BioCore Exploring Scientific Community

International Journal of Pharma Sciences and Scientific Research

ISSN 2471-6782

Research Article Open Access

Phytochemical profiling and evaluation of antioxidant and antidiabetic activity of methanol extract of spinach (spinacia oleracea l.) Leaves

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Abstract

The purpose of this study was to investigate the presence of major phytochemicals and the anti-diabetic and antioxidant effects of the methanolic extract of the plant Spinacia oleracea L. leaves. Phytochemical analysis of Spinacia oleracea L. displayed the presence of alkaloids, saponinsand tannin types of compounds. The anti-oxidative effect was evaluated using DPPH free radical scavenging activity method. The methanolic extract of Spinacia oleracea L. leaves is found to have no antioxidant activity. The $IC_{50}$of the extraction is 211081.58 µg /ml. In case of anti-diabetic activity test good anti-diabetic activity was observed. Mice treated with extract Group (250 mg/kg) showed decrease (from 21.7 mM ± SEM to 14.1mM ± SEM) ($p<0.05$) in blood glucose concentration at 120 min and extract group (500 mg/kg) showed decrease (from 24.9 mM ± SEM to 14.3 mM ± SEM) ($p<0.05$) at 120 min compared with Standard Group (from 25.6 mM ± SEM to 11.2mM ± SEM) at 120min.

Keywords: Phytochemical Profilining, Antioxidant, Antidiabetic, DPPH, Alloxan, Carrageenan

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Citation: Sharif Mohammad Shaheen et al. (2018), Phytochemical profiling and evaluation of antioxidant and antidiabetic activity of methanol extract of Spinach (Spinacia oleracea L.) leaves. Int J Pharm Sci & Scient Res. 4:1, 24-27

Received: December 26, 2017
Accepted: January 06, 2017
Published: March 07, 2018

Introduction

Considerable portion of current diseases are caused due to the 'oxidative stress' which results in enormous amount of free radicals, causing tumor, atherosclerosis and cardiovascular illnesses (Braca et al., 2002). Cells of the human body ensure themselves against harm caused by free radicals by catalysts such as ascorbic acid, tocopherol and glutathione (Braca et al., 2016). Cell reinforcement supplements are imperative to battle oxidative harm. That is why much consideration has been taken towards the improvement of ethnomedicine with solid cell reinforcement properties with low cytotoxic effects.

Diabetes mellitus (DM) is a metabolic and endocrine disorder which currently affects more than 100 million people all over the world and the number of affected people is increasing due to aging, increasing prevalence of obesity and physical inactivity (Nair et al., 2006; Sarah et al., 2004; Safdar et al., 2004; Umara et al., 2010). According to recent studies, approximately by the year 2030, 438 million people all over the world are expected to have diabetes (Mojekwu et al., 2011; Noor et al., 2008; Rehman et al., 2011). The worldwide cost to control diabetes and associated complications exceeds $100 billion per year and complications are far less common and less severe in people who have well controlled blood sugar levels (Chattopadhyay, 1999; Sokeng et al., 2001).The treatment of diabetes with synthetic drugs is generally not preferred because of its high cost and side effects, for this reason, it is necessary to develop alternative medicines of plant based origins with anti-diabetic properties (Emmanuel et al., 2010; Tanko et al., 2008).

Spinacia oleracea L. (Spinach) is a leafy green vegetable that came originally from southwestern Asia and is now grown in most parts of the world. Its leaves, which are broad and smooth and about ten inches long, Spinach, especially raw, is a very good source of folic acid, Spinach leaves sare rich in vitamin C and E, which are antioxidant. These are

supposed to lower risks of heart disease, stroke and cancer. The high amount of vitamin A in spinach may protect against eye degeneration. The potassium helps prevent and regulate high blood pressure. The plant is carminative and laxative. Inexperiments it has been shown to have hypo-glycemic properties. The aim of this study was to determine the presence of major phytochemicals in methanolic extract of Spinaci oleracea L. leaves and to investigate its anti-oxidative and anti-diabetic effects.

Materials and methods

Plant Materials

Spinacia oleracea L. leaves were collected from Mahammadpur, Townhall Kacha Bazar, Dhaka and the plant authentically was confirmed form the Bangladesh National Herbarium,

Drying and Grinding

The collected plants were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of Plant Extract

About 300 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1500 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained. Fine powders of the flowering plant of Spinacia oleracea L. leaves are dissolved in 90% methanol and then evaporation the solvent.

Phytochemical Screening

Phytochemical studied of methanolic extract of plant material extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book (Trease and Evans, 1983; Mohammed Ali, 2012; Abdul Ghani, 2005).

Antioxidant tests (Proctor, 1989; Hennekens et al., 1994; Clarkson, 1995)

Stock solution of the plant extract was prepared in methanol (10mg/ml) from which a serial dilution was carried out. At first 6 volumetric flasks are taken to make 6 different types of concentration 1, 5, 10, 50, 100 and 500 µg/ml. Test tubes and volumetric flasks are rapped with foil paper. In 6 volumetric flasks serial dilution of extract is done and marked them respectively.2ml of sample from each concentration and 2 ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. 2ml of sample from each concentration and 2 ml of 0.004% DPPH solution is taken with the help of pipette in 6 test tubes respectively. Then solution is kept in dark place for 30 minutes with raping each test tube with foil paper. In another test tube 2ml 0.004% DPPH & 2ml methanol is taken to prepare blank solution. Then absorbance is taken by UV Spectroscopy. The percent of inhibition is calculated by using following formula

$$\% \text{ of inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

Drugs and chemicals

Carrageenan was purchased from Otto chemicals, India. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh. Acetic acid, methanol and other chemicals supplied from laboratory of Bangladesh University were analytical grade.

Experimental animals

Eight week-old Swice albino mice (27-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

Method for Evaluation of Hypoglycemic Activity

Oral Glucose Tolerance Test (OGTT) in diabetic mice

After fasting 16hr, diabetes was induced into mice by in intra-peritoneal injection (i.p.) of alloxan monohydrade (90 mg/kg) disolved in saline. After 48hrs, plasma glucose levels were measured by glucometer (Tyson, Taiwan) using a blood sample from tail-vein of mice. Mice with blood sugar higher than11.5 mmol/l were considered as diabetic. All the mice were divided into 4 groups, each group containing 5 mice. The divided groups are NC (normal control), DC (diabetic control), STD (diabetic mice receiving Metformin), ME (diabetic mice receiving methanolic extract). The mice were fasted over-night and next day blood samples were taken from all groups of animals to estimate fasting blood glucose level (0 min). All mice received 1gm /kg glucose. Without delay extract and were given per oral and three more blood samples were collected at 30, 90 and 120 minutes intervals and blood glucose level was estimated in all the experiments by using glucometer (Hossain et al., 2011).

Result and discussion

Phytochemical Screening

Phytochemical screening of Methanolic Extract of Spinaciaoleracea L.leaf is displayed in Table-1

Chemical Groups	Methanolic Extract of *Spinacia oleracea L.* leaf
Saponin	+
Glycoside	-
Flavonoids	-
Tannin	+
Alkaloids	+

Table 1: Results of Phytochemical Screening

Note: (+) = Indicates the presence and (−) = Indicates the absence of the tested group.

Result of Anti-oxidants test

DPPH scavenging assay was used to determine the antioxidant activity

Conc. (µgml)	Absorbance (nm)		% of Inhibition	
	Blank	Ascorbic Acid	*Spinacia oleracea L.*	Ascorbic Acid	*Spinacia oleracea L.*
1	0.902	0.863	0.872	4.3	3.3
5		0.780	0.864	13.6	4.2
10		0.509	0.849	43.6	5.9
50		0.047	0.834	94.8	7.5
100		0.034	0.797	96.2	11.6
500		0.023	0.598	97.5	33.7

Table 2: % Inhibition of Ascorbic acid and Spinacia oleracea L.

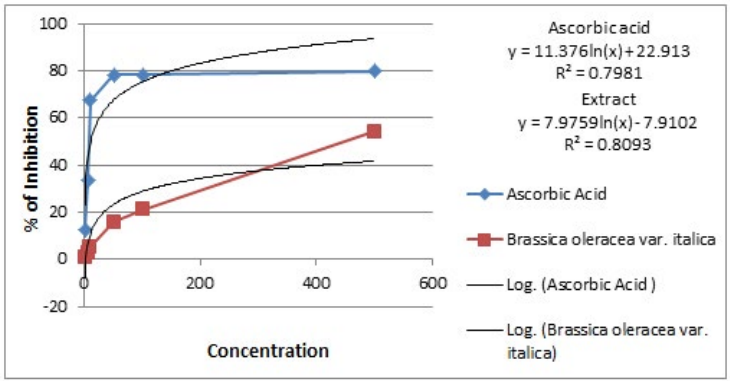

Fig 1. Anti-oxidant activity of Ascorbic acid and Spinacia oleracea L

Test Samples	Regression line	R2	IC_{50}µg/ml
Ascorbic Acid	y = 18.114ln(x) + 2.0487	R² = 0.8726	14.15
Spinacia oleracea L.	y = 4.2575ln(x) - 2.196	R² = 0.6898	211081.58

Table 3: IC_{50} values of the extracts of Ascorbic Acid and Spinacia oleracea

The IC_{50} of Spinacia oleracea L. is 211081.58µg/ml, whereas IC_{50} of Ascorbic Acid is 14.15µg/ml.

Results of Hypoglycemic Activity

Animal Group	0 min	30 min	60 min	90 min	120 min
Control Group	18.5±1.09	21.3±0.53	19.7±0.44	16.6±0.66	15.2±0.69
Standard Group	25.6±0.44***	28.5±0.38***	16.3±0.59	12.8±0.69**	11.2±0.74***
Extract Group (250 mg/kg)	21.7±0.33*	27.4±0.36***	21.8±0.49*	18.6±0.52	14.1±0.41
Extract Group (500 mg/kg)	24.9±0.51***	27.8±0.38***	21.2±0.64	17.9±0.53	14.3±0.31

Table 4: Oral Glucose Tolerance Test (OGTT) of Spinacia oleracea L. leaf extract in alloxan-induced diabetic (mM/L) in mice

Experimental data were presented as mean ± SEM. By using the Dunnett test significant differences (*p<0.05, **p<0.01, ***p<0.001) between the means were determined compare to control group where n=04. For statistical evaluation IBM-SPSS software version 20 was utilized.

Discussion

This study demonstrated the findings of Phytochemical studies, anti-oxidant and anti-diabetic activity of methanolic extract of Spinacia oleracea L. leaves by severel in vivo and in vitro method. Phytochemical observations have revealed the presence of several phytochemicals including alkaloids, saponins, and tannins.

The reducing power of a compound may act as a momentous indicator of its potential antioxidant activity (Pal R et al., 2011). Samples with elevated reducing power are better capable to donate the electron and free radical from stable substance by accepting the donated electrons, resulting in the termination of radical chain reaction (Deori M et al., 2014).

The experiment showed with IC_{50} of the extract is 211081.58µg/ml, whereas IC_{50} of Ascorbic acid is 14.15µg/ml. When the IC_{50} value of extract compared with the standard ascorbic acid it seems a large value. Which unfortunately poses that, the extract offers no antioxidant activity (Wasim M et al., 2015). But, the extract presents a great positive effect on mice with alloxen induced disturbance in glucose tolerance.

Alloxan is a popular diabetogenic agent hydrophilic in nature and chemically unstable pyrimidine derivative, which harms pancreatic β-cells because it can generate toxic free oxygen radicals during redox cycling in the presence of reducing agents such as glutathione and cysteine (Wasim M et al., 2015).

Experimental data were presented as mean ± SEM. By using the Dunnett test significant differences (*p<0.05, **p<0.01, ***p<0.001) between the means were determined compare to control group where n=04. For statistical evaluation IBM-SPSS software version 20 was utilized.

Conclusion

Phytochemical analysis of Spinacia oleracea L. displayed the presence of alkaloid, saponin and tannin types of compounds. The results stated above showed that the methanolic extract of Spinacia oleracea L. possessed no antioxidant effect and very good anti-diabetic properties. However, this can't be confirmed without further higher and specific tests. So, further researches should be conducted to get information about these activities.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

The authors are grateful to Bangladesh University for providing the space for research and necessary lab equipments.

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