ORIGINAL ARTICLE

Nutritional profile of spinach and its antioxidant & antidiabetic evaluation

Anil Kumar Sah[1], Shivangni Raj[1], Gopal L. Khatik[2], Manish Vyas[1]

[1]Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Abstract

Background: A proper diet is the best source of complete nutrition which provides strength, complexion, and vitality. Nutraceuticals and dietary supplement are the functional food that promotes the health and manage the disease. Spinach (*Spinacia oleracea*) is a leafy vegetable and considered a good source of nutrients. **Objective:** The objective of the study is to determine the nutritional value and evaluate *in vitro* antioxidant as well as antidiabetic potential of spinach. **Materials and Methods:** The methanolic extract of spinach was prepared using Soxhlet extraction technique which was subjected for physicochemical, nutritional value determination along with the OH - scavenging, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and α-amylase inhibition activity. **Result and Discussion:** The results suggested the good nutritional values such as total crude fiber 4.55 ± 0.244% w/w, proteins 0.052 ± 0.0068% w/w, oils and fats 0.72 ± 0.036% w/w, carbohydrate 61.95 ± 0.382% w/w, Vitamins A 26.85 ± 0.154 µg, and Vitamins C 19.66 ± 0.21 µg. Further, methanolic extract of spinach showed the antioxidant activity and antidiabetic effect with an inhibitory concentration of 3.03 µg/mL, 6.03 µg/mL and 3.046 µg/mL for OH- scavenging, DPPH inhibition and α-amylase inhibition, respectively. **Conclusion:** Results revealed the potential nutraceutical values of spinach which can further explored to effectively use it by preparing suitable formulation.

Key words: Amylase, antidiabetic activity, antioxidant activity, nutraceutical, *Spinacia oleracea*

INTRODUCTION

"Ayurveda" is a science of life and longevity[1] it helpful in the maintenance of the health of the healthy individual and treatment of diseases[2] Ayurveda has three modes of treatment, i.e. Hetu (cause), Ling (symptom), and Aushadh (medicine).[3] Aushadh is incorporated in all branches of Ayurveda as a mode of treatment including Rasayana.[4,5] Rasayana is the branch of Ayurveda which deals with the nourishment of the body and tissues to enhance memory, intelligence, luster, complexion, and voice and reduce the effect of aging.[6] Acharya Charak,[7] Acharya Sushruta,[8] and Acharya Dalhan have described the different types of Rasayana. Ajasrika is a type of Rasayana described by the Acharya Dalhan deals with a daily intake of proper diet (Aahar) specifically milk and ghee[9] Proper diet is the best source of complete nutrition which provides strength, complexion, and vitality.[10] Drugs and their formulations have also been prescribed by the different scholars of Ayurveda if Aahar is not able to deliver the required amount nutrition. These drugs and formulations can be correlated with the nutraceuticals as these are also used to provide the complete nutrition to the body.

Nutraceuticals and dietary supplement are the functional food that promotes the health and manage the disease.[11] These are utilized for the development of the body and boost the immunity from decades. Nutraceuticals have an advantage due to their natural sources and polyherbal combinations.[12] The Indian pharmaceutical industry is rapidly growing, in the field of nutraceuticals with the growth rate of 19.5% per year.[13] The global market of nutraceuticals was 142.1 billion USD in 2011 and is expected to reach up to 204.8 billion USD in 2017. The

Address for correspondence:
Dr. Manish Vyas, Department of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara - 144 411, Punjab, India.
Phone: +91-9780210783. E-mail: vymanish@gmail.com

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Transparency Market Research, Albany, New York, has reported growth in the market of nutraceuticals at a growth of 6.3%.[14] The increasing demand leads to the new scope of research and drug development in the field of nutraceuticals. Besides, Ayurveda has great potential to provide established and time-tested drugs to develop potent nutraceuticals. Some examples of such drugs are liquorice, ginseng, onion, ginger, spinach, aloe, and turmeric.[12] Spinach contains various amino acids, vitamins, carbohydrates, fats and fatty acids, micro and macronutrients, and sterols, etc.[15] Hence, the present study was designed to develop an analytical and nutritional profile of the spinach. In addition to this, *in vitro* antioxidant and antidiabetic activities were also performed.

MATERIALS AND METHODS

Plant Material

Fresh spinach (*Spinacia oleracea*) [Figure 1] was procured from the local market of the Phagwara (Punjab) and authenticated by the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar.

Chemical

All the chemicals and reagents were of analytical grade. Ferrous sulfate ($FeSO_4$), 2, 2-diphenylpicrylhydrazyl (DPPH) are from choline dehydrogenase, 30% hydrogen peroxide, sodium salicylate (LOBA Chemie laboratory reagents and fine chemical), α- amylase, ascorbic acid (Titan Biotech Ltd.), iodine (Sigma-Aldrich), etc. The solutions were prepared using double distilled water and stored at 30°C until analysis and were diluted with distilled water just before measuring.

Figure 1: Whole plant of spinach

Physicochemical Analysis of Spinach

Various physicochemical parameters were analyzed to find out the identity, purity, and strength of the spinach, i.e. foreign matter, loss on drying (LOD), ash value, acid insoluble ash, water soluble extractive value, and alcohol soluble extractive value.[16,17]

Qualitative Analysis of Spinach

Various chemical constituents were analyzed by the phytochemical screening to establish a chemical profile of spinach which is the test for alkaloid, glycoside, reducing sugar, monosaccharides, amino acid, steroids, and proteins.[18]

Proximal Value of Spinach

The quantitative analysis was performed to determine the actual percentage of the different parameters such as moister content, total crude fiber, proteins, oils and fats, carbohydrate, minerals, and vitamins. Nutritional value of the spinach was important as a nutraceutical which was established by the presence of proteins, oils and fats, carbohydrate, minerals, vitamins, etc.[19,20]

Extraction of the Spinach

The fresh leaves of spinach were crushed to make a fine paste and extracted with ethanol using Soxhlet extraction method for 72 h at 50-60°C with continuous stirring. The obtained extract was filtered, and filtrate was evaporated on a rotary evaporator. The crude obtained was partitioned using 70 mL methanol and 20 mL n-hexane and methanol layer was collected and concentrated till dryness on rotary evaporator.[21]

High Performance Thin Layer Chromatography (HPTLC) Analysis

HPTLC was used for the qualitative and quantitative analysis by enhancing the separation and resolution of the compounds with a fine particle size of stationary phase.[22] The mobile phase was used as a mixture of chloroform: Isopropyl alcohol: Acetic acid (12:8:1)[23] and the prepared methanolic extract was analyzed.

Preparation of Sample

About 10 mg of dry methanolic extract of spinach was taken and dissolved in 10 mL of the methanol. Thereafter, it was filtered using Whatman filter paper, and the filtrate was concentrated on a water bath and stored in the closed container.

In Vitro Antioxidant Activity

OH- scavenging assays: OH- radicals were generated from $FeSO_4$ and H_2O_2 mixture and scavenging efficiency

was detected by their ability to hydroxylate the salicylate. A 3 mL of reaction mixture was prepared using 1 mL of $FeSO_4$ (1.5 mM), 0.7 mL H_2O_2 (6 mM), 0.3 mL sodium salicylate (20 mM), and 1 mL of different concentrations of the methanolic extract. Then, these mixtures were subjected for the incubation of 1 h at 37°C. After incubation, the absorbance of hydroxylated salicylate complex was measured at 562 nm for different samples,[24] and % inhibition was calculated by following formula.

$$\%\ \text{Inhibition}\ (\%\ \text{I}) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

DPPH radical scavenging assay: The DPPH radical scavenging assay was performed with 700 µL of sample (methanolic extract of spinach) and MeOH (control) was added to the same volume of a methanolic solution of a 100 µM DPPH. Mixtures were shaken vigorously and left to stand in the dark at room temperature for 20 min and then absorbance was read at 515 nm, using a ultraviolet spectrophotometer.[25] Antioxidant activity was expressed in % inhibition and calculated using the following formula.

$$\%\ \text{Inhibition}\ (\%\ \text{I}) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

Table 1: Physicochemical analysis of spinach

Physical parameters	Results (% w/w)
Foreign matter	0
LOD	9.476±0.119
Ash value	29.65±0.720
Acid insoluble ash	6.01±0.244
Water soluble extractive value	54.02±0.574
Alcohol soluble extractive value	45.49±0.576

LOD: Loss of drying

In Vitro α-amylase Inhibition Assays (Antidiabetic Activity)

Starch iodine method was used for the determination of α-amylase inhibition activity. A 10 µL of α-amylase solution (0.025 mg/mL) was mixed with 390 µL of phosphate buffer containing different concentrations of methanolic extract of spinach. After incubation at 37°C for 10 min, 100 µL of the 1% starch solution was added and re-incubated for 1 h. After reincubation 0.1 mL of 1% iodine solution was added, and further, it was diluted with 5 mL distilled water. The absorbance's of all the solutions were measured at 565 nm[24], and % inhibition was calculated by following formula.

$$\%\ \text{Inhibition}\ (\%\ \text{I}) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

The identity, purity, and strength of spinach crude drug was evaluated using six different batches and variation percentage w/w determined by various parameters [Table 1], i.e., LOD 9.476 ± 0.119% w/w, ash value 29.65 ± 0.720% w/w, acid insoluble ash 6.01 ± 0.244% w/w, water soluble extractive value 54.02 ± 0.574% w/w, and alcohol soluble extractive value 45.49 ± 0.576.

Phytochemical screening is the qualitative analysis of the spinach indicates the presence of saponin glycosides, reducing sugars, monosaccharides, steroids, and proteins [Table 2]. These constituents are the main components of the good nutraceuticals.

Six different batches were used for the proximate analysis of spinach contains the nutritional value in % w/w [Table 3], moisture content 1.97 ± 0.053, total crude fiber 4.55 ± 0.244, protein 0.052 ± 0.0068, oils and fats 0.72 ± 0.036, carbohydrates 61.95 ± 0.382, Vitamins A 26.85 ± 0.154, and Vitamins C 19.66 ± 0.21.

Table 2: Qualitative analysis of spinach

Test	Chemical tests	Result	Observation
Alkaloids	Mayer's reagent	Negative	NA
	Wagner's reagent	Negative	NA
	Dragendorff's reagent	Negative	NA
	Hager's reagents	Negative	NA
Saponin glycosides	Foam test	Positive	Foam appear
Reducing sugars	Benedict's reagents	Positive	Green color
	Fehling's test	Positive	Brick red color
Monosaccharides	Barfoed's test	Negative	NA
Amino acids	Ninhydrin test	Negative	NA
Steroids	Salkowski reaction	Positive	Greenish yellow fluorescence
Proteins	Biuret test	Positive	Voilet color

Table 3: Nutritional value investigation of spinach

Nutritional value	Results (w/w)
Moister content (%)	1.97±0.053
Total crude fiber (%)	4.55±0.244
Proteins (%)	0.052±0.0068
Oils and fats (%)	0.72±0.036
Carbohydrates (%)	61.95±0.382
Vitamins A (µg)	26.85±0.154
Vitamin C (µg)	19.66±0.21

Table 4: Rf value of the spinach

Rf	Standard (Rf)
0.10	0.38 (reference 8)
0.13	
0.14	
0.25	
0.38	
0.44	
0.52	
0.60	
0.69	

Rf: Retention factor

Methanolic extract of spinach was analyzed by HPTLC using mobile phase as chloroform: Isopropyl alcohol: Acetic acid (12:8:1). Nine different compounds were detected with retention factor (Rf) 0.10, 0.13, 0.14, 0.25, 0.38, 0.44, 0.52, 0.60, and 0.69 [Table 4 and Figure 2]. Among them, peak 5 with Rf 0.38 was identified as 20-hydroxyecdysone which is an important chemical constituent in spinach as reported in the literature.[8]

Different concentration varying 10-1500 µg/mL of standard antioxidant, i.e., ascorbic acid and the extract of the spinach were prepared for evaluation of antioxidant activity using OH- scavenging assays. The result showed that extract was found comparable with ascorbic acid from concentration varying from 20 to 100 µg/mL with the inhibition approximately 50-60%. The inhibitory concentration (IC_{50}) of the standard and extract was calculated 3.23 µg/mL and 3.03 µg/mL, respectively. Extract has good OH- scavenging antioxidant activity which is comparable with ascorbic acid [Table 5 and Figure 3].

DPPH scavenging assay was performed using a different concentration of 10-300 µg/mL of standard antioxidant, i.e., ascorbic acid and the extract. The result showed that extract was less effective at low concentration whereas found comparable at a 200 µg/mL with ascorbic acid. IC_{50} of the standard and extract was calculated as 3.58 µg/mL and 6.03 µg/mL, respectively. It showed that extract has

Figure 2: Thin layer chromatography plate of extract

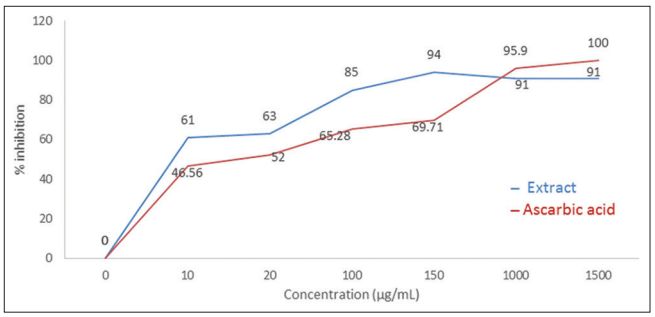

Figure 3: Comparative plot of OH- scavenging % inhibition by extract and ascorbic acid

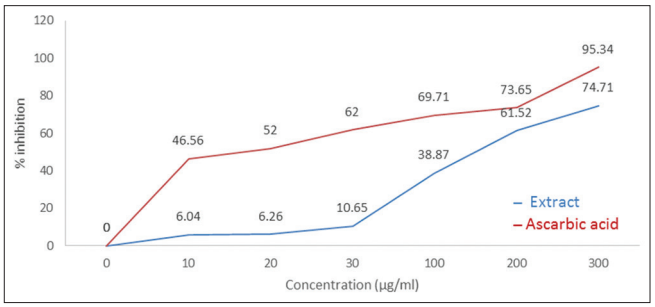

Figure 4: Comparative plot of 2,2-diphenyl-1-picrylhydrazyl % inhibition by extract and ascorbic acid

moderate DPPH scavenging activity compared to ascorbic acid [Table 6 and Figure 4].

To evaluate the antidiabetic potential of spinach, we performed alpha-amylase inhibition using different concentration 10-200 µg/mL of the standard antidiabetic agent, i.e., acarbose and the extract of spinach. The results showed that extract was found comparable with acarbose from concentration varying 10-100 µg/mL with the % inhibition approximately 40-80%. Both standard and extract showed the 100% inhibition at 200 µg/mL concentration. IC_{50} of the standard and extract was calculated as 3.82 µg/mL and 3.046 µg/mL, respectively. It suggests

Table 5: OH- scavenging assays

Concentration (µg/mL)	% Inhibition by extract	% Inhibition by standard (ascorbic acid)
0	0	0
10	61	46.56
20	63	52
100	85	65.28
150	94	69.71
1000	91	95.9
1500	91	100

Table 6: DPPH scavenging assay

Concentration (µg/mL)	% Inhibition by extract	% Inhibition by standard (ascorbic acid)
0	0	0
10	6.04	46.56
20	6.26	52
30	10.65	62
100	38.87	69.71
200	61.52	73.65
300	74.71	95.34

DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 7: Antidiabetic effect of spinach

Concentration (µg/mL)	% Inhibition of extract	% Inhibition of standard (acarbose)
0	0	0
10	8.1	11.62
15	50.54	23.54
25	82.43	48.43
30	100	68.23
100	100	87.71
200	100	100

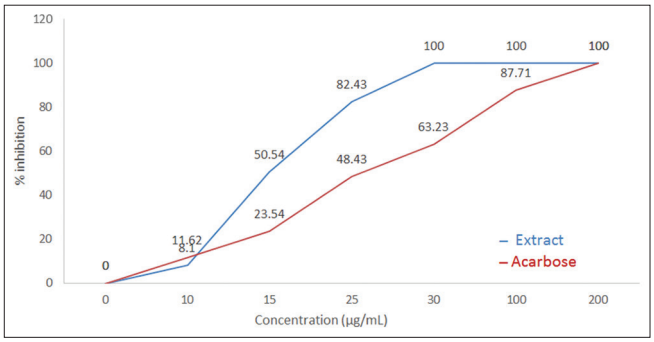

Figure 5: Comparative plot of α-amylase % inhibition by extract and acarbose

that extract is comparable with acarbose and can be a good antidiabetic agent [Table 7 and Figure 5].

CONCLUSIONS

Leafy vegetables are the good source of nutrients. Spinach is an important leafy vegetable described in ayurvedic texts in Shak Varga. It was reported for various pharmacological activities such as hepatoprotective, clastogenic, central nervous system depressant, antitumor. The present study was focused on evaluating its nutritional potential and antioxidant as well as antidiabetic effect. The crude drug showed % w/w of LOD 9.476 ± 0.119, ash value 29.65 ± 0.720, acid insoluble ash 6.01 ± 0.244, water soluble extractive value 54.02 ± 0.574, and alcohol soluble extractive value 45.49 ± 0.576. Some nutritional values observed are such as total crude fiber 4.55 ± 0.244% w/w, proteins 0.052 ± 0.0068% w/w, oils and fats 0.72 ± 0.036% w/w, carbohydrate 61.95 ± 0.382% w/w, Vitamins A 26.85 ± 0.154 µg, and Vitamins C 19.66 ± 0.21 µg. Moreover, the antioxidant activity and antidiabetic effect of spinach were comparable with IC_{50} of 3.03 µg/mL, 6.03 µg/mL, and 3.046 µg/mL for OH- scavenging, DPPH inhibition, and α-amylase inhibition, respectively.

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