ISSN 1810-3030 (Print) 2408-8684 (Online)
Journal of Bangladesh Agricultural University
Journal home page: http://baures.bau.edu.bd/jbau, www.banglajol.info/index.php/JBAU

Cardioprotective molecule and bioactive compounds of some selected vegetables available in Bangladesh

Md. Arif Sakil[1], Mohammed Arif Sadik Polash[2], Md. Tahjib-Ul- Arif[1], Md. Afzal Hossain[2], Md. Alamgir Hossain[2]

[1]Department of Biochemistry & Molecular Biology and [2]Department of Crop Botany, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ARTICLE INFO

Article history:
Received: 13 March 2018
Accepted: 16 April 2018

Keywords:
Cardio protective molecule, Bioactive compounds, Antioxidant, Diphenylamine, Dietary nitrate

Correspondence:
Md. Alamgir Hossain
(alamgir.cbot@bau.edu.bd)

Abstract

Cardiovascular disease (CVD) is one of the leading causes of death and morbidity as well as imposing a huge economic burden at both country and household level. Upto 90% cardiovascular disease may be preventable if established risk factors are avoided. In this context, dietary nitrate from vegetables was highlighted as a potential candidate for cardio protection. Hence, by supplying dietary nitrate and other bio-active compounds to ease the risk of cardiovascular disease, present research work was carried out. Among the tested vegetables, the highest nitrate content (745 mg/100g) was determined in Indian spinach. In addition, Indian spinach also possesses more chlorophyll (150mg/100g), beta carotene (40mg/100g) and lycopene (34mg/100g) than other tested vegetables in the current study. In case of anti-oxidant content, Indian spinach showed the highest (103mg/100g) vitamin C content. Taken all together, Indian spinach may be a good and cheapest source of cardio protective molecule (nitrate) bioactive compounds to avoid risk of cardiovascular disease.

Introduction

Cardiovascular disease (CVD) is the number one cause of death globally (Shanthi *et al*., 2011). According to the heart disease and stroke statistics, both continue to be the top two killers worldwide. The burden of cardiovascular disease, especially the coronary artery disease (CAD) is increasing at a greater rate in South Asia than in any other part in the world. In Bangladesh, NCDs (Non communicable diseases) represent 59% of the total deaths; Cardiovascular disease was the single-most important contributor, being responsible for 17% of the country's deaths (WHO, 2014). The increasing pattern of cardiovascular disease in Bangladesh may be the result of socioeconomic transition; Bangladesh adopts a Western lifestyle, as well as, changing its dietary pattern. High dietary intakes of saturated fat, trans-fats and salts, and low intake of fruits, vegetables and fish are linked to cardiovascular risk.

Upto 90% of cardiovascular disease may be preventable if established risk factors are avoided (McGill *et al., 2008)*. There are many preventive approaches to reduce the risk of cardiovascular diseases. Among them, one of the best approaches is the supplement of heart healthy diet, such as the dietary supplement of nitrate. In mouth, dietary nitrate is reduced to nitrite by anaerobic bacteria (Duncan *et al.,* 1995). In acidic stomach, nitrite further reduces to nitric oxide (so-called nitrate-nitrite-nitric oxide pathway). This is an important non-enzymatic pathway (Govoni *et al*., 2008; Petersson *et al*., 2009; Webb *et al*., 2008). Interestingly, 'nitric oxide' (NO) can prevent even reverse heart disease and strokes. Being unstable and short-lived, nitric oxide must be renewed continuously in our body. In mammals, spontaneous nitric oxide generation takes place in the vascular endothelium due to the action of nitric oxide synthase (eNOS) on L-arginine (Ignarro, 2002). Unfortunately, most people over the age of 40 have some degree of endothelial dysfunction, which is related to decrease in the nitric oxide production via eNOS route.

Recently, a fundamentally different pathway for nitric oxide generation was discovered in humans that does not involve nitric oxide synthases (Weitzberg and Lundberg, 1998). Successive reduction of dietary nitrate generates nitric oxide, is one of the alternatives (Lundberg and Govoni, 2004). Jonvik *et al.,* (2016) reported that nitrate-rich vegetables beverage effectively increases postprandial plasma nitrate and nitrite concentration and lowers blood pressure to a greater extent than sodium nitrate. Therefore, dietary nitrate is receiving increased attention due to its cardio protective properties. Hypoxia and acidosis conditions favor the conversion of nitrite to nitric oxide. Exercise may induce hypoxia and promote nitric oxide generation. On the other hand, dietary vitamin C makes the stomach acidic which is favorable for generation of nitric oxide. Vitamin C is also an effective reducing agent to convert nitrite to nitric oxide and preventing nitrosamine formation *in vitro* and *in vivo*. (Mirvish, 1983; Mirvish *et al.,* 1972) and vegetables are the rich source of vitamin C. In addition,

vegetables are also the potential source of bioactive compounds, minerals, crude fibre, crude fat, proteins and carbohydrates. Some bioactive compounds (chlorophyll, carotene, lycopene etc.) possess the cardio protective properties.

Considering all, nitrate rich vegetables may be an alternative source of nitric oxide generation in the body and subsequently improve cardiac health. Despite the physiologic roles for nitrate and nitrite in vascular and immune function, nitrate rich vegetables act as healthful dietary component, have received little attention a few decades ago. Therefore, the current research work was designed to determine the content of nitrate, bioactive compound and antioxidant of selected vegetables available in Bangladesh.

Materials and Methods

Mustard, leaf mustard, radish, cabbage, lettuce, Indian spinach, spinach, coriander leaf, water spinach, Amaranth, jute leaf, gourd leaf and turnip were the experimental materials of this study which were collected at 30 days after sowing (DAS) from different cultivated area of Mymensingh, Bangladesh.

Details of experimentation

The experiment was conducted in Food Biochemistry laboratory, Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh from August 2017 to October 2017. Among the listed vegetables six vegetables (Leaf mustard, cabbage, coriander leaf, lettuce, Indian spinach and spinach) were selected by performing Diphenylamine (DPA) field kit test (Roberts, 1994) based on intensity of blue color. Then selected vegetables were analyzed for nitrate (NO_3^-), bio-active compounds, anti-oxidant (Vitamin C). Each sample was analyzed three times.

Nitrate (NO_3^-) content: Nitrate (NO_3^-) content from fresh vegetables was measured by "Improved Diphenylamine-based Spectrophotometric Method" (Omar *et al*., 2013). Nitrate calibration curve (Fig. 1) was prepared by using potassium nitrate (KNO_3) as standard.

Content of bioactive compounds: The leaves of selected vegetables (1 g) were extracted in 10 ml of chilled acetone solution in dark. After centrifugation at 4000 rpm for 10 minutes at room temperature the absorbance of supernatants was taken at 453, 505, 645 and 663 nm wave length. Contents were calculated according to the equation used in Barros *et al*., (2010).

Ascorbic acid content: Ascorbic acid was determined following a procedure previously described by Xaio *et al*. (2012), with 2.6-dichloroindophenl and measuring the content by titrimetric method. The results were expressed as mg of ascorbic acid per 100g of fresh weight.

Data analysis

The collected data were statistically analyzed by using Minitab 17 statistical Computer Package Programmer in accordance with the principles of Completely Randomized Design. Duncan's Multiple Range Test (DMRT) was used to compare variations among the treatments.

Results

Diphenylamine (DPA) field kit test: Intensity of blue color indicates the concentration of nitrate. Accordingly, leaf mustard, Cabbage, Coriander leaf, Indian Spinach, Lettuce, Spinach were selected based on intensity of blue color (Fig 2).

Nitrate (NO_3^-) content: Nitrate content varied significantly in the leaves of selected six vegetables (Fig. 3). Indian spinach showed the highest nitrate content (745 mg) and coriander leaf showed the lowest (352mg) in 100g samples. However, the nitrate content in the leaves of mustard, lettuce, cabbage and spinach was 574, 537, 490 and 355 mg respectively.

Chlorophyll content: Chlorophyll content also varied significantly among the tested vegetables (Table 1). The highest chlorophyll content (150 mg) was found in Indian spinach and lettuce contained the lowest (19 mg). The other vegetables such as, leaf mustard, cabbage, coriander leaf and spinach contained 82, 63, 47 and 73 mg chlorophyll respectively. Among the tested vegetables, chlorophyll a was always higher than that of chlorophyll b.

Table 1. Chlorophyll a, chlorophyll b and total chlorophyll (mg/100g) of the fresh leaves of some selected vegetables at 30 DAS

Vegetables	Chlorophyll a	Chlorophyll b	Total chlorophyll
Leaf mustard	68.70±1.99	15.50±1.85	82.40±2.95
Cabbage	50.40±2.08	12.30±1.27	62.70±2.32
Coriander leaf	45.70±1.56	11.20±1.50	46.90±2.67
Indian spinach	104.30±1.56	46.10±1.79	150.40±2.83
Lettuce	14.40±1.39	4.40±0.31	18.80±2.00
Spinach	58.00±2.89	14.80±1.10	72.80±2.76

Carotene content: In case of carotene, a significant difference was found in the tested vegetables (Fig. 4). The highest amount of carotene was 40 mg in Indian spinach and the lowest was in spinach (7 mg). In addition 25, 20, 17 and 9 mg carotene was found in leaf mustard, cabbage, coriander leaf and lettuce, respectively.

Lycopene content: The variation of lycopene content was statistically significant among the tested vegetables (Fig. 5). The highest lycopene content (34 mg) was found in Indian spinach. The lycopene content was found decrease in leaf mustard, cabbage, coriander leaf and lettuce about 20, 17, 15 and 12 mg respectively. The lowest lycopene (8 mg) was found in spinach.

Ascorbic acid content: Variation of Vitamin C content was statistically significant in the selected vegetables (Fig. 6). The highest vitamin C (102.67 mg) was found in Indian spinach, followed by leaf mustard (98 mg). Cabbage, spinach and lettuce contained 46, 28.33 and 25 mg, respectively. The lowest vitamin C (24 mg) was found in coriander leaf.

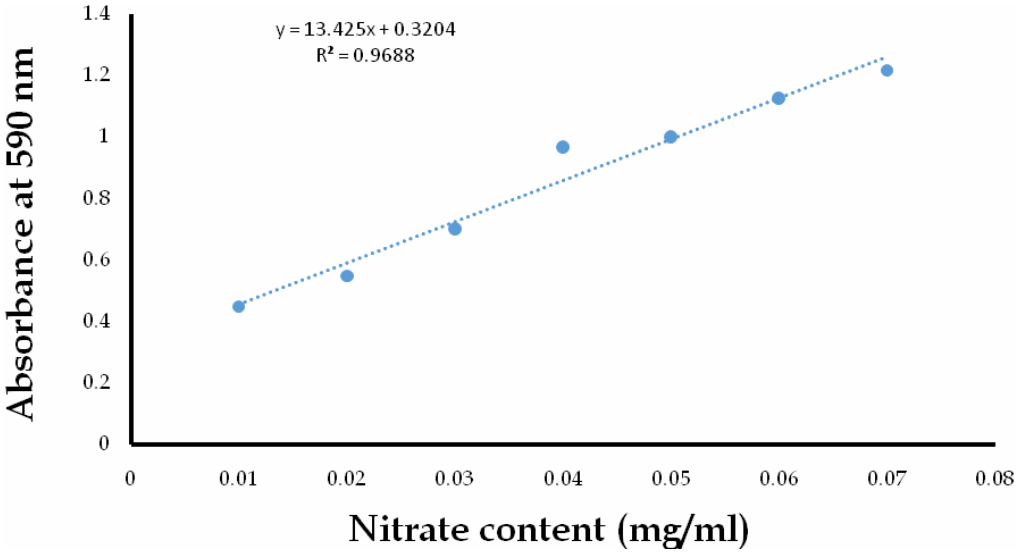

Figure 1: Nitrate (NO_3^-) calibration curve

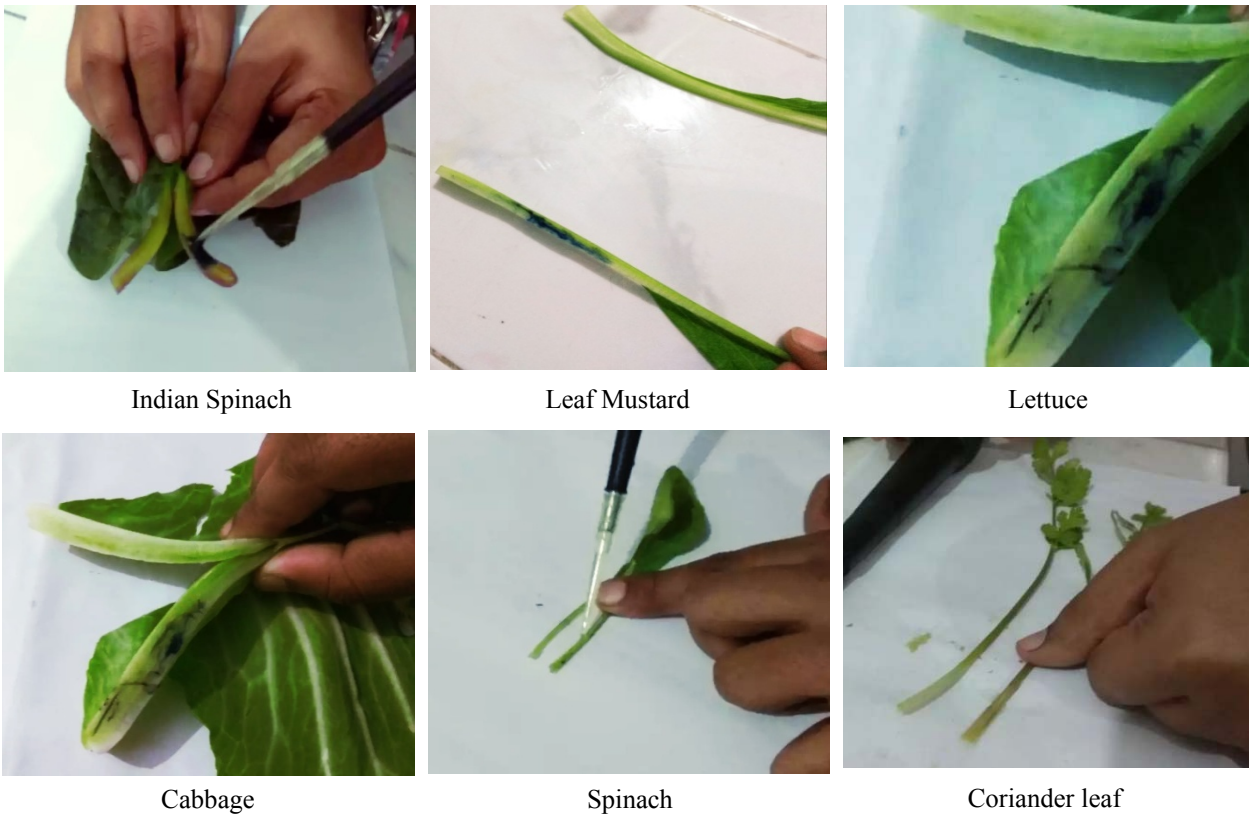

Figure 2. Pictorial view of selected vegetables based on DPA field kit test

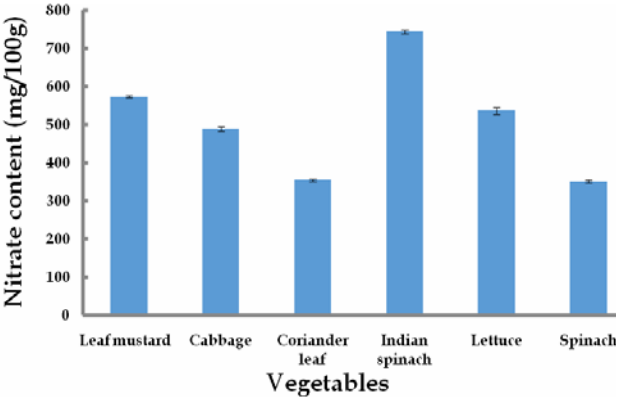

Figure 3. Nitrate (NO_3^-) content of the fresh leaves of some selected vegetables at 30 DAS
The vertical bars represent the mean ±SE (n=3)

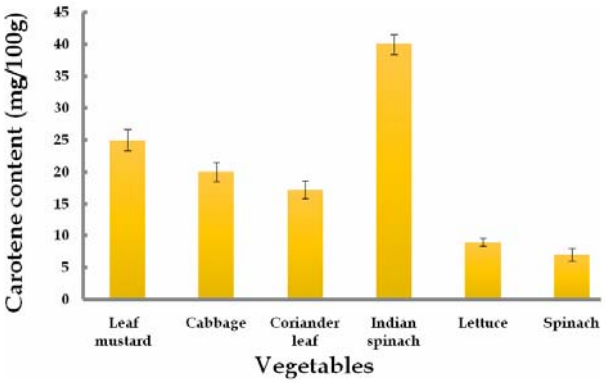

Figure 4. Carotene content of the fresh leaves of some selected vegetables at 30 DAS.
The vertical bars represent the mean ±SE (n=3).

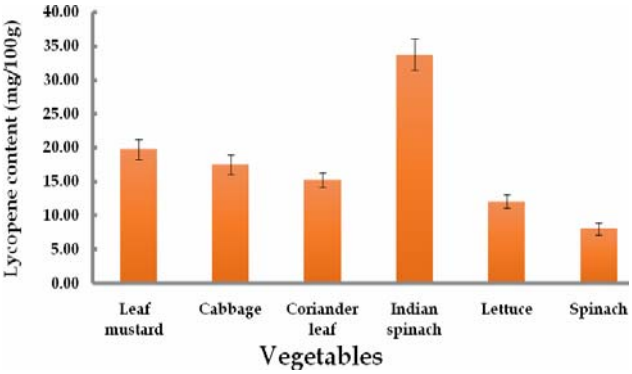

Figure 5. Lycopene content of the fresh leaves of some selected vegetables at 30 DAS.
The vertical bars represent the mean ±SE (n=3).

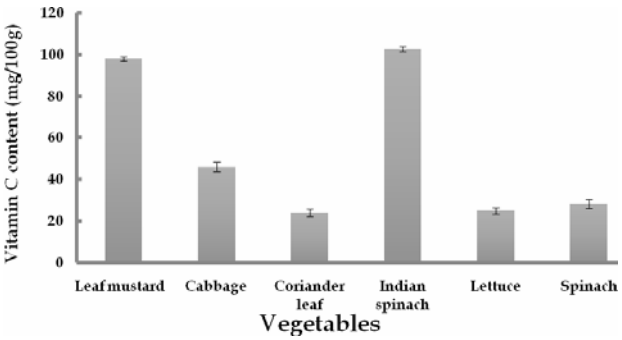

Figure 6. Vitamin C content of the fresh leaves of some selected vegetables at 30 DAS.
The vertical bars represent the mean ±SE (n=3).

Discussion

The vegetable samples were selected by performing diphenylamine (DPA) field kit test (Fig. 2). The diphenylamine field kit test was performed with 13 vegetables that are available in the local markets and which are eaten mostly by consumers. Out of 13 vegetables, 6 were selected based on the color intensity, which is related to nitrate content for further analysis.

In the present study, six selected vegetables contained significant amount of nitrate, of which Indian spinach showed the highest nitrate content followed by leaf mustard (Fig. 2). So, selected vegetables, especially Indian spinach may be an important organic source(s) of nitrate. In general, all cardiovascular disease patients in the globe as well as in our country are prescribed by their physician for taking NO_3^- containing inorganic chemicals/drugs (trinitroglycerine). However, dietary and organic nitrate was also worked as a potential candidate for cardio protection (Lundberg *et al.,* 2006). Nitrate helps in lowering of blood pressure, improvement of endothelial function, protection against ischemia reperfusion injury, reduction in platelet aggregation and improvement of exercise performance with nitrate intake (Joshipura *et al.,* 1999; Joshipura *et al.,* 2001). Therefore, selected vegetables, especially green leafy vegetables contain substantial amount of nitrate (Fig. 2), which may provide the greatest protection from cardiovascular disease in a cheaper and the healthiest way. It is reported that dietary nitrate is not particularly toxic to cattle or infants (6 months age). The potential hazard of nitrate in either food/feed or water occurs after bioconversion of nitrate to nitrite. The nitrite binds with hemoglobin (methemoglobin) and oxidizes ferrous iron in hemoglobin to the ferric state. The resulting compound, methemoglobin, is incapable for carrying molecular oxygen (WHO, 1999, 2007). Thus, nitrate poisoning is created in cattle and infants via blood circulation. However, nitrate toxicity is not included in the present investigation since infants/children are not the target subject in this study.

Vegetables are packed with antioxidants and nutrients that are important for eyes, skin, bones, healthy digestion, reducing inflammation, preventing cardiovascular disease, fighting cancer, and strengthening the immune system**.** Recent report demonstrated that the selected vegetables contain higher amounts of phytonutrients such as chlorophyll, ascorbic

acid (vitamin C), β-carotene (vitamin A), α-tocopherol (vitamin E) and phylloquinone (vitamin K) (Pinto *et al.,* 2015; Xiao *et al.,* 2012; Xiao *et al.,* 2014**).** In the present study, Indian spinach and leaf mustard vegetables possess more chlorophyll than derived from others (Table 2). Interestingly, chlorophyll has anti-inflammatory, antioxidant, and wound-healing properties. Chlorophyll is a good source of antioxidant (Lanfer-Marquez *et al.,* 2005). Chlorophyll is an efficient deliverer of magnesium and helps the blood to carry oxygen to the cells and tissue. Chlorophyll assists in the chelation of heavy metals (Hosikian *et al.,* 2010). Chlorophyll has been studied for its potential in stimulating tissue growth and in stimulating red blood cells in connection with oxygen supply. Chlorophyll may reduce the binding of carcinogens to DNA in the liver and other organs (Ferruzzi and Blakeslee, 2007).

Vegetables are loaded with β-carotene (Brazaityte *et al.,* 2015). In the present study, all tested vegetables contained a good amount of β-carotene and lycopene (Fig. 4 and Fig. 5). Both are beneficial for human health. Beta (β)-carotene (precursor of Vitamin A) helps to maintain the health of our skin, eyes and immune system. It also acts as an antioxidant, protecting our body from disease-causing free radicals. Lycopene is a potent anti-oxidant that reduces the chronic diseases such as cancer and cardiovascular diseases (Kang *et al.,* 2003).

Vitamin C is Abundant in fresh vegetables. In the present study, Indian spinach contained the highest amount of vitamin C (Fig. 6). Generally, fresh edible leaves are a good source of vitamin C, an antioxidant that helps protect our body from the harmful effects of free radicals. Vitamin C prevents the formation of peroxynitrite (ONOO) by scavenging the superoxide (O_2^-) and ensures bioavailability of NO in our body.

Conclusion

Based on results, Indian spinach contained the highest amount of nitrate which may acts as a vasodilator and helps in reduction of blood pressure and cardiovascular diseases after bioconversion of nitrate as NO in our body. Further research is needed to clarify the issue by using human subjects and/or lab animals.

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