibit key enzymes of glucose metabolism such as α -glucosidase and α -amylase, which contributes to **diabetes control** [74]. Capsaicin reduced glucose intolerance and fasting glucose levels in experimental mice, demonstrating antihyperglycemic and antidiabetic activities [75]. The consumption of RC powder has also been found to be associated with lowered blood triglycerides, increased plasma high-density lipoprotein (HDL) levels and enhanced liver function while reducing glucose, alanine aminotransferase, and glutamic-oxaloacetic transaminase (GOT).

Discussion and Conclusion

Black carrots, which contain a number of useful bioactive compounds, are underutilized due to a limited shelf life. Kanji drink is an effective way to utilize the rich nutritional value and health benefits of these black carrots in the form of a commercial non-dairy-based probiotic drink. Phytochemicals contained in black carrots include carotenoids, anthocyanins, polyacetylenes and phenolics, all of which have immense benefits for health. Many studies have demonstrated the strong free radical scavenging properties of black carrots [76,77]. Black carrot extracts can thus protect against oxidative stress [14]. Furthermore, it has been reported that black carrots possess higher antioxidant capacity than orange carrots [78].

The process of fermentation aids in the release of the phytoactive compounds into the aqueous medium, making the Kanji drink an assemblage for good health. In Indian culture, Kanji is a well-known home remedy for various gastro-intestinal disturbances like anorexia, bloating, and liver disorders. Its probiotic nature is effective in promoting gut health and aiding digestion [79]. The lactic acid bacterial strain, *Pediococcus acidilactici*, isolated from Kanji, is a potential candidate for novel indigenous starter cultures in fermentation studies. *Pediococcus* sp. can support the microbiological safety of the beverage due to its bacteriocin production ability [80]. Bacteriocin is an anti-

microbial agent. Research findings have demonstrated that Kanji LAB strains exhibited promising potential as probiotics. The beneficial probiotic attributes include the resilience to tolerate acid and bile salts, assimilate cholesterol, and execute antimicrobial activities against food-relevant pathogenic bacteria [81]. Additionally, *Lactobacillus plantarum* from Kanji has been identified as a promising source of Vitamin B12 [82].

Brassica nigra (black mustard) seeds, an integral part of black carrot Kanji, exhibit a broad spectrum of therapeutic potential. Their extracts have demonstrated significant anticancer and antimicrobial activity, suggesting potential applications in treating infections. The seeds' extracts also show promising antimalarial properties, effectively combating *Plasmodium berghei* infection in mice. The prophylactic anti-malarial activity of Kanji can prove very beneficial in tropical climates such as northern India, which are endemic regions for malaria. Widespread use of Kanji drink can be an effective public health measure against this deadly disease. Moreover, *Brassica nigra* has exhibited notable antiviral activity against SARS-CoV-2, highlighting its potential in COVID-19 treatment strategies. Additionally, these seeds possess protective properties against oxidative stress and tissue damage induced by toxic substances like cadmium chloride. Furthermore, the antihyperglycemic and antioxidant properties of *Brassica nigra* seed oil and extracts offer promising avenues for managing type 2 diabetes and its complications. Hence, we see that Black carrot Kanji is a phytochemical and prebiotic powerhouse loaded with exceptional nutritional content, which could be highly significant for a diverse range of preventive and therapeutic options.

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