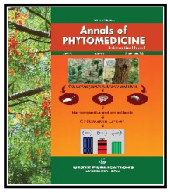
Annals of Phytomedicine 11(1): 53-67, 2022

DOI: http://dx.doi.org/10.54085/ap.2022.11.1.6

Annals of Phytomedicine: An International Journal

http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Review Article : Open Access

Role and importance of spinach fortification in Indian traditional foods

Divya◆

Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara-144411, Punjab, India

Article Info

Article history

Received 25 April 2022

Revised 14 June 2022

Accepted 15 June 2022

Published Online 30 June 2022

Keywords

Disorders

Folic acid

Fortification

Health benefits

Spinach

Traditional foods

Abstract

From the historical time in India, most of the population depends upon locally available traditional foods, but the traditional food are deficient in some essential nutrients which in turn give rise to many nutritional deficiencies, for example folic acid deficiency. Folic acid deficiency mainly targets low income group which may leads to many disorders. Folic acid deficiency can be overcome by the use of spinach fortified traditional foods because spinach is an easily available and economic green leafy vegetable which enhance the nutritive value of food and provide some additional nutrients like folic acid, lutein, calcium and magnesium which may or may not be present in general traditional foods of various region. The folic acid is an essential, organic, water soluble, micronutrient. It is not synthesized in the human body, so it is taken from the external sources in the form of folates. The daily requirement of the folic acid is too low but the effects of its deficiency are long lasting. So, the selection of the right food is important to overcome the deficiency related issues. The main objective of this review is to study the nutritional value of traditional food and by fortification enhancement of nutritional content of traditional food as wella as supply the information related to the chemical composition, natural and synthetic sources, significance way of enhancement and various deficiency related issues of the folic acid, for example spina-bifida, neurological disturbances, cancer, microcytic anaemia, *etc*. It also provides information about the adequate value of intake and daily value per cent on per serving of variety of food items. Current days most of the population consume nutritionally deficient diet or consume fast food which becomes the major problem and the addition of green leafy vegetable in the traditional food can become a defendable solution to combat the folic acid as well as iron deficiency.

1. Introduction

India is well known for its uniqueness in different languages and culture because of people belongs from diverse socio-traditional backgrounds. Different people in different regions and community have different dressing styles and languages also consume different type of food which varies regionally and all regions have their own unique traditional food which are consumed on different occasions (Krishnakumar, 2019). All the traditional foods are affected by the traditions, cultural choices and religion because the food represents the culture of that society (Hoque and Taufique, 2019). There is diversity in consumption and preparation of traditional foods from region-to-region in India and most of the population consume plant based diet as medicine or their nutritional requirements (Majeed, 2017). For example *idli, dosa* with *sambar* are the traditional food consumed in Southern region which are good source of carbohydrates and protein (Ramalingam *et al*., 2019). In Northern and Western region *chapattis, dal, paranthas, puri, curd* are most commonly consumed and are good source of free fibre, zinc, carbohydrates as well as vitamins (Roughani and Miri, 2019). *Fish* with *Rice, rasgolla, gulab jamun* (milk based sweets) are good sources of amino acid, protein, calcium, carbohydrates and consumed as traditional foods in eastern region (Mohanty *et al.*, 2014). Further, more traditional foods have good taste and many nutritional qualities besides various health benefits like they supports immunity, anti-inflammation and brain functioning in the body, but most of these food are also deficient in many vitamins and minerals. Also, naturally occurring folate get destroyed during cooking and processing activities, so to overcome this deficiency in food, we can use the method of fortification which includes the process of addition of the extra nutrients in to the food which may or may not be present in it. Fortification is a strategy in the welfare of public health to enhance the nutrients intake in a population and it is effective in reducing the risk of the nutrient deficiency such as goitre, pellagra, spinabifida, malnutrition, anaemia (Olson *et al.*, 2021). As per the regionally available variety of fruits and vegetables spinach is considered as the most commonly available alternative to be used in fortification of food to combat folic acid deficiency related disorder. Spinach (*Spinacia oleracea* L.) belongs to the family Amaranthaceae and it is an annual plant and grows on the loamy, moist as well as well-drained soil. It is a green leafy vegetable which mainly originated in Persia (Gunnars, 2019). But, its use is quite common among the Indian traditional foods. Spinach leaves are edible and are ovate to triangular in shape, flat and tender, with variability in their sizes (2-30 cm length and 1-15 cm breadth). Flat leaf spinach has become a mainstay in the supermarkets and salad bars because of its mode of eating in raw or partly cooked form. In India, there are three different varieties known as smooth leafed spinach, semi savoy spinach and savoy spinach (Tewani *et*

Corresponding author: Ms. Divya

Department of Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara-144411, Punjab, India

E-mail: divyad7246@gmail.com

Tel.: +91-8307239054

al., 2016). A cartoon character Popeye was attributed for his capacity to eat the spinach and a high iron content was shown in it. But, apart from the iron, spinach was found to be rich supplier of vitamins K,C,A,E,B_2,B_6 manganese, magnesium, folic acid, iron as well as potassium, flavonoids and omega-3 fatty acid. It also consists a rich amount of dietary fibre which lowers the cholesterol level (Roughani and Miri, 2019). It contains antioxidants as well as anti-cancerous agents with good amount of vitamin K and carotenoids (Maeda *et al*., 2010). It has many health benefits including bone protection, eyesight protection and muscular degeneration (Al-Qumboz and Abu-Naser, 2019). Spinach is a fine source of calcium as well as vitamin A which prevent acidosis and night blindness (Tewani *et al*., 2016). In each per 100 g, spinach consists about 22-23 kcal, 91% water, 2.9 g protein, 3.6 g CHO, 0.4 g sugar, 2.2 g fibre and 0.4 g lipids (Gunnars, 2019). So, the fortification of spinach in Indian traditional food can enhance the nutritional quality in present scenario. One of the most widely accepted method for the treatment of disease related to the nutritional deficiency is the supplementation of medicine, but as per the increasing demand and acceptance of the traditional food among the local people, the fortified food can be a better alternative to combat the nutritional deficiency related disorder among the people of every age group and economic class. For example, *idli* is a good source of calcium and carbohydrate but fortification of spinach add some additional benefits of folic acid in it.

Table 1: Importance and nutritional value of traditional food per 100 g

Nutrients	Idli	Dosa	Bajra khichdi	Dhokla	Rasgulla	Makki ki roti	Sarson saag	Pongal
Moisture (%)	-	-	-	-	45-55	-	-	-
Energy (kcal)	33	60	252	34	106	90	64	212
Carbohydrate (g)	6-10	8.3	38-41	6.9	25	14-21	4-7	29
Protein (g)	2-3	0.7	6-12	1	1.7	1-7	1-5	5.5
Fat (g)	0.1	2.6	1-6	0.3	1.8	2-3	4-5	8.2
Folic acid (µg)	3.6	0.9	59	0.1	4.6	4.7	105.5	21.8
Iron (mg)	3.6	0.1	3.5	0	0.1	0.5	2.4	0.8
Vitamin A (µg)	0.8	22.8	91	2.8	66.6	108.1	4776.9	68.8
Vitamin B_1 (mg)	-	-	0.2	-	-	-	-	0.1
Vitamin B_2 (mg)	0	0	0.1	00	0.1	0	0.2	0
Vitamin B_3 (mg)	00.3	00.2	1.4	1.9	0.1	00.4	0.5	0.8
Vitamin C (mg)	-	-	-	-	2	-	25.6	-
Vitamin E (mg)	0	0	0.1	0	0	0.1	1.7	0
Calcium (mg)	4	10.9	36.3	6	105.1	1.3	91.9	13.5
Magnesium (mg)	7.5	9.6	74	0.2	6.6	17.5	57	47.4
Phosphorus (mg)	19.2	17.1	81.4	8.9	69	51.7	26.1	55.2
Sodium (mg)	1.1	0.7	12	201.2	28.8	25.7	50.7	5.6
Zinc (mg)	0.2	0.1	1.8	0	0.2	0.3	0.3	0.9
Fibre (g)	0.3	0.4	2.2-5.8	0	0	1.4-3.0	4.8	2.2
Reference	(Krishna moorthy *et al.*, 2013)	Anonymous, 2020a)	(Anonymous, 2019)	(Anonymous, 2020b)	(Gurveer and Goswami, 2017)	(Anonymous, 2020c)	(Anonymous, 2020d)	(Anonymous, 2021a)

2. Review of literature

2.1 Nutritional importance of traditional food

Traditional foods are the choice for exceptionally nutritious foods which adds functional and biological values to the health of people. They are consumed regularly in urban and rural region of India (Gudulkar *et al*., 2020). These are embedded with the capacity to manage body with blood sugar, immunity, bodyweight, *etc*., the major functional properties includes the chemicals with body healing capabilities, antioxidants, probiotics and dietary fibres. Role of traditional foods is also exclusive in combating the vitamin, proteins and mineral deficiency related disorder because these foods are enriched with all the nutrients. For example in Table 1, the nutritional values of some traditional foods has been mentioned (*idli, dosa, rasgulla, bajra khichdi, makki di roti, sarson ka saag* and *pongal*) (Sarkar *et al*., 2015). *Idli* is a fermented product prepared by steam cooking. *Idli* is a spongy product, white in colour, small in size, round in shape. It is a famous food of south India and is a finest source of minerals, carbohydrates, proteins, phosphorus, *etc*., but it is not a good supplier of vitamins like thiamine, riboflavin, ascorbic acid, vitamin A as well as folic acid. Consumption of one *idli* provides about 2% of the total calorie requirement of the standard adult diet. *Dosa* is also another highly famous food of south India and also consumed highly in the other areas of the country. It is also good

source of the proteins, carbohydrates and fat (Krishnamoorthy *et al*., 2013; Anonymous, 2020 a).

Bajra khichdi is a one of the commonly consumed traditional food of Haryana and Rajasthan, it provides 13 per cent of the daily calorie requirement. It is a rich source of protein for the people who consume vegetarian diet besides rich in magnesium which lowers the insulin resistance that is good for people suffering from diabetes and the *yellow moong daal* used in this prevents the deposition of the bad cholesterol as well as enhance the protein quality (Joshi and Shinde, 2009). *Dhokla* is mainly consumed in Gujarat as probiotic breakfast food. It is prepared by using bengal gram and rice mixture with the help of fermentation process. *Dhokla* provides 2% of daily calorie requirement as well as easy to digest and good source of carbohydrate and folic acid (Sarkar *et al*., 2015). *Rasgulla* is traditional food of eastern part of India and one serving of it provide 5% of total daily calorie requirement. It is a milk based desert and nice source of carbohydrate, protein, calcium, but it is not good for diabetics, heart patients or weight loss, so it is traditional food but not a healthy food (Gurveer and Goswami, 2017). *Sarson da saag* and *makki di roti* are the traditional foods of Punjab and are good source of iron, folic acid besides rich in vitamin A, C, carbohydrate and calcium. *Pongal* is rich source of protein, folic acid, magnesium and thiamine.

2.2 State wise traditional food of India

Indian traditional food represents its culture, cast, religion, group identification (Srinivas, 2011) as well as finest source of antioxidants, probiotics and dietary fibre, so it is reported to have positive effects on human health (Srinivasan, 2010). Table 2 represents different regions of India prefer different type of foods, some people consume vegetarian diet which is plant based diet or some prefer non-vegetarian (animal based) diet (Ananthanarayan *et al*., 2019). The traditional foods consumed in east, west, north and south region as represented in Figure 1 are quite different because every region have their own cooking style, climatic factor and availability of food (Hoque and Taufique, 2019).

Table 2: Traditional food consumed in different Indian states

State	Traditional food
Andhra Pradesh	Rice, dosa, vada and idli
Arunachal Pradesh	Rice, fish, meat, boiled rice cake wrapped in leaves, thukpa
Assam	Fish, chickens, ducks, pigeon, insects
Bihar	Litti chokha, dalpuri, malpua, balushahi
Chhattisgarh	Rice
Delhi	Rajma chawal, paranthas, kulfi
Goa	Sea food, meat, rice, fish
Gujarat	Kesari, dhokla
Haryana	Kadhi, pakora, bajra roti, churma, bathua rayta, bajra khichdi, tamatar chutney
Himachal Pradesh	Lentils, rice, sidu, chukh, rajma and til chutney
Jharkhand	Dhuska, mitha pitha, roti
Karnataka	Bisi bele bath, idli, mysore masala dosa, ragi roti, kootu, kesari bhath, uppittu
Keral	Sadya, idli, appam, puttu, biryani, coconut, sea fish
Madhya Pradesh	Daal bafla, rogan josh, biryani, jalebi, Ladoos
Maharashtra	Rice, masala bhat, pav bhaji, wada pav, aloo poha, shrikhand
Manipur	Black rice, fish, bamboo shoots fermented
Meghalaya	Spiced meat from pigs, fowl, ducks, chicken
Mizoram	Rice, spinach, eggplant, beans
Nagaland	Rice, meat, stewed and steamed vegetable
Odisha	Fish and other sea food, dali, anna
Punjab	Stuffed paratha, dairy product, makki di roti and sarson ka saag, tandoori food
Rajasthan	Daal- baati, raabdi, ghevar, laapsi, kadhi and boondi, daalbati churma, imarti
Sikkim	Dhindo, daal bhat
T N (Tamil Nadu)	Saapadu, dosa, sambar, idli, pongal
Telangana	Sarva pindi, pachi pulusu
Tripura	Mui borok
Uttaranchal	Rabri, arsa, gulthiya
UP (Uttar Pradesh)	Dal, roti, rice, chaat, gulab jamun, kheer, ras malai, pooris
West Bengal	Fish, rice, shondesh, rasgulla
Reference	Hoque and taufique, 2019; Krishnamoorthy *et al*., 2013; Krishnakumar, 2019

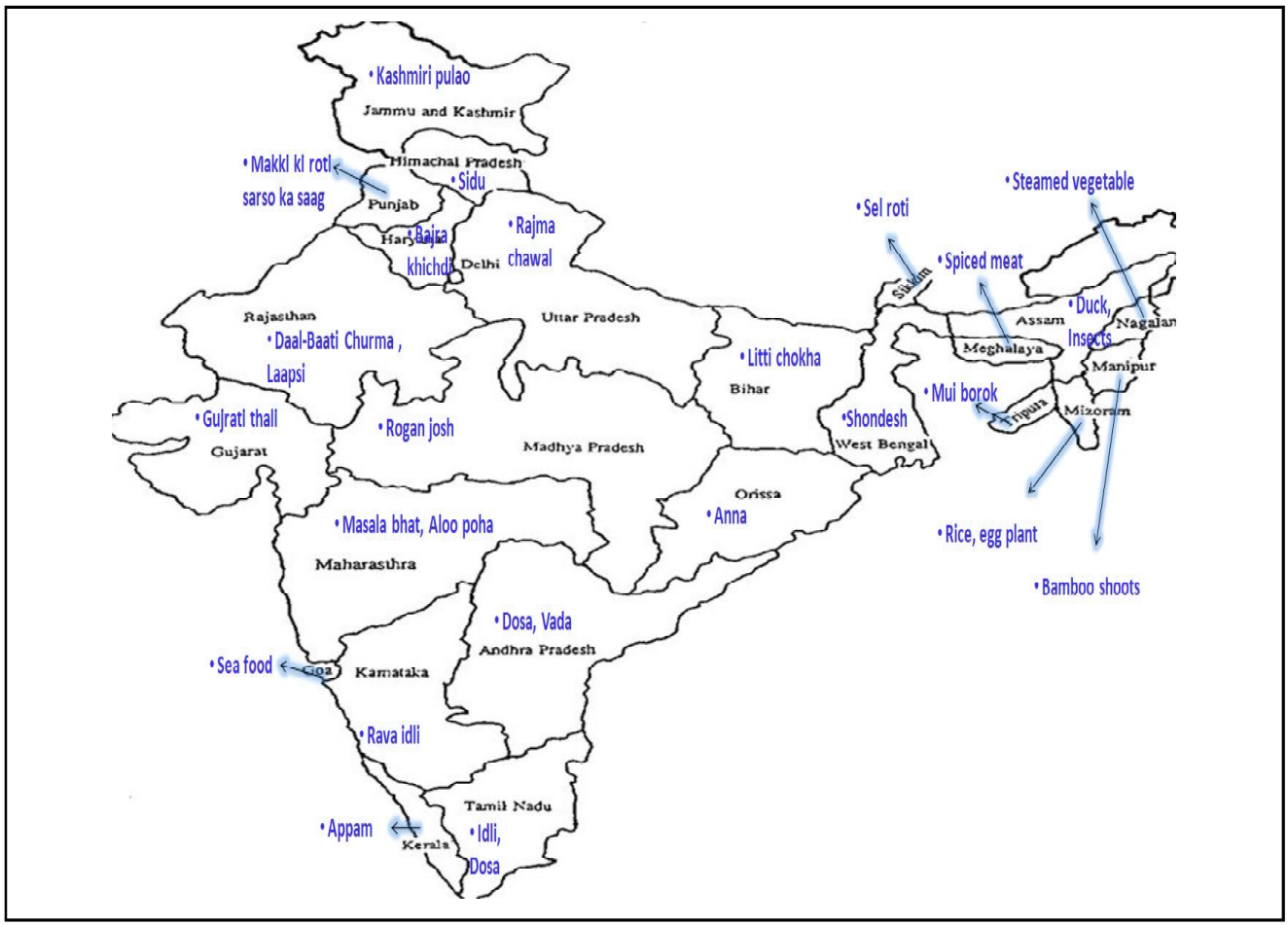


Figure 1: State wise traditional food consumed in India.

2.3 Nutritive value of spinach

Processing is directly going to affect the amounts of macronutrients, vitamins and minerals between raw, cooked, boiled and powdered form of spinach (per 100g). Spinach is a nice source of protein, carbohydrate as well as high in fibre content. Spinach is rich in minerals like iron, calcium, potassium, magnesium, sodium, zinc and manganese. It has high content of vitamins A, E, K, C, B2 and B6. Spinach is also a acceptable food for diabetic as well as obese individuals because of its low fat and high chlorophyll level which helps in digestion (Roughani and Miri, 2019). Spinach also contains vitamin K, fibre and thiamine and it is a plant based source of iron which helps in oxygen transportation in blood and magnesium present in spinach helps in energy metabolism as well as maintaining muscle. Spinach also contains lutein, zeaxanthin, nitrates which are helpful in maintaining eye health to reduce inflammation. Spinach is highly nutritious vegetable which contains high amount of phytochemicals, vitamins as well as minerals and the value of all these nutrients are different in different form of spinach like the calorie value of fresh, boiled or cooked spinach is 20-30 kcal (Holmes and Kennedy, 2000). The powder form of spinach reported to contains 2-3 g of fat which is quite higher than the fresh form. The boiled and cooked form of spinach reported to contains 0-0.8 g of fat; on the other hand, the fresh spinach has 0.4-0.7 g of fat. The high carbohydrate content is reported in the powdered form of spinach (14-36 g) as compare to fresh form (92-3.6 g),boiled form (0-2 g) and cooked form (2-4 g). The powder form of spinach also reported to contain 29-31 g of protein which is quite higher than fresh form (2-2.9 g) and also the boiled (2.2 g) or cooked form (2-3 g) (El-Sayed, 2020). The cholesterol amount is negligible in all groups. The fresh form reported to contain less fibre content which is 1-2.2 g and the boiled and cooked form of spinach also contain the similar amount (2-2.4 g), but the powder form of spinach consists higher amount of fibre (22-24 g). The sugar content is almost same in all groups and the value is between 0-4 g per 100 g. Moisture content is 90-92 per cent in fresh, boiled and cooked form which is quite higher than powder form which contains only 5-6% of moisture (Tewani *et al*., 2016). Spinach is a nutritious and plant based source of iron and the fresh spinach contain 2-11 mg per 100 g; on the other hand, powdered form consists 26-27 mg of iron and boiled or cooked form consist in between the range of 1-4 mg. Fresh form of spinach contains less amount of calcium (73-99 mg) than powdered, cooked or boiled form. The boiled or cooked form of spinach consist the calcium in the range of 73-160 mg/100 g and the powder form contains 2988 mg of calcium. The powdered form of spinach is also rich in potassium and magnesium while the fresh form of spinach consists only 558 mg of potassium and 74-79 mg of magnesium (Ramdas *et al*., 2018; Tewani *et al*., 2016).

Table 3: Comparison of nutritive value of fresh, boiled, cooked and powder form (100g) of spinach on the basis of fresh weight

Nutrients	Fresh spinach (raw)	Spinach powder	Boiled spinach	Cooked spinach
Energy (kcal)	20-23	-	20-22	20-23
Fat (g)	0.4-0.7	2-3	0-0.8	0-0.3
Carbohydrate (g)	2-3.6	14-36	0-2	2-4
Protein (g)	2-2.9	29-31	2.2	2-3
Fibre (g)	1-2.2	22-24	2.1	2.4
Sugar (g)	0-0.4	0-4	0.8	0.4
Moisture (%)	90-92	5-6	91.8	90-92
Iron (mg)	2-11	26-27	1.6	3.6
Calcium (mg)	73-99	2988	160	73-136
Potassium (mg)	558	3334	230	146-470
Magnesium (mg)	74-79	1428	34	24-87
Phosphorus (mg)	45-49	-	28	21-56
Sodium (mg)	75-79	45-70	120	60-70
Zinc (mg)	0-0.5	323	0.5	0-0.8
Copper (mg)	0-0.1	0.718	0.01	0-0.2
Manganese (mg)	0-0.9	4-5	500	0-0.9
Selenium (mg)	1	-	0-0.38	1-1.5
Vitamin A (µg)	524	3752	635-640	524
Vitamin C (mg)	27-28.1	225	5-8	9-10
Thiamine B^+ (mg)	0.1	-	0.06	0.1
Riboflavin B, (mg)	0.2	-	0.05	0.2
Niacin B_3 (mg)	0.6-0.7	-	0.9	0.5
Vitamin B_5 (mg)	0.1	-	-	0.1
Vitamin B^+ (mg)	0.2	-	0.09	0.2
Folate B% (µg)	156-194	1552	140	127-146
Choline (mg)	18-19.3	-	-	19-20
Beta-Carotene (µg)	5626	-	3840	6288
Vitamin E (mg)	2	-	1.7	2-2.5
Vitamin K (µg)	482.9	100.47	-	493.6
References	(Holmes and Kennedy, 2000; Aslam *et al*., 2017; Tewani *et al*., 2016)	(El-Sayed, 2020)	(Hedges and Lister, 2007; Gunnars, 2019; Ramaiyan *et al*., 2020)	(Panda and Shinde, 2017; Ramdas *et al*., 2018)

The fresh spinach contains almost 49 mg of phosphorus but the boiled and cooked form of spinach contains the range of phosphorus between 21-56 mg so, the fresh form of spinach is rich source of phosphorus it also contains sodium in good amount which is almost 79 mg and the cooked form of spinach is also contain about 70 mg of sodium. The fresh form of spinach is not containing zinc, copper and manganese in higher amount and all these nutrients are also present in small amount in other form. Fresh form is also nice source of various minerals, vitamins as well as compounds that may have many health benefits including boost the immune system and digestive system. So, it is better to consume raw form of spinach because the most of the nutrients are affected by the cooking and processing methods.

2.4 Potential health benefits of spinach

Spinach is an important green leafy vegetable and is an annual herb. It is cultivated for its high nutritive value. It consumed in different form for, example raw, dry or cooked form (Raughani and Miri, 2019). Each form of spinach have its own importance and provide many health benefits which includes improvement in eyesight, healthy blood pressure (BP), bone mineralization, iron deficiency (anaemia),

good for pregnant women as well as prevent neural tube defects (NTD) (Liu *et al*., 2004). Spinach is reported to contain high potassium and low sodium level, hence helps in maintaining blood pressure. It is very useful for patients having high BP because the high potassium level decreased the BP. The concentration of folate in spinach is helpful in decreasing the hypertension as well as release the blood vessels to maintain blood flow. The cardiovascular pressure is also decreased which helps in proper functioning (Jovanovski *et al*., 2015). It is reported to be a good source of vitamin K which helps in the retention of calcium in the bone matrix which leads to bone mineralization as well as having minerals like manganese, magnesium, copper, zinc, phosphorous which helps in building strong bones, prevent from osteoporosis and also important for nails as well as teeth (Karpouzos *et al*., 2017). Spinach reported to contain high content of essential nutrients for example, iron which helps in the prevention of anaemia as well as required for energy. It also helps in the oxygen transportation in body because it is a component of haemoglobin (Taneja *et al*., 2020). Spinach is beneficial for pregnant women because it is having many nutrients which are required by developing foetus. It also increases the quantity of milk in lactating mother (Tewani *et al*., 2016). There were several experiments which were performed on human being and the animals for example, rat and rabbits to analyse the benefits of using spinach under certain conditions on the health related aspect of human being like insulin resistance, inflammation, cardiovascular diseases, diabetes, anti-ageing effect, *etc*. There is an experiment performed on elderly women in which they were feed with coconut beverage and spinach, so that the production of antioxidant repetitions were improved (Cao *et al*., 1998). In a certain analytical studies on the rabbits, they were induced with the certain dose of glucose due to which rabbits acquired diabetes, then for the treatment of the diabetes, spinach tea was fed to the rabbits. The tea was processed by adding 10 g of spinach in 100 ml of water and it was boiled for 30 min till the 90% of water get evaporated. This concentrated solution of spinach was fed to the rabbit before 6 h. of glucose monitoring and they were found with normal level of glucose. So, the regulated consumption of spinach tea helps in regulation of glucose level in blood (Kuti and Torres, 1996). Spinach consumption is also helpful in the improvement of motor learning. This is proved in the experiment performed on the aged rats feed with AIN-93 diet supplemented with spinach abstract (Bickford *et al*., 2000) and also the rats feed with NIH-31 rodent diet supplemented with powdered form of spinach shows improvement in learning delay classical eye blink (Cartfort *et al*., 2002). In an another experiment performed by Umar (2007) in which rats were fed with a dose of 0.5 ml of 1,2,3 and 4% of n-hexane in 99, 98, 97, 96 ml of olive oil, respectively, for regular 30 days by Ryle's tube. Due to which, the effect of arsenic get reduced in the body tissues like spleen, liver, kidney, *etc*., hyperlipidaemia Increase in the blood cholesterol level was achieved by feeding the rats with HFCD (high fat cholesterol diet). The high HFCD feed supplemented with 5% dried spinach powder leads to the improvement of the enzymatic antioxidants status in the body, protection of DNA damage was also observed (Ko *et al*., 2014). The diet supplemented with spinach powder also improve the healing of wound and also reduce the time of healing process in diabetic rats (Rahati *et al*., 2016). Another case for the improvement of cardiovascular state under the hypertensive condition was treated with a diet of spinach soup rich in inorganic nitrate among rats. A course of seven days was observed in which improvement in arterial stiffness and blood pressure was observed (Jovanovski *et al*., 2015).

Table 4: Potential health benefits of different form of spinach

Type/ health status	Spinach part/extract used/ dose/time (days)	Potential health benefits	References
Healthy elder women	Coconut beverage (250 ml) with 294 g raw spinach in breakfast for 10 weeks	Improvement in antioxidant capacity of of serum in females and this directly indicates the direct absorption and increased production of antioxidants.	Cao *et al*., 1998
Rabbits: diabetic	Feed with spinach tea (900 ml) 6 h before blood glucose sampling. (10 g fresh leaves boiled in 100 ml water until the volume of water reduced to 90%)	The blood glucose level of diabetic rabbit gets normalized.	Kuti and Torres, 1996
Aged rats : motor learning	Aged rats were fed modified AIN-93 diets supplemented with spinach extract 1000 g/kg (8 weeks)	The spinach feed animals can reach a better level of performance (motor learning task).	Bickford *et al*., 2000
Rat : learning Delay classical eyeblink	Rats are feed with rodent diet (NIH-31) supplemented with dry spinach (0.02%) for 6 weeks	The aged spinach feed diet rats show improvement in eye blink conditioning.	Cartfort *et al*., 2002
Rats: arsenic in tissues	Rats are feed with 1-4% n-hexane (0.5 ml spinach extrat) in olive oil (99-96 ml) by Ryle's tube (30 days)	Treatment with extract decreased the arsenic level to (1.1-1.4 µg/g).	Umar, 2007
Rat : Hyperlipidaemia	Rats are feed with HFCD supplemented freeze-dried spinach powder (5%) for 6 weeks.	Spinach extract may improvement in efficiency of enzymatic antioxidant system it also reduced DNA damage in leukocyte of rat and improved antioxidant status.	Ko *et al*., 2014
Diabetic rats with *streptozotocin* : wound healing	Rats were feed with 10% concentration of 100 g spinach powder in 1 lt. of water for 30 days	Partial weight loss, decreased blood sugar level, improved diabetic-wounds as well as the healing time get shorten.	Rahati *et al*., 2015

Human: Hypertension	Spinach soup rich in inorganic nitrate 500 ml/d for 7 days	The dietary nitrate improve vascular health which decrease arterial stiffness and central BP leads to good cardio vascular health.	Jovanovski *et al*., 2015
Mice: high fat or fructose treated mice.	Mice were feed with nitrate rich spinach powder 60 mg/kg for 28 days	Helps in the regulation of glucose as well as lipid metabolism positively, insulin resistance and endothelial dysfunction markers in mice.	Li *et al*., 2016
Rats: metabolic syndrome	Rats were feed with spinach powder extracted with methanol water, natural antioxidants (200-400 mg/kg, p.o) and (60 mg/kg, p.o) gemfibrozil for 45days	Improve glucose tolerance, enhance the enzymatic process in lipid metabolism.	Panda *et al*., 2017
Rat: spinal cord ischemia-reperfusion injury	A single dose of 20 mg per kg and 50 mg per kg spinach extract were injected intraperitoneally to rats (before 30 min of operation)	After 72 h of spinal cord ischemia rats are found with better hind limb motor function as well as less grey matter injury (spinach extract group).	Farjah *et al*., 2018
Rat: obese	Rats are feed withdiet (high fat) supplemented with spinach powder (5%) for 4 weeks	The spinach diet feed rat shows improvement in weight loss of testes and epididymis.	Iqbal*et al*., 2018
Rat: NAFLD	Feed with pulverized pellets of the standard diet or the high fat diet with freeze dried spinach powder (2.5%, 5%) for 5 weeks	Consumption of spinach decrease cholesterol level in liver and increase MUFA and PUFA, it also increase the level of protein which involve in fatty acid metabolism.	Elvira-Torales *et al*., 2019
Rats : hepatic steatosis	Rats are feed with standard powder diet with spinach powder (12.75%) as well as tomato powder (25.5%) for 35 days	Overweight people suffering from type 2 diabetes can be cured with an improvement rate of 58% for the metabolic regulations when fed with diet supplemented with spinach it reduce 5-8% 0f body weight of rats.	Elvira-Torales *et al*., 2020
Rats with PCOS	Rats are feed with Letrozole (1mg/kg) for first 28 days and then with met formin (150 mg/kg), caraway (3 g/kg) and thylakoid 6 mg chlorophyll orally for 8 weeks	Improved the result of fasting blood sugar, luteinizing hormone, the volume of corpus luteum increased as well as the atretic follicles get reduced.	Sherafatmanesh *et al*., 2020
Obese women with PCOS: cardiometa bolic risk factor	Feed with hypo caloric diet with 5 g/day thylakoid-rich spinach extract powder for 12 weeks	Improvement in the reduction of abdominal obesity indicator, diastolic blood pressure, So the diet shows improvement in the risk factor of CMRF in obese women with PCOS.	Tabrizi *et al*., 2020

Swiss-Kunming mice were treated with high fat as well as fructose diet, then they were feed with nitrate rich spinach powder dissolved in 5% carboxymethylcellulose sodium and 0.4 ml m/v give orally once in a day for 28 days. After that, the mice found with a regulation of glucose as well as lipid metabolism. The study also shows many good outcomes on inflammation on high fructose diet feed mice. It also provides information about the benefits of spinach resist the disease persuaded by high fat as well as fructose (Li *et al*., 2016). One study found that feeding spinach powder when extracted with methanol and water as diet therapy to a female rat suffering from metabolic syndrome in the ratio of 70:30 exposed positive outcomes. Glucose intolerance, lipid metabolism and diabetes were among the common disease to be treated (Panda *et al*., 2022; Panda *et al*., 2017). The effectiveness of low carbohydrate spinach diet was also observed in the improvement of the enzymatic process of lipid metabolism. Spinach extract is also proved beneficial in an experiment on rats with spinal cord ischemia, reperfusion injury. They are injected with spinach extract intra, peritoneal and reported to found with improved hind limb motor function as well as less grey matter injury (Farjah *et al*., 2018). The obese rats are also feed with high fat diet supplemented with spinach powder shows improvement in weight loss of epididymis (Iqbal *et al*., 2018). Diet supplemented with spinach also helpful in NAFLD and also increase the level of protein which involve in the fatty acid metabolism as well as help to treat hepatic steatosis (Elvira-Torales *et al*., 2019). Spinach extract is also good for biological and stereological factors in rats with PCOS and improve fasting blood sugar, luteinizing hormone (Sherafatmanesh *et al*., 2020). In obese women with PCOS, the diet supplemented with 5 g spinach extract per day results in the decrease of cardiometabolic risk factors (Tabrizi *et al*., 2020). Another health benefits are help improve vision, cognitive function, treats anaemia, prevent cancer further more boost immunity (Figure 2).

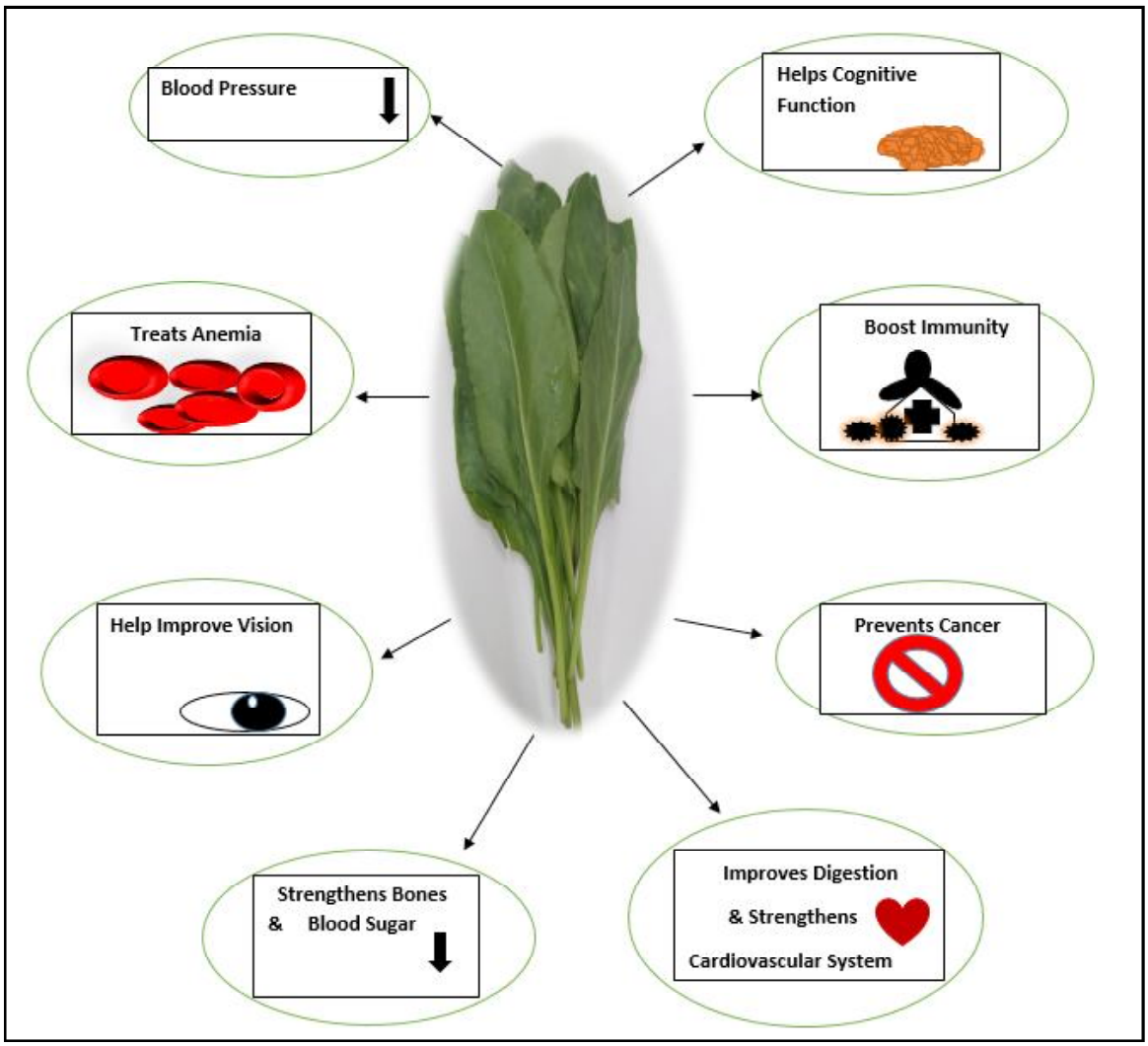


Figure 2: Health benefits of spinach.

2.5 Traditional food fortified with spinach and their nutritional value

In many countries (low and middle-income groups), women and young ones are diagnosed with various kind of micronutrient deficiencies because there diet is not sufficient to full fill the daily needs of micronutrients (Tyagi, 2017). There are some ways to raise the micronutrient level like pharmaceutical supplements, bio-fortification and food fortification. Among all these type of foods, fortification is a good way to raise the micronutrient level (Dwyer *et al*., 2015). So, to address all these deficiencies, traditional foods are supplemented with micronutrients which may or may not be present in it with the help of fortification process (Dary and Hurrell, 2006). There are many traditional foods which are fortified with green leafy vegetable like spinach to address the deficiencies like folate, iron, calcium, potassium, magnesium, zinc and vitamin C (Morelock and Correll, 2008). Fortification of Indian traditional food with locally available green leafy vegetables is suitable, healthy and economic because green leafy vegetables (spinach) are the finest source of micronutrient. From generation to generation, plants are consumed as food as well as used as medicine to cope up with various nutritional deficiencies (Warrier, 2021).The appropriate method of preparation and preservation of spinach can preclude the wastage and enhance the availability in diet throughout the off season (Chadare *et al*., 2019). The preserved spinach in powdered form can be used in different concentrations in the preparation of Indian traditional food of various region where spinach availability is very low. For example, spinach fortified chapatti are prepared with wheat flour containing (1-10%) of spinach powder, but chapatti prepared with 5% spinach powder are very nutritious and it may increase all the nutrients like carotenoids, calcium, vitamin C, Folate (Khan *et al*., 2015; Waseem *et al*., 2021). Spinach fortified biscuits are also prepared with 5, 10 and 15% supplementation of spinach powder in bakery flour, but the most acceptable group of biscuits are made up with 5% supplementation of spinach powder. The nutritional value of these biscuit is also improved after supplementation (Galla *et al*., 2017). Apricot snack bar are also fortified with spinach (powder) to enhance the nutritional value and their storage can be done up to 3 months. The storage time period did not affect the nutritional value of snack food bar (Munir *et al*., 2019). Traditional food, *vada* ingredient like bengal gram, soybean are fortified with fresh spinach leaves in the ratio of 50:50 + 100 and namakparas ingredients like refined wheat flour, semolina are fortified with spinach powder in the ratio of 60 : 20 : 20 (Tyagi, 2017). In India, salted wheat flour noodles are also

consumed as traditional food, but the noodles lost many nutrients during the wheat refinement and lack in many nutrient like dietary fibre, vitamins and minerals. To enhance the nutritive value ,noodles are supplemented with 10, 20 and 30% of spinach paste and the most acceptable supplementation is 20% which results in improved and additional nutritional value with increased protein, fat and ash level (Ramu *et al*., 2016). To increase the folate content of bread (white, whole-grain), fortification is done with 20 g/100 g or 40 g/ 100 g spinach paste and it was reported that the most acceptable group of spinach paste supplementation is 40 g/100 g with increased folate content in bread (López-Nicolás *et al*., 2014). Rice flour pasta is a great source of starch, but is not a good supplier of protein and micronutrients. To improve the micronutrient value, rice flour is supplemented with 03, 05, 07, 10% of spinach powder and the most acceptable group is 5% which improve the nutritional value (Trivedi, 2018). The traditional foods of many regions of India is deficient in folic acid naturally or some content of folic acid get destroyed during the cooking and processing methods, so the lack of folic acid in diet may leads to anaemia as well as folic acid deficiency related disorders. The process of spinach fortification in Indian traditional foods may prevent all these micronutrient deficiency (Crider *et al*., 2011).

2.6 Significance of spinach fortification to combat folic acid deficiency

The basic need of body is most commonly met through the food which is not limited to any single category or region. We consume a wide variety of traditional foods to meet our daily nutritional needs and also supplement the daily diet with additional nutrient with the help of fortification process to address the nutrient deficiency. Folic acid is a man-made form of folate and is required to prevent nutritional anaemia, neural tube defect during prenatal period, skin related disorders, certain cancers (Kondo *et al*., 2009; Liu *et al*., 2004) as well as Alzheimer's disease (Kruman *et al*., 2002). Folate is also essential for pregnant women for proper growth and development of foetus. Inadequate intake of folic acid may leads to serious birth abnormalities like spina bifida and also leads to anaemia in pregnant female (Mitchell *et al*., 2004; Lassi *et al*., 2013). As all these disorders are related to folic acid deficiency, there is a need to take some additional amount of folic acid in natural or synthetic form for prevention. According to the RDA suggested by ICMR, the daily requirement of folic acid for Indians is different for every age group as represented in the Table 5.

Table 5: Recommended dietary allowance for Indians as per ICMR 2020

Group	Particulars	Body weight (kg)	Free folate µg/day
Adult (man)	Sedentary		
	Moderate	65	300
	Heavy		
Adult (women)	Sedentary		
	Moderate	55	220
	Heavy		
	Pregnant Women	50+10	570
	Lactating (0-6 mth)		330
	(6-12 mth)		
New born (infants)	(0-6 mth	5.8	25
	(6-12 mth)	8.5	85
Children	1-3 years	11.7	110
	4-6years	18.3	135
	7-9 years	25.3	170
Boy	10-12 years	34.9	220
Girl	10-12 years	36.4	225
Boy	13-15 years	50.5	285
Girl	13-15 years	49.6	245
Boy	16-18 years	64.4	340
Girl	16-18 years	55.7	270
Reference	(Anonymous, 2020e)		

Folate can be acquired by outside source and gut microbes because it is not innate. Folate is a raw form of folic acid present in sevral fruits and vegetables (green leafy), for example, spinach, fenugreek, brussels sprouts, orange, papaya, legumes (Iyer and Tomar, 2009). For example, per 100 g of vegetables contain different composition of folic acid like spinach (51.0 µg), mint (9.7 µg), cabbage (13.3 µg), amaranth (41.0 µg). All the fruits and vegetable containing folic acid have different daily value (DV) and dietary folate equivalent (DFE) as shown in the Table 6.

Table 6: Dietary folate equivalent per serving (25 g or 1 bowl) and daily value of different food items.

Food	DFE per serving microgram (µg)	Percentage DV
Spinach leaves	131.0	33.0
Peas (black eyed)	105.0	26.0
Rice (white)	90..0	22.0
Brussels sprouts	78.0	20.0
Spaghetti	74.0	19.0
Avocado	59.0	15.0
Fresh broccoli	52.0	13.0
Mustard (greens)	52.0	13.0
Peas (green)	47.0	12.0
Beans (kidney)	47.0	12.0
Turnip (greens)	32.0	8.0
Papaya	27.0	7.0
Reference	(Anonymous, 2021b)	

Table 7: Bioactive compounds in spinach and their health benefits

Bioactive compounds	Therapeutic use	References
Flavonoids	Cancer, anti-inflammatory, antidiabetic, antiobesity, protect cardiovascular system, antidiarrhoea	Bergquist, 2006; Bottino *et al*., 2009; Manzoor *et al*., 2021; Kanabur and
Carotenoids	Eye disease, Anticancer	Reddy, 2014; Singh *et al*., 2018;
Ascorbic acid	Anti-aging, growth and development of healthy bones, teeth, gums	Ballard and Junior, 2019;
Folic acid	Neural tube defect, megaloblastic anaemia	Patel, 2008;
Phenolic acid	Obesity, cardiovascular disease, anti-inflammatory	Krinsky and Johnson, 2005;
Peptides	Lower high blood pressure, anti-inflammatory, prevent formation of blood clots	Ravetti *et al*., 2019; Field and Stover, 2018;
Saponins	Decrease blood lipid, cancer, lower blood glucose	Bento-silva *et al*., 2020;
Alkaloids	Anticancer, antimicrobial, pain relief, antifungal	Cassidy, 2018;
Terpenoids	Antimicrobial, antiviral, antiallergenic, anti-inflammatory	Granado *et al*., 2003;
Tannins	Antibacterial, heart disease, anti-inflammatory, cancer	Craig, 2004;
Defensins	Antibacterial, antiviruses, antifungal	Ruxto, 2004; Gunnars, 2019;
Flavonols	Anticancer, anti-inflammatory, antioxidant	Duthie, 1999; Altemimi *et al*., 2017
Lutein	Cataracts, anticancer	
Betaine	Prevent steatosis, cardiovascular disease, Alzheimer disease	
Omega-3 fatty acid	Depression, anti-inflammatory, cardiovascular disease, behavioural problem	
Zeaxanthin	Eye health	
Nitrates	Cardiovascular disease	
Quercetin	Anti-inflammation, antidiabetic	
Kaempferol	Anticancer, chronic disease	

Along with the natural source, there are many synthetic forms of folic acid and are consumed in the forms of various vitamins like B9. vitamin B9 is a type of synthetic form of the folic acid. It is mainly obtained from the supplements and the fortified food. Many of the foods which are produced on commercial basis like as bread and cereals used in breakfast generally lacks the folic acid. The foods fortified with folic acid which are generally high in the folic acid are actually consisting the synthetic forms of it, as these foods are industrially processed or usually made artificially (Siciñska and Pelc, 2011). The average amount of folic acid in the supplement is about 400-1000 micrograms. The absorbability of the synthetic form of folic acid is about 100 per cent when taken empty stomach and it lowers to about 85 per cent when taken in the form of fortified foods (DeSoto and Hitlan, 2012). High intake of synthetic folic acid may leads to over supplementation issue and cause many health effect like autism. So, the traditional food fortified with spinach is a finest way to amplify the nutritive value of food.

2.7 Bioactive compounds in spinach

A copious consumption of different type of fruits and vegetables provides many health benefits as well as the green vegetable like spinach are good source of bioactive compounds. The bioactive compounds provide many additional health benefits; for example, spinach is a good source of flavonoids which helps against diseases like cancer, diabetes, cardiovascular disorder (Bergquist, 2006). Carotenoid have anticancer property and also provide good eye health (Eggersdorfer and Wyss, 2018).

Bioactive compounds like ascorbic acid have anti-aginge effect and also helps in growth and development of healthy bones and teeth (Ravetti *et al*., 2019). Consumption of spinach provide folic acid which prevent neural tube defect in new-borns and spinach is also good source of phenolic acid which has anti-inflammatory property and prevent obesity. Folate is also essential for a healthy pregnancy. During the prenatal stage, folic acid aids cell formation, production of brain cells, blood cells and DNA/RNA during pregnancy, which helps to prevent serious birth abnormalities to the brain and spinal cord. Folic acid, when consumed in sufficient amounts, diminish the risk of neural tube abnormalities in young one. Simple deficiency of folic acid cause interruption in DNA biosynthesis and due to which neural tube does not close properly and leads to neural tube defect. Folic acid consumption by fortification of spinach in traditional foods decreases the prevalence of NTD (Scott *et al*., 1994). Flavonols also have anti-inflammatory and anticancer property which is found in high number of cases in developing countries (Gezici, 2018). Lutein present in spinach prevent cataract and betaine prevent liver disorder as well as Alzheimer disease. The consumption of omega-3 fatty acid in spinach prevent depression and behavioural problem, it also have anti-inflammatory property (Ruxto, 2004). Zeaxanthin promotes good eye health and the nitrates prevent from cardiovascular disease. The quercetin and kaempferol have anticancer as well as anti-inflammatory property and also prevent from chronic disease (Gunnars, 2019).

2.8 Antimicrobial activity of spinach

Spinach leaves are the appropriate source of minerals, antioxidants and antimicrobials in human diet. As a result, spinach leaves are beneficial to one's health and have been used to treat human disorders since antiquity (Altemimi *et al*., 2017).The extract from all the plants has antimicrobial activity (Rex *et al*., 2019). Spinach is used as a material for food fortification because it has a lot of beneficial health effect which prevent from various pathogenic microorganisms as shown in Table 8. Various microorganism like *Streptococcus mutans, S. salivarious, Salmonellatyphimurium, Pasteurella multocida, Escherichia coli, Lactobacillus bulgaricus, Micrococcus luteus, Proteous vulgaris, Klebsiella pneumonia, Staphylococcus aureus* and *Staphylococcus epidermidis* almost all the microorganisms growth were inhibit by various spinach extract.

Table 8: Antimicrobial activity of spinach against various micro-organism

Part used	Extract preparation	Micro-organism	Antimicrobial activity (Inhibition zone mm)
Leaves of spinach, neem and tulsi	In the extraction process shade drying was done (1 month) and dried material converted in coarse powder and microwave extraction was done (ethyl acetate). For the preparation of herbal mouthwash spinach, neem and tulsi extract (4ml + 2ml + 2ml) were dissolved in 10 ml distilled water and peppermint and methyl paraben added as preservatives (Patil *et al*., 2020).	*S. mutans* *S. Salivarious*	18 20
Fresh spinach	Fresh spinach crushed and soaked in ethanol (24 h) and blended then the extract was filtered and centrifused at 400 rmp (30 min). Extract was concentrated in evaporator (40°C) and stored for further analysis (4°C) (Iqbal *et al*., 2012).	*S. aureus; S. typhimurium* *E. coli; P. multocida* *L. bulgaricus; M. luteus* *K. pneumonia; P. vulgaris* *S. epidermidis*	4.3 10.0 6.0 3.3 4.66 2.0 4.0 5.3 6.6
Spoiled spinach	Spoiled spinach crushed and soaked in ethanol (24 h) and blended then the extract was filtered and centrifused at 400 rpm (30 min), concentrated in evaporator (40°C) and stored for further analysis (4°C) (Iqbal *et al*., 2012).	*S. aureus* *S. typhimurium* *E. coli* *P. multocida* *L. bulgaricus* *M. luteus* *K. pneumonia* *P. vulgaris* *S. epidermidis*	6.66 7.3 14.3 3.66 3.33 3.0 4.33 4.0 6.0

3. Conclusion

Historically, India is relay on traditional foods because as per the easy or local availability and economical nature. The traditional foods lack many nutrients due to which people are affected from generation-to-generation with many serious micronutrient deficiency disorders like folic acid deficiency which may cause NTD, spina bifida, anaemia, *etc*. People of every age group which includes neonates, adults, pregnant females and geriatric were suffering from nutritional deficiency because there regionally and traditional foods are not sufficient to fulfil their nutritional requirement. Deficiency related disorders are not genetically inheritable but still people of every generation are suffering from it as they are transferring their cultural habit of consumption of traditional food through one generation to other. As all the people are not socially or economically aware about supplementation of food with essential nutrients which becomes a major cause for persistence of these disorders in their body. Fortification of traditional foods improve their nutritive value as well as help in to combat with many disorders. In order to cope up with micronutrient deficiencies like folic acid, fortification of spinach in Indian traditional food in fresh, dried or cooked form is a better manner to enhance the nutritional value as well as reduce the risk of NTD of spina bifida in new bourn. So that, if the people aware about the supplementation of food with the help of fortification process, the cases of many nutritional deficiency related disorders get reduced and the health status of people of new generation can also improve.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Al-Qumboz, M. N. A. and Abu-Naser, S. S. (2019). Spinach expert system: Diseases and symptoms. International Journal of Academic Information Systems Research, **3**(3):16-22.

Altemimi, A.; Lakhssassi, N.; Abu-Ghazaleh, A. and Lightfoot, D. A. (2017). Evaluation of the antimicrobial activities of ultrasonicated spinach leaf extracts using rapd markers and electron microscopy. Archives of Microbiology, **199**(10):1417-1429.

Ananthanarayan, L.; Dubey, K. K.; Muley, A. B. and Singhal, R. S. (2019). Indian traditional foods: Preparation, processing and nutrition. In Traditional Foods, Springer, Cham., pp:127-199.

Anonymous (2019). Nutritional fact of bajra khichdi,https://www.tarladalal.com/calories-for-bajra-khichdi-rajasthani-3894, Date of accession, 10 May, 2022.

Anonymous (2020a). Nutritional facts of dosa, https://www.tarladalal.com/calories-for-dosa-south-indian-recipe-32927, Date of accession, 16 April, 2022.

Anonymous (2020b). Nutritional facts of dhokla, https://www.tarladalal.com/calories-for-khaman-dhokla-soft-gujarati-khaman-dhokla-33269, Date of accession, 17 April, 2022.

Anonymous (2020c). Nuritional facts of makki ki roti, https://www.tarladalal.com/calories-for-makki-ki-roti-punjabi-makki-di-roti-recipe-30908, Date of accession, 21 April, 2022.

Anonymous (2020d). Nutritional facts of sarson ka saag, https://www.tarladalal.com/calories-for-sarson-ka-saag-punjabi-sarson-ka-saag-recipe-32800, Date of accession, 20 April, 2022.

Anonymous (2021a). Nutritional facts of pongal, https://www.tarladalal.com/calories-for-ven-pongal-south-indian-breakfast-32896, Date of accession, 25 April, 2022.

Anonymous (2021b). Vitamin B6 health sheet for health professionals, https://ods.od.nih.gov/factsheets/VitaminB6-HealthProfessional/#disc, Date of accession, 28 March, 2022.

Anonymous (2020e). Nutrient requirements for Indian https://www.nin.res.in/RDA_short_Report_2020.html, Date of accession, 22 April, 2022.

Aslam, F.; Muhammad, S. M.; Aslam, S. and Irfan, J. A. (2017). Vitamins: Key role players in boosting up immune response: A mini review. Vitamins and Minerals, **6**(01):8.

Ballard, C. R. and Junior, M. R. M. (2019). Health benefits of flavonoids. In Bioactive Compounds, Woodhead Publishing, pp:85-201.

Bento-Silva, A.; Koistinen, V. M.; Mena, P.; Bronze, M. R.; Hanhineva, K.; Sahlstrøm, S.; Kitryte, V.; Moco, S. and Aura, A. M. (2020). Factors affecting intake, metabolism and health benefits of phenolic acids: Do we understand individual variability?. European Journal of Nutrition, **59**(4):1275-1293.

Bergquist, S. (2006). Bioactive compounds in baby spinach (*Spinacia oleracea* L.) Acta Universitatis Agriculturae Sueciae, pp:62.

Bickford, P. C.; Gould, T.; Briederick, L.; Chadman, K.; Pollock, A.; Young, D.; Shukitt-Hale B. and Joseph, J. (2000). Antioxidant-rich diets improve cerebellar physiology and motor learning. Brain Research, **866**(1-2):211-217.

Bottino, A.; Degl'Innocenti, E.; Guidi, L.; Graziani, G. and Fogliano, V. (2009). Bioactive compounds during storage of fresh-cut spinach: The role of endogenous ascorbic acid in the improvement of product quality. Journal of Agricultural and Food Chemistry, **57**(7):2925-2931.

Cao, G.; Russell, R. M.; Lischner, N. and Prior, R. L. (1998). Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. The Journal of Nutrition, **128**(12):2383-2390.

Cartford, M. C.; Gemma, C. and Bickford, P. C. (2002). Eighteen-month-old Fischer 344 rats fed a spinach-enriched diet show improved delay classical eyeblink conditioning and reduced expression of tumor necrosis factor á (TNFá) and TNFâ in the cerebellum. Journal of Neuroscience, **22**(14):5813-5816.

Cassidy, A. (2018). Berry anthocyanin intake and cardiovascular health. Molecular Aspects of Medicine, **61**:76-82.

Chadare, F. J.; Idohou, R.; Nago, E.; Affonfere, M.; Agossadou, J.; Fassinou, T. K.; Kenou, C.; Honfo, S.; Azokpota, P.; Linnemann, A. R. and Hounhouigan, D. J. (2019). Conventional and food to food fortification: An appraisal of past practices and lessons learned. Food Science and Nutrition, **7**(9):2781-2795.

Craig, S. A. (2004). Betaine in human nutrition. The American Journal of Clinical Nutrition, **80**(3):539-549.

Crider, K. S.; Bailey, L. B. and Berry, R. J. (2011). Folic acid food fortification, its history, effect, concerns, and future directions. Nutrients, **3**(3):370-384.

Dary, O. and Hurrell, R. (2006). Guidelines on food fortification with micronutrients. World Health Organization, Food and Agricultural Organization of the United Nations: Geneva,Switzerland, pp:1-376.

DeSoto, M. C. and Hitlan, R. T. (2012). Synthetic folic acid supplementation during pregnancy may increase the risk of developing autism. Journal of Pediatric Biochemistry, **2**(4):251-261.

Duthie, S. J. (1999). Folic acid deficiency and cancer: mechanisms of DNA instability. British Medical Bulletin, **55**(3):578-592.

Dwyer, J. T.; Wiemer, K. L.; Dary, O.; Keen, C. L.; King, J. C.; Miller, K. B.; Philbert, M. A.; Tarasuk, V.; Taylor, C. L.; Gaine, P. C.; Jarvis, A. B. and Bailey, R. L. (2015). Fortification and health: Challenges and opportunities. Advances in Nutrition, **6**(1):124-131.

Eggersdorfer, M., and Wyss, A. (2018). Carotenoids in human nutrition and health. Archives of Biochemistry and Biophysics, **652**:18-26.

El-Sayed, S. M. (2020). Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. Heliyon, **6**(1):e03278.

Elvira-Torales, L. I.; Martín-Pozuelo, G.; González-Barrio, R.; Navarro-González, I.; Pallarés, F. J.; Santaella, M.; Navarro –Gonzalez, I.; Garcia-Alonso, J.; Sevilla, A. and Periago-Castón, M. J. (2019). Ameliorative effect of spinach on non-alcoholic fatty liver disease induced in rats by a high-fat diet. International Journal of Molecular Sciences, **20**(7):1662.

Elvira-Torales, L. I.; Navarro-González, I.; Rodrigo-García, J.; Seva, J.; García-Alonso, J. and Periago-Castón, M. J. (2020). Consumption of spinach and tomato modifies lipid metabolism, reducing hepatic steatosis in rats. Antioxidants, **9**(11):1041.

Farjah, G. H.; Pour, M. M. and Hassan, M. (2018). Protective effect of aqueous spinach (*Spinacia oleracea* L.) extract on spinal cord ischemia: Reperfusion injury in rats. In Veterinary Research Forum, **9**(2):187-191.

Field, M. S. and Stover, P. J. (2018). Safety of folic acid. Annals of the New York Academy of Sciences, **1414**(1):59-71.

Gudulkar, S.; Rajbhar, K.; Dawda, H. and Mukundan, U. (2020). Screening of selected plants for their effectiveness in the treatment of kidney stone. Ann. Phytomed., **9**(1):213-217.

Gezici, S. (2018). Promising anticancer activity of lavender (Lavandula angustifolia Mill.) essential oil through induction of both apoptosis and necrosis. Ann. Phytomed., **7**(2):38-45.

Galla, N. R.; Pamidighantam, P. R.; Karakala, B.; Gurusiddaiah, M. R. and Akula, S. (2017). Nutritional, textural and sensory quality of biscuits supplemented with spinach (*Spinacia oleracea L.*). International Journal of Gastronomy and Food Science, **7**:20-26.

Granado, F.; Olmedilla, B. and Blanco, I. (2003). Nutritional and clinical relevance of lutein in human health. British Journal of Nutrition, **90**(3):487-502.

Gunnars, K. (2019). Spinach 101: Nutrition facts and health benefits. Iceland: Health-line Newsletter. https://www. healthline. com/nutrition/foods/spinach, Date of accession- 12 May, 2022.

Gurveer, K., and Goswami, T. K. (2017). Rasgulla: A review. Dairy and Veterinary Science Journal, **2**(3):555-589.

Hedges, L. J. and Lister, C. E. (2007). Nutritional attributes of spinach, silver beet and eggplant. Crop Food Res. Confidential Rep., **1**(15):1-29.

Holmes, R. P. and Kennedy, M. (2000). Estimation of the oxalate content of foods and daily oxalate intake. Kidney International, **57**(4):1662-1667.

Hoque, A. and Taufique, M. (2019). Mouth-watering traditional cuisines of India: A study of cultural geography. Open Learning, **2**:34-45.

Iqbal, M.; Ghous, T.; Khan, A. N. and Akhtar, K. (2012). Evaluation of antimicrobial activity of extracts of fresh and spoiled *Spinacia oleracea* against some mammalian pathogens. African Journal of Microbiology Research, **6**(29):5847-5851.

Iqbal, S.; Ali, S. and Siddiqui, A. (2018). Antioxidant effects of spinach *(Spinacia Oleracea)* on testicular and epididymal weight of obese spragu dawley rats. Journal of Islamic International Medical College, **13**(3):157-161.

Iyer, R. and Tomar, S. K. (2009). Folate: A functional food constituent. Journal of Food Science, **74**(9):R114-R122.

Joshi, S. R. and Shinde, J. (2009). Dietary consideration in diabetes. Elsevier, a division of Red Elsevier India Private Limited, pp:75-91.

Jovanovski, E.; Bosco, L.; Khan, K.; Au-Yeung, F.; Ho, H.; Zurbau, A.; Jenkins, A. L. and Vuksan, V. (2015). Effect of spinach, a high dietary nitrate source, on arterial stiffness and related hemodynamic measures: A randomized, controlled trial in healthy adults. Clinical Nutrition Research, **4**(3):160-167.

Kanabur, V. and Reddy, R. (2014). Bioactive components of spinach and their effect on some patho physiological conditions: A review. International Journal of Current Research and Review, **6**(9):156-166.

Karpouzos, A.; Diamantis, E.; Farmaki, P.; Savvanis, S. and Troupis, T. (2017). Nutritional aspects of bone health and fracture healing. Journal of Osteoporosis,https://doi.org/10.1155/2017/4218472, Date of accession- 16 May, 2022.

Khan, M. A.; Mahesh, C.; Semwal, A. D. and Sharma, G. K. (2015). Effect of spinach powder on physicochemical, rheological, nutritional and sensory characteristics of chapati premixes. Journal of Food Science and Technology, **52**(4):2359-2365.

Ko, S. H.; Park, J. H.; Kim, S. Y.; Lee, S. W.; Chun, S. S. and Park, E. (2014). Antioxidant effects of spinach *(Spinacia oleracea* L.*)* supplementation in hyperlipidemic rats. Preventive Nutrition and Food Science, **19**(1):19.

Kondo, A.; Kamihira, O. and Ozawa, H. (2009). Neural tube defects: Prevalence, etiology and prevention. International Journal of Urology, **16**(1):49-57.

Krinsky, N. I. and Johnson, E. J. (2005). Carotenoid actions and their relation to health and disease. Molecular Aspects of Medicine, **26**(6):459-516.

Krishnakumar, T. (2019). Traditional foods of India. Geoscience. DOI:10.13140/RG.2.2.18647.11684 , Date of accession- 28 April, 2022.

Krishnamoorthy, S.; Kunjithapatham, S. and Manickam, L. (2013). Traditional Indian breakfast (idli and dosa) with enhanced nutritional content using millets. Nutrition and Dietetics, **70**(3):241-246.

Kruman, I. I.; Kumaravel, T. S.; Lohani, A.; Pedersen, W. A.; Cutler, R. G.; Kruman, Y.; Haughey, N.; Lee, J.; Evans, M. and Mattson, M. P. (2002). Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. Journal of Neuroscience, **22**(5): 1752-1762.

Kuti, J. O. and Torres, E. S. (1996). Potential nutritional and health benefits of tree spinach. Progress in New Crops, **13**(5):516-520.

Lassi, Z. S.; Salam, R. A.; Haider, B. A. and Bhutta, Z. A. (2013). Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. Cochrane Database of Systematic Reviews, (3). https://doi.org/10.1002/14651858.CD006896.pub2, Date of accession- 10 May, 2022.

Li, T.; Lu, X.; Sun, Y. and Yang, X. (2016). Effects of spinach nitrate on insulin resistance, endothelial dysfunction markers and inflammation in mice with high-fat and high-fructose consumption. Food and Nutrition Research, **60**(1):32010.

Liu, S.; West, R.; Randell, E.; Longerich, L.; O'connor, K. S.; Scott, H.; Crowley, M.; Lam, A.; Prabhakaran, V. and McCourt, C. (2004). A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. BMC Pregnancy and Childbirth, **4**(1):1-10.

López-Nicolás, R.; Frontela-Saseta, C.; González-Abellán, R.; Barado-Piqueras, A.; Perez-Conesa, D. and Ros-Berruezo, G. (2014). Folate fortification of white and whole-grain bread by adding Swiss chard and spinach. Acceptability by consumers. LWT-Food Science and Technology, **59**(1):263-269.

Maeda, N.; Yoshida, H. and Mizushina, Y. (2010). Spinach and health: Anticancer effect. In: Bioactive Foods in Promoting Health, Academic Press, pp: 393-405.

Majeed, M. (2017). Evidence-based medicinal plant products for the health care of world population. Ann. Phytomed., **6**(1):1-4.

Manzoor, M. F.; Xu, B.; Khan, S.; Shukat, R.; Ahmad, N.; Imran, M.; Rehman, A.; Karrar, E.; Aadil, R. M. and Korma, S. A. (2021). Impact of high-intensity thermosonication treatment on spinach juice: Bioactive compounds, rheological, microbial, and enzymatic activities. Ultrasonics Sonochemistry, **78**:105740.

Mitchell, L. E.; Adzick, N. S.; Melchionne, J.; Pasquariello, P. S.; Sutton, L. N. and Whitehead, A. S. (2004). Spina bifida. The Lancet, **364**(9448):1885-1895.

Mohanty, B.; Mahanty, A.; Ganguly, S.; Sankar, T. V.; Chakraborty, K.; Rangasamy, A.; Paul, B.; Sarma, D.; Mathew, S.; Asha, K. K.; Behera, B.; Aftabuddin, Md.; Debnath, D.; Vijayagopal, P.; Sridhar, N.; Akhtar, M. S.; Sahi, N.; Mitra, T.; Banerjee, S.; Paria, P.; Das, D.; Das, P.; Vijayan, K. K.; Laxmanan, P. T. and Sharma, A. P. (2014). Amino acid compositions of 27 food fishes and their importance in clinical nutrition. Journal of Amino Acids, pp:7.

Morelock, T. E. and Correll, J. C. (2008). Spinach. In Vegetables I, Springer, New York, NY, pp:189-218.

Munir, M.; Ahad, A.; Gull, A.; Qayyum, A.; Siddique, N. R.; Mumtaz, A.; Safdar, N.; Ali, B.; Nadeem, M. and Qureshi, T. M. (2019). Addition of spinach enhanced the nutritional profile of apricot based snack bars. Pakistan Journal of Agricultural Research, **32**(3):490.

Olson, R.; Gavin-Smith, B.; Ferraboschi, C. and Kraemer, K. (2021). Food fortification: The advantages, disadvantages and lessons from sight Xand life programs. Nutrients, **13**(4):1118.

Panda, V. and Shinde, P. (2017). Appetite suppressing effect of *Spinacia oleracea* in rats: Involvement of the short term satiety signal cholecystokinin. Appetite, (113):224-230.

Panda, V.; Bhandare, N.; Mistry, K. and Dande, P. (2022). Cardioprotective potential of *Spinacia oleracea* (Spinach) against isoproterenol-induced myocardial infarction in rats. Archives of Physiology and Biochemistry, **128**(1):101-110.

Panda, V.; Mistry, K.; Sudhamani, S.; Nandave, M. and Ojha, S. K. (2017). Amelioration of abnormalities associated with the metabolic syndrome by *Spinacia oleracea* (spinach) consumption and aerobic exercise in rats. Oxidative Medicine and Cellular Longevity, (15), https://doi.org/10.1155/2017/2359389, Date of accession- 17 May, 2022.

Patel, J. M. (2008). A review of potential health benefits of flavonoids. Lethbridge Undergraduate Research Journal. URI: https://hdl.handle.net/10133/1220, Date of accession, 23 May, 2022.

Patil, S.; Yadav, A.; Chopade, A. and Mohite, S. (2020). Design, development and evaluation of herbal mouthwash for antibacterial potency against oral bacteria. Journal of University of Shanghai for Science and Technology, **22**(11):881-898.

Rex, B.; Prabhu, S. and Kumar, J. S. (2019). Antifungal efficacies of plant extracts against *Alternaria solani* (Ellis and Martin) jones and groutunder *in vitro* condition. Annals Phytomed., **8**(1):1-5.

Rahati, S.; Eshraghian, M.; Ebrahimi, A. and Pishva, H. (2016). Effect of spinach aqueous extract on wound healing in experimental model diabetic rats with streptozotocin. Journal of the Science of Food and Agriculture, **96**(7):2337-2343.

Ramaiyan, B.; Kour, J.; Nayik, G. A.; Anand, N. and Alam, M. S. (2020). Spinach (*Spinacia oleracea* L.). In: Antioxidants in Vegetables and Nuts-Properties and Health Benefits. Springer, Singapore, pp:159-173.

Ramalingam, S.; Kandasamy, S.; Bahuguna, A. and Kim, M. (2019). Nutritional and health benefits of idli and dosa. In: Fermented Food Products CRC Press, pp:181-194.

Ramdas, W. D.; Schouten, J. S. and Webers, C. A. (2018). The effect of vitamins on glaucoma: A systematic review and meta-analysis. Nutrients, **10**(3):359.

Ramu, L. and Maloo, S. R. I. N. I. V. A. S. (2016). Physical, chemical and sensory properties of spinach paste fortified Instant noodles. International Journal of Innovative Technology and Research, **4**:5318-5322.

Ravetti, S.; Clemente, C.; Brignone, S.; Hergert, L.; Allemandi, D. and Palma, S. (2019). Ascorbic acid in skin health. Cosmetics, **6**(4):58.

Roughani, A. and Miri, S. M. (2019). Spinach: An important green leafy vegetable and medicinal herb. In The 2nd International Conference on Medicinal Plants, Organic Farming, Natural and Pharmaceutical Ingredients pp:1-6.

Ruxto, C. (2004). Health benefits of omega-3 fatty acids. Nursing Standard, **18**(48):38-42.

Sarkar, P.; DH, L. K.; Dhumal, C.; Panigrahi, S. S. and Choudhary, R. (2015). Traditional and ayurvedic foods of Indian origin. Journal of Ethnic Foods, **2**(3):97-109.

Siciñska, E. and Pelc, A. (2011). Fortified food products as a potential source of folic acid in human nutrition. Roczniki Panstwowego Zakladu Higieny, **62**(2):209-214.

Scott, J. M.; Weir, D. G.; Molloy, A.; McPartlin, J.; Daly, L. and Kirke, P. (1994). Folic acid metabolism and mechanisms of neural tube defects. In Neural Tube Defects. CIBA Foundation Symposium, **181**:180-191.

Sherafatmanesh, S.; Ekramzadeh, M.; Tanideh, N.; Golmakani, M. T. and Koohpeyma, F. (2020). The effects of thylakoid-rich spinach extract and aqueous extract of caraway (*Carum carvi* L.) in letrozole-induced polycystic ovarian syndrome rats. BMC Complementary Medicine and Therapies, **20**(1):1-13.

Singh, J.; Jayaprakasha, G. K. and Patil, B. S. (2018). Extraction, identification, and potential health benefits of spinach flavonoids: A review. Advances in Plant Phenolics:From Chemistry to Human Health, (6):107-136. DOI: 10.1021/bk-2018-1286.ch006.

Srinivas, T. (2011). Exploring Indian culture through food. Education about Asia, **16**(3):38-41.

Srinivasan, K. (2010). Traditional Indian functional foods. Functional Foods of the East, Nutraceutical Science and Technology. Series, **10**:51-76.

Tabrizi, F. P. F.; Farhangi, M. A.; Vaezi, M. and Hemmati, S. (2020). The effects of thylakoid-rich spinach extract combined with the calorie-restricted diet on cardiometabolic risk factors in obese women with polycystic ovary syndrome: A Randomized Controlled Clinical Trial, https://doi.org/10.21203/rs.3.rs-29599/v1.

Taneja, D. K.; Rai, S. K. and Yadav, K. (2020). Evaluation of promotion of iron-rich foods for the prevention of nutritional anemia in India. Indian Journal of Public Health, **64**(3):236.

Tewani, R.; Sharma, J. K. and Rao, S. V. (2016). Spinach (Palak) natural laxative. International Journal of Applied Research and Technology, **1**(2):140-148.

Trivedi, P. (2018). Development of fortified rice based pasta incorporated with spinach powder. Food Science, **9**(1):85-87.

Tyagi, S. (2017). Micronutrient fortification of traditional Indian food preparations by incorporation of fresh as well as dehydrated spinach leaves.International Journal of Sciences and Applied Research, **4**(7):67-70.

Umar, B. U. (2007). Effect of hexane extract of spinach in the removal of arsenic from rat. Bangladesh Journal of Pharmacology, **2**(1):27-34.

Warrier, R. R. (2021). Authentication of herbal products to attract global markets. Annals of Phytomedicine, **10**(2):1-3.

Waseem, M.; Akhtar, S.; Manzoor, M. F.; Mirani, A. A.; Ali, Z.; Ismail, T.; Ahmad, N. and Karrar, E. (2021). Nutritional characterization and food value addition properties of dehydrated spinach powder. Food Science and Nutrition, **9**(2):1213-1221.

Citation **Divya (2022). Role and importance of spinach fortification in Indian traditional foods. Ann. Phytomed., 11(1):53-67. http://dx.doi.org/10.54085/ap.2022.11.1.6.**