Research Article

Improvement of Antioxidant Activity and Related Compounds in Fenugreek Sprouts through Nitric Oxide Priming

Palash Mandal*, Saran Kumar Gupta
Plant Physiology and Pharmacognosy Research Laboratory, Department of Botany, University of North Bengal, Siliguri, India.
***Corresponding author's E-mail:** nbubotanypm@gmail.com

Accepted on: 07-03-2014; **Finalized on:** 30-04-2014.

ABSTRACT

Nitric oxide (NO) is an important signalling molecule in plants. In the present work, the effect of some nitric oxide donors namely, sodium nitroprusside (SNP), sodium nitrite (NN) and potassium ferricyanide (FCN) on the antioxidant activity of *Trigonella foenum-graecum* L. was studied during germination. The treated seeds were germinated in dark for 72 h and the antioxidant activity were determined at interval of 24 h up to 3 days. The *in vitro* antioxidant activity was determined spectrophotometrically by the following methods, DPPH, ABTS, nitric oxide, anti-lipid peroxidation, beta-carotene bleaching, ferric reducing power and metal chelating as well as phytochemicals such as phenol, flavonol and carotene content were also evaluated. The results demonstrated that the seeds treated with nitric oxide donors showed gradual increase in their antioxidant potential from 24 h to 48 h and then declined at 72 h stages. Overall, the seeds treated with sodium nitroprusside and potassium ferricyanide showed higher antioxidant potential particularly at concentration 80 mM and 40 mM respectively, when compared with control. Our data supported the hypothesis that NO is a signalling molecule that plays an important role as an antioxidant component in plants.

Keywords: Antioxidant, Germination, Nitric oxide, Phenolics, *Trigonella foenum-graecum.*

INTRODUCTION

Trigonella foenum-graecum commonly known as fenugreek, being rich in antioxidants and phytochemicals has been traditionally used as a food, forage and medicinal plant.[1-2] The pharmacological and folkloric uses of different plant parts of fenugreek have been reported by different researchers. Its seeds have been reported to have anti-diabetic,[3-4] anti-cancerous,[5] anti-inflammatory[6] and antioxidant activity.[7] Its leaves have been reported to possess potential anti-bacterial activity,[8] anti-diabetic[9] and antioxidant property.[10] Randhir *et al.,*[11] have also reported about the presence of potential antioxidant activity in the sprouts of fenugreek.

Nitric oxide is a short- lived bioactive molecule,[12] which is considered to function as prooxidant as well as antioxidant in plants.[13] Nitric oxide molecule is now recognized as an important signaling molecule and reported to be involved in various key physiological processes such as abiotic stress tolerance,[14] plant defense mechanism,[15] germination,[16] growth and development of plants[17] etc. In the cited study it was also shown that plant response to such stressors like drought, high or low temperature, salinity, heavy metals and oxidative stress, is regulated by NO. [13, 18]

Free radicals, such as reactive oxygen and nitrogen species, are an integral part of normal physiology. An over-production of these free radicals occurs, due to increase in oxidative stress brought by the imbalance of the bodily antioxidant defence system and free radical formation.[19] These highly reactive species on reaction with biomolecules can cause cellular injury and even death. This may lead to the development of several disorders and chronic diseases such as cancers, Alzheimer's and Parkinson's diseases and those related to cardiac and cerebrovascular systems.[20]

Antioxidant compounds play vital role in protecting cell against destructive chemical compounds such as free radicals and reactive oxygen species (ROS) that are constantly produced by the cell metabolism and their concentration increases under stress conditions.[21] Phenolics and flavonoids are considered to be very important antioxidant components, which play very important roles in the prevention of human oxidative damages. [22-23]

In recent times seed sprouting is gaining more significance commercially because it enhances the nutritional value of the seed. A large number of chemical changes occur to mobilize the stored carbohydrates and protein reserve into the germinating sprout.[11,24] Sprouting also removes some anti-nutritive factors such as enzyme inhibitors from the seed that make sprouts safe for consumption. Sprouting in fenugreek is known to improve its soluble protein and fibre content and reduce the phytic, tannic acid and trypsin inhibitors.[24]

The purpose of the present study was to evaluate the effect of nitric oxide donor namely, sodium nitroprusside, potassium ferricyanide and sodium nitrite on the antioxidant activity of *Trigonella foenum-graecum* sprouts germinated under dark condition. To our knowledge, this is the first report on the effect of nitric oxide donors in counteracting oxidative stress in fenugreek sprouts.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

MATERIALS AND METHODS

Materials and treatment

The fenugreek seeds were sterilized with 4% sodium hypochlorite solution. The sterilized seeds were treated with the solutions of nitric oxide donors: sodium nitroprusside (SNP), potassium ferricyanide (FCN), and sodium nitrite (NN) at five different concentrations: 10 mM, 20 mM, 40 mM, 80 mM and 120 mM and kept in rotary shaker for 24 hours. For control set, seeds were primed with normal water and kept in rotary shaker along with the treated seeds. After treatment the seeds were washed thrice with sterile water and kept in the seed germinator for 72 hours.

Preparation of extract

The sprouts of fenugreek of 3 different stages: 24h, 48h & 72h were crushed in mortar-pestle and processed through soxhlet extraction apparatus with methanol. The refluxed samples were separated from the residues by filtering through Whatman No. 1 filter paper and the extract was concentrated to a definite concentration of 1g/ml using a vacuum rotary evaporator. The obtained methanolic extracts were stored in brown bottles and kept in refrigerator for further experimental analysis.

Animal material

Goat liver, used for anti-lipid peroxidation assay, was collected from slaughter house immediately after slay and the experiment was conducted within an hour after collection.

Chemicals and reagents

Methanol, 2.2-diphenyl-1-picryl hydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), phenazine methosulphate (PMS), sulphanilamide, glacial acetic acid, napthyl ethylenediamine dihydrochloride, potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), thiobarbituric acid (TBA), $FeSO_4.7H2O$, potassium hydroxide (KOH), potassium dihydrogen phosphate (KH_2PO_4), ethylene-diamine tetra acetic acid (EDTA), 2-deoxyribose, potassium ferricyanide, ferric chloride ($FeCl_3$), ferrous chloride ($FeCl_2$), ferrozine, hydrogen peroxide (H_2O_2), sodium nitroprusside, gallic acid, quercetin, catechol, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), hydrochloric acid, potassium chloride, sodium acetate, β-carotene, chloroform, Tween 20 and linoleic acid were either purchased from Sigma Chemicals (USA), or of Merck analytical grade.

DPPH based free radical scavenging activity (DPPH)

The radical scavenging activity of the sprout extracts were measured by DPPH method.[25] The reaction mixture contained 1.8 ml of 0.1mM DPPH and 0.2 ml of methanolic extracts. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was considered as control.

Radical scavenging activity was expressed as percent inhibition from the given formula:

Percentage inhibition of DPPH radical = $[(A_0 - A_1)/A_0] \times 100$.

Where, A_0: absorbance of the control and A_1: absorbance of the extract or standard. Then percentage inhibitions were plotted against concentration and from the graph, IC_{50} was calculated.

Reducing antioxidant power (FRAP) Assay

The reducing antioxidant power of methanolic extracts was determined by the standard method.[26] Different concentrations of 1 ml of methanolic extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis. Increased absorbance of the reaction mixture indicates increase in reducing power.

Metal chelating activity (MC)

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al.*, [27] with slight modification. To 0.4 ml of methanol extract, 1.6 ml of methanol was diluted and mixed with 0.04 ml of $FeCl_2$ (2 mM). After 30s, 0.8 ml ferrozine (5 mM) was added. After 10 min at room temperature, the absorbance of the Fe^{2+}–Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as

Chelating rate (%) = $(A_0 - A_1) / A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in presence of the extract.

Nitric oxide scavenging activity (NOS)

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction.[28] 320 µL methanol extract, 360 µL (5 mM) sodium nitroprusside-PBS solution, 216 µL Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthyl ethylenediamine dihydrochloride) was mixed and incubated at 25°C for one hour. Lastly 2 ml water was added and absorbance was taken at 546 nm.

Radical scavenging activity was expressed as percent inhibition from the given formula: percentage inhibition of NO radical = [(Abs. of control – Abs. of sample) / Abs. of control] x 100.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

$ABTS^+$ radical cation(s) decolorization assay

The spectrophotometric analysis of $ABTS^+$ radical cation(s) scavenging activity was determined according to Re *et al.*[29] method with some modification. This method is based on the ability of antioxidants to quench the $ABTS^+$ radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of BHT. The $ABTS^+$ was obtained by reacting 7 mM $ABTS^+$ radical cation(s) in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 6 hrs. Before usage, the $ABTS^+$ solution was diluted to get an absorbance of 0.750 ± 0.025 at 734 nm with sodium phosphate buffer (0.1 M, pH 7.4). Then, 2 ml of $ABTS^+$ solution was added to 1 ml of the methanolic extract. After 30 min, percentage inhibition at 734 nm was calculated for each concentration, relative to a blank absorbance. Solvent blanks were run in each assay.

The $ABTS^+$ scavenging was calculated using the following formula:

$ABTS^+$ scavenging effect (%) = (1- As/Ac) × 100

Where A_C is the initial concentration of the $ABTS^+$ radical cation(s) and A_S is absorbance of the remaining concentration of $ABTS^+$ radical cation(s) in presence of the extract.

Anti-lipid peroxidation (ALP) assay

The anti-lipid peroxidation activity of the methanolic extracts of different developmental stages of sprouts was determined by the standard method[30] followed by slight modification with the goat liver homogenate. 2.8 ml of 10% goat liver homogenate, 0.1 ml of 50 mM $FeSO_4$ and 0.1 ml extract was mixed. The reaction mixture was incubated for 30 minutes at 37°C. 1 ml of reaction mixture was added with 2 ml 10% TCA-0.67% TBA in acetic acid (50%) for terminating the reaction. Mixture was boiled for 1 hour at 100°C and centrifuged at 10,000 rpm for 5 minutes. Supernatant was taken for absorbance at 535 nm.

ALP % was calculated using the following formula:

$$\text{ALP percent} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{Abs. of control}} \times 100$$

β-Carotene–linoleate bleaching (BCB) assay

The antioxidant activity was evaluated based on the β-carotene bleaching protective method developed by Velioglu *et al.* [31] β-Carotene (0.2 mg in 1 ml chloroform), linoleic acid (0.02 ml) and Tween 20 (0.2 ml) were transferred into a round bottomed flask. Chloroform was totally evaporated at room temperature under vacuum at reduced pressure using a rotary evaporator. After evaporation, 50 ml of distilled water was added to the mixture, and then shaken vigorously to form an emulsion. 2 ml of emulsion was then added to 0.2 ml of methanolic extract or methanol (as control) into test tubes and immediately placed in a water bath at 50 °C. The absorbance was read at 30 min intervals for 2 h at 470 nm. Degradation rate (DR) was calculated according to first order kinetics, based on equation; [32]

$$\text{Ln } a/b \times 1/t = DR_{sample} \text{ or } DR_{standard}$$

where ln is natural log, *a* is the initial absorbance (470 nm) at time 0, *b* is the absorbance (470 nm) at 30, 60, 90 or 120 min and t is the initial absorbance (470 nm) at time 0.

Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula:

$$AA = \frac{DR_{control} - DR_{sample \text{ or } standard}}{DR_{control}} \times 100$$

Total phenolic content (TPC)

Total phenolic contents of sprout extracts were determined according to the standard protocol. [33] 1 ml of the various methanolic extracts was mixed in a test tube containing 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin-Ciocalteu reagent. The resultant mixture was allowed to react for 5 min and 1 ml of 5% Na_2CO_3 was added. It was mixed thoroughly and placed in dark for 1h. Finally the absorbance of coloured reaction product was measured at 765nm against the reagent blank. The total phenolic content was expressed as mg of gallic acid equivalent per gram fresh weight.

Total flavonoid content (TFC)

The flavonoid contents were measured following a standard Spectrophotometric method.[34] 1 ml of methanolic extracts was diluted with water (4ml) in a 10 ml volumetric flask. Initially, 5% $NaNO_2$ solution (0.3ml) was added to each volumetric flask; at 5 min, 10% $AlCl_3$ (0.3ml) was added; and then after 6 min, 1M NaOH (2ml) was added. Next water (2.4ml) was added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm. The total flavonoid content in different extracts was calculated as quercetin equivalent (QE) per gm fresh weight.

Total carotene estimation

Total carotene contents (TCC) were determined according to the standard protocol.[35] Firstly, the methanolic sample was dried and converted to aqueous extract which was further partitioned thrice with equal volume of peroxide free ether using a separating funnel. The ether layer was collected and evaporated and then after reconstituted with ethanol. To the ethanolic extract 0.1 ml of 60% aqueous KOH was added and heated for 5 min with test tubes covered with marbles. The mixture was kept in dark for overnight. Next day again the mixture was partitioned with ether, the ether layer was evaporated and reconstituted with ethanol and the absorbance of the ethanolic mixture was recorded at 450nm. The carotene

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

content was calculated using a calibration curve prepared against pure β-carotene.

Statistical Analysis

Each experiment was performed in triplicate. The software package, MS Excel 2007 (Microsoft, Redmond, WA, USA) was used for comparing the antioxidant attributes of different developmental stages of the fenugreek sprouts. The different group means were compared by Duncan's Multiple Range Test (DMRT) through DSAASTAT software (version 1.002; DSAASTAT, Peruglia, Italy); $p < 0.05$ was considered significant in all cases. Smith's Statistical Package version 2.5 (prepared by Gary Smith, CA, USA) was used for determining the IC_{50} values of antioxidants and their standard error of estimates (SEE). Correlation coefficient analysis was conducted using SPSS (Version 12.00, SPSS Inc., Chicago, IL, USA) to indentify the relationship patterns between antioxidant activity and phytochemical attributes.

RESULTS AND DISCUSSION

The health benefits of phenolic compounds have been widely investigated. In human beings phenolics are known to exhibit strong antioxidant activity, which is involved in prevention of oxidative damage to biomolecules like DNA, proteins and lipids that may result in chronic diseases, such as cancer and cardiac disorders.[36] The present research aimed at improving the phenolic content and related antioxidant properties of fenugreek through elicited sprouting. Antioxidant compounds are very much effective in inhibiting the processes involved in peroxidation such as: neutralizing existing free radicals in the system, inhibiting the peroxidation catalysis by metal ions and also through breaking the lipid-radical chain reactions. [37]

Antioxidant activity of the sprouts was measured in terms of their free radicals such as DPPH, ABTS, and nitric oxide scavenging potential; metal chelation, lipid peroxidation, reducing power and beta-carotene protective activity. The determination of antioxidant activity is important for assessing the nutritional value of the extracts.[38] The antioxidant activity in elicited fenugreek sprout extract was significantly improved over control. The elicitation showed a gradual increase in free radical scavenging activity for DPPH and ABTS from 24h stage to 48h stage. In comparison to control set the best IC_{50} value was exhibited by sodium nitroprusside (80mM) and potassium ferricyanide (40mM) (Table 1-3). In case of ABTS assay, sodium nitroprusside (80mM) and potassium ferricyanide (20mM) exhibited the highest scavenging activity among all other treatments in all 3 stages (Table: 1-3). In case of nitric oxide radical scavenging assay, all the treated sprouts showed zero activity at initial stage (24hrs) later it was enhanced but pulsing with sodium nitroprusside showed better scavenging properties. The result demonstrated that the antioxidant activity of the sprouts gradually increased from 24h stage to 48h stage and there after decreased towards 72h stage.

The reducing capacity of a biological compound acts as a significant indicator of its antioxidant potential. In the reducing power assay, the antioxidant components convert the oxidized form of iron (Fe^{+3}) from ferric to ferrous (Fe^{+2}) chloride.[39] The reducing ability of the sprouts was determined with ascorbic acid equivalent. Higher ascorbic acid equivalent value indicates higher reducing capacity of samples. It was observed (Table: 1-3) that the sprouts treated with sodium nitroprusside (80mM) and potassium ferricyanide (40mM) showed enhanced reducing activity with ascorbic acid equivalent value 768.30 mg/g FW (48h) and 1017.40 mg/g (fresh weight) FW (48h) respectively when compared to that of control with 689.80 mg/g FW (48hrs).

Another important antioxidative mechanism involves the chelation of transition of metal ions such as copper and ferrous ions, which prevent the participation of these metal ions in Fenton and Haber–Weiss reactions further inhibiting the generation of highly reactive hydroxyl radicals.[40] The antioxidant components which are efficient in ferrous ion chelation helps in mobilization of iron present in tissues by forming soluble, stable complexes that are excreted along with the faeces and urine.[41-42] Hence chelation therapy has been implemented in reducing iron-related complications such as thalassemia which is characterized by iron overload in vital body organs.[43-44] Here the sprouts treated with SNP at 40mM concentration exhibited the best metal chelating activity among all the treatments and control on day 2 (48h).

The β-carotene bleaching assay estimates the ability of the extract to function at a lipid water interface to inhibit the bleaching of β-carotene by H_2O_2 catalyzed oxidation.[45] The antioxidant activity of the fenugreek sprouts was evaluated by BCB method; it was found that 20mM SNP primed sprouts showed the best β-carotene protective capacity at 24h (12.39%), 48h (47.72%) and 72h (23.42%) stages. Lipid peroxidation is the oxidative degradation of lipids through peroxide formation which induces severe damage to adjacent bio-molecules in cells causing cell death. Thus, the inhibition of such destructive processes is very much important for the protection of the living system.[46] The sprouts elicited with 10mM potassium ferricyanide exhibited the highest activity against lipid peroxidation on day 1 (24h) and day 3 (72h), though other treatments also showed significant enhancement in comparison to control sprouts.

For phytochemical analysis, total phenol, flavonol and carotene content were determined. The total phenol and flavonol content of fenugreek sprouts was estimated for 3 days of dark germination. The highest stimulation was observed at 72h stage for control as well as the different elicitors used in this study. The significant enhancement was observed at different concentrations for the different elicitors. Among the different concentrations applied, sodium nitroprusside at 120mM, 10mM potassium ferricyanide and 40mM sodium nitrite exhibited the

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

highest phenolic content which was 11%, 28 % and 29% higher than control on 72 hours germination phase respectively (Table 1-3). From flavonol estimation, it was observed that sodium nitroprusside concentration treated 80mM exhibited the highest flavonol content on day 3 of germination. Maximum flavonol stimulation was observed for the 20 mM potassium ferricyanide and 80mM sodium nitrite treatment on day 3 of dark germination that too was higher than control on the same day (Table 1-3). For all treatments the total phenol and flavonol contents were higher compared to control on 3 days germination stage. The phenol and flavonol content of all treatments and control fluctuated throughout all stages of germination except in case of potassium ferricyanide where gradual increase was observed throughout germination phases.

Table 1: Antioxidant activity and phytochemical constituents of fenugreeks sprouts treated with different nitric oxide donors at 24 h stage

24 H STAGE GERMINATION										
TREATMENT	DPPH(mg/ml)	ABTS(mg/ml)	MC(mg/ml)	FRAP (mg AAE/gm FW)	NOS(mg/ml)	ALP(mg/ml)	BCB (%)	TPC (mg GAE/gm)	TFC(mg QE/gm)	TCC(mg BCE/gm)
HYDRO	101.04±9.55b	95.52±8.63ef	159.94±17.22abc	633.90±65.44bc	3057.06±298.65b	584.40±54.22ef	6.58±0.912ef	0.0739±0.002g	0.008±0.002ab	NA
SNP-10	65.78±6.23a	57.47±6.25cd	196.89±19.67c	797.30±71.02a	1992.93±191.26a	205.51±21.77ab	11.06±0.23ab	0.084±0.003efg	0.007±0.002b	NA
SNP-20	53.90±5.11a	22.96±3.16a	144.57±13.43ab	754.50±69.87ab	NA	230.50±21.09ab	12.39±0.259a	0.0791±0.002fg	0.008±0.002ab	NA
SNP-40	67.31±6.47a	49.58±5.79bc	388.41±36.56e	622.00±59.08bc	NA	285.41±26.45bc	6.82±0.83def	0.088±0.003def	0.011±0.003ab	0.0374±0.004b
SNP-80	52.62±5.59a	10.85±9.41a	179.38±18.24bc	664.10±63.15b	NA	285.63±27.12bc	10.4±0.92abc	0.095±0.003cde	0.010±0.003ab	NA
SNP-120	61.52±6.92a	30.14±3.12ab	197.34±21.01c	693.70±68.12ab	NA	361.94±27.66cd	NA	0.095±0.003cde	0.007±0.002b	0.0036±0.001d
FCN-10	58.15±5.87a	97.99±9.55ef	286.41±25.34d	653.80±62.13b	2037.55±198.23a	180.78±17.56a	8.89±0.73bcd	0.1185±0.111a	0.011±0.003ab	0.0145±0.002c
FCN-20	55.05±4.97a	79.09±8.33de	124.49±12.44a	669.70±65.24b	1991.98±193.44a	196.90±18.33ab	10.2±1.26abc	0.1124±0.010ab	0.013±0.003a	0.0507±0.005a
FCN-40	54.69±5.58a	114.43±12.4fg	185.82±19.13bc	666.80±67.11b	NA	395.87±38.22d	10.2±1.72abc	0.106±0.00398bc	0.007±0.002b	NA
FCN-80	58.15±5.83a	95.07±9.35ef	175.17±18.55bc	643.38±61.42b	NA	511.11±52.87e	8.7±0.752cde	0.091±0.003def	0.006±0.001b	0.0325±0.002b
FCN-120	86.63±9.25b	109.45±10.1fg	171.71±19.02abc	517.48±49.01c	NA	427.47±41.28d	7.85±0.516de	0.101±0.004bcd	0.007±0.002b	NA
NN-10	53.20±5.33a	122.21±12.66g	205.47±22.31c	650.50±58.12b	NA	351.94±36.21cd	11.85±1.713a	0.1114±0.005ab	0.007±0.002b	0.0508±0.002a
NN-20	62.05±6.88a	161.06±15.81h	267.45±25.15d	345.64±32.11d	NA	278.02±28.34bc	8.99±0.76bcd	0.101±0.005bcd	0.009±0.003ab	NA
NN-40	65.60±7.12a	155.75±14.92h	168.54±16.13abc	140.39±13.09e	NA	410.96±42.44d	10.2±1.81abc	0.1060±0.004bc	0.011±0.003ab	NA
NN-80	57.83±5.54a	153.75±16.24h	162.34±15.32abc	121.22±14.25e	NA	549.51±53.89ef	NA	0.085±0.003efg	0.007±0.002b	0.0544±0.004a
NN-120	66.68±7.01a	151.77±15.75h	174.98±18.78bc	166.96±17.13e	NA	625.00±70.11f	5.64±0.632f	0.096±0.004cde	0.007±0.002b	NA

Results are expressed as Mean ± SEM of triplicate determinations. Values with different letters (a, b, c, etc.) differ significantly ($p \leq 0.05$) by Duncan's Multiple Range Test (DMRT).

In a nut shell, it was observed that among all treatments, sodium nitroprusside and potassium ferricyanide elicited the higher total phenolic content which was higher than control. Sodium nitroprusside and potassium ferricyanide treated sprouts showed consistency in their antioxidant activity but sodium nitrate treated sprouts exhibited relatively inconsistent antioxidant activity as we can see absence of beta-carotene protective activity at 80mM concentration at each stage (Table 1-3).

It is found that the efficiency of plant phenolics in protection against oxidative stress depends on their reactivity towards reactive oxygen species (ROS). Reduction of phenoxyl radicals by the intracellular reductants is well-known to recycle phenolic antioxidants, thus enhancing antioxidant property.[47] Sprouting significantly enhanced the antioxidant activity for control and all treatments. Highest antioxidant activity was observed during 48h stage confirming that the molecules

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

responsible for antioxidant activity were elicited appropriately at this stage. Another reason could be that, during initial germination stages there is a higher oxygen demand and therefore phenolics might be involved in protecting the cells from possible oxidation-induced damages.[48] Higher antioxidant activity was observed in treatments compared to control during late stages of germination indicates that most phenolics under natural conditions are diverted towards lignification with growth which was also observed during elicitation of corn.[49] Whereas in the case of treatments with nitric oxide elicitor, perhaps the stress imposed by the elicitors reduces the amount of phenolics being partitioned for lignification because some are utilized for antioxidant function against the ROS generated. It may also be same phenomena for the sprouts of 72h exhibited considerably high phenolics content but low antioxidant activity compared to 48h sprouts; it may be due to less utilization of phenolics against oxidative stress and mostly for secondary growth.

Table 2: Antioxidant activity and phytochemical constituents of fenugreeks sprouts treated with different nitric oxide donors at 48h stage

48 H STAGE GERMINATION										
TREATMENT	DPPH(mg/ml)	ABTS(mg/ml)	MC(mg/ml)	FRAP (mg AAE/gm FW)	NOS(mg/ml)	ALP(mg/ml)	BCB (%)	TPC (mg GAE/gm)	TFC(mg QE/gm)	TCC(mg BCE/gm)
HYDRO	77.08±6.53c	73.93±8.03hi	141.69±14.26abc	689.80±65.99cde	12884.8±256.65h	315.91±17.34bc	20.64±3.92de	0.0735±0.007bc	0.0046±0.0003g	0.0330±0.0029gh
SNP-10	107.43±11.2de	36.19±3.23bcd	161.94±17.47bc	660.97±64.02de	5963.48±118.26d	272.00±13.02ab	45.67±4.23a	0.0607±0.006c	0.005±0.003fg	0.2222±0.0261a
SNP-20	92.90±8.11d	30.25±3.56bc	254.01±23.52d	746.50±73.66bcd	7346.93±146.26g	428.76±21.09e	47.72±5.29a	0.0613±0.006c	0.004±0.003gh	0.0360±0.0033gh
SNP-40	59.58±5.62b	24.15±2.69ab	118.25±17.77a	666.60±65.19de	4312.35±89.39c	814.55±38.64g	27.19±2.82a	0.0756±0.007bc	0.005±0.004fg	0.1791±0.0168abc
SNP-80	33.55±3.52a	9.67±1.24a	145.20±15.32abc	768.30±68.15bcd	6947.67±139.42f	296.53±14.18b	32.32±3.91cd	0.0936±0.008b	0.008±0.005cd	0.0027±0.0002h
SNP-120	54.53±5.11b	52.92±5.62efg	142.32±15.61abc	710.50±69.12cd	NA	519.39±25.36f	29.77±3.26bc	0.0866±0.008b	0.007±0.005de	0.0222±0.0023gh
FCN-10	68.16±6.07bc	62.67±6.55fgh	158.14±15.23bc	825.27±82.13bc	NA	382.92±18.29de	25.69±2.33bc	0.0887±0.008b	0.008±0.005cd	0.0448±0.0047gh
FCN-20	38.00±4.32a	44.41±3.35cde	154.81±14.23abc	868.10±85.17b	NA	320.89±14.54bc	37.41±4.23cd	0.1410±0.012a	0.013±0.011a	0.1399±0.0129cd
FCN-40	33.55±4.17a	48.92±4.38def	124.12±12.09ab	1017.40±97.19a	6714.48±136.33e	494.71±24.27f	30.65±3.22b	0.1410±0.013a	0.011±0.009b	0.1095±0.0915def
FCN-80	34.74±3.23a	69.23±7.35h	148.01±13.72abc	729.30±71.33bcd	NA	959.84±46.89h	45.19±5.75bc	0.1604±0.017a	0.009±0.008c	0.0056±0.0002h
FCN-120	64.49±6.25bc	73.07±8.16hi	171.92±17.32c	759.90±69.21bcd	1946.11±46.63a	993.33±45.43h	13.20±1.52a	0.0784±0.008bc	0.007±0.005de	0.0595±0.0026fgh
NN-10	97.23±9.33de	108.30±12.01j	142.60±14.09abc	556.50±54.12ef	NA	522.00±23.28f	29.62±2.13e	0.0721±0.007bc	0.003±0.002h	0.0076±0.0003h
NN-20	111.40±12.88e	87.18±7.91i	135.28±13.15abc	492.90±48.19f	NA	397.67±19.54de	20.53±3.65de	0.0734±0.007bc	0.006±0.004ef	0.2095±0.0197ab
NN-40	76.39±7.32c	66.48±6.52gh	151.85±16.02abc	470.00±45.08f	2344.52±41.55b	266.66±11.71ab	27.88±3.82cd	0.0765±0.008bc	0.005±0.003fg	0.1595±0.0139bcd
NN-80	65.59±6.13bc	86.59±9.35i	133.28±13.11ab	448.72±44.32f	NA	222.00±12.25a	NA	0.0762±0.008bc	0.011±0.010b	0.0664±0.0043efg
NN-120	66.88±6.81bc	129.13±12.32k	158.08±16.29bc	201.77±21.24g	NA	360.32±17.11cd	34.27±4.62bc	0.0834±0.008b	0.005±0.003fg	0.117±0.0098de

Results are expressed as Mean ± SEM of triplicate determinations. Values with different letters (a, b, c, etc.) differ significantly ($p \leq 0.05$) by Duncan's Multiple Range Test (DMRT).

Previous studies have already reported that the antioxidant activity is very well correlated with the content of phenolic components. [31, 50] As demonstrated in Table: 4, our results showed strong correlation between total phenol and flavonoid with antioxidant activities such as DPPH scavenging, metal chelating and β- carotene protective activity of the sprouts. Similar trend was reported by different authors in their respective studies. [51-52] These results suggest that phenolics are efficient scavenger of free radicals as well as transition metal ion chelating agents. Flavonoids possess a chemical structure with particular hydroxyl position in the molecule that is considered to be involved in proton donating and radical scavenging mechanism.[53] However no significant correlation was found between free-radical scavenging and total carotene content of the sprouts except with β-carotene protective activity (r^2= 0.481**)** which showed highly positive correlation (Table 4).

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Table 3: Antioxidant activity and phytochemical constituents of fenugreeks sprouts treated with different nitric oxide donors at 72 h stage

72 H STAGE GERMINATION										
TREATMENT	DPPH(mg/ml)	ABTS(mg/ml)	MC(mg/ml)	FRAP (mg AAE/gm FW)	NOS(mg/ml)	ALP(mg/ml)	BCB (%)	TPC (mg GAE/gm)	TFC(mg QE/gm)	TCC(mg BCE/gm)
HYDRO	97.65±6.53bc	73.73±5.03ef	153.13±14.3bc	530.7±51.99ab	1417.5±39.65b	501.14±50.34de	14.36±0.52de	0.4479±0.00412f	0.0171±0.0013jk	0.0289±0.026c
SNP-10	150.88±11.23ef	66.12±4.23de	125.57±9.47a	539.7±52.02ab	1890.13±91.3d	462.13±46.02cde	16.19±0.63cd	0.3082±0.00294k	0.022±0.0021hij	0.0265±0.024c
SNP-20	117.68±8.11cd	36.12±2.56a	239.65±23.52e	549.6±55.66ab	2213.85±118.2ef	398.57±39.09bcd	23.42±1.29a	0.4664±0.00425e	0.0191±0.0015ij	0.0567±0.053ab
SNP-40	115.19±9.62bcd	42.81±2.69abc	151.14±16.8bc	590.38±61.19a	2550.9±149.39g	364.77±35.64bc	8.95±3.82h	0.3753±0.00371i	0.0231±0.0021hij	NA
SNP-80	161.00±13.52f	38.28±2.24ab	220.75±19.3de	519.36±50.2ab	2587.94±155.4g	529.64±51.18e	19.67±1.01b	0.4815±0.00449d	0.0342±0.0032cde	0.0628±0.062a
SNP-120	115.89±9.11bcd	47.60±3.62abc	223.13±21.6de	460.63±46.12b	2428.8±141.6fg	354.47±35.36bc	1.14±0.16jk	0.4993±0.00489c	0.0296±0.0028efg	0.0483±0.045b
FCN-10	99.27±6.07bc	87.56±6.55fg	421.17±35.23h	519.83±52.1ab	1045.30±23.24a	211.29±20.99a	10.06±0.93gh	0.5738±0.00581a	0.0323±0.0029def	0.0531±0.051ab
FCN-20	94.18±4.92b	51.46±4.35bc	184.15±17.2cd	542.57±55.2ab	1782.25±75.42cd	321.33±31.54b	13.05±0.13ef	0.405±0.00397g	0.0411±0.0039ab	NA
FCN-40	69.27±4.17a	56.31±4.38cd	102.13±10.09a	520.56±52.2ab	1097.64±27.33a	371.33±36.27bc	11.67±0.12fg	0.3302±0.00331j	0.0395±0.0037bc	0.0217±0.019c
FCN-80	71.92±4.23a	41.71±3.35ab	204.09±19.7de	592.82±58.33a	1476.66±45.63b	308.80±29.89ab	5.14±0.55i	0.5083±0.00517c	0.0374±0.0034bcd	0.0338±0.032c
FCN-120	130.53±10.25de	94.89±8.16g	294.15±27.32f	517.85±51.2ab	4277.18±191.53i	525.93±53.02e	1.06±0.12jk	0.5582±0.00548b	0.0281±0.0025fgh	0.0024±0.020d
NN-10	110.02±7.33bcd	76.45±7.01ef	131.60±12.1ab	612.93±59.12a	1615.14±59.71de	522.76±51.98e	17.53±0.63bc	0.3868±0.00379h	0.0121±0.0012k	NA
NN-20	121.79±12.88d	86.19±7.91fg	129.09±11.2ab	541.36±53.2ab	2005.81±104.29de	876.94±88.54g	18.65±0.75b	0.3803±0.00037hi	0.0243±0.0024ghi	0.0072±0.069d
NN-40	197.07±17.32g	149.68±12.52h	362.5±26.02g	324.12±32.08c	1133.33±22.55a	690.66±68.71f	4.84±0.42i	0.5769±0.00568a	0.0221±0.002hij	NA
NN-80	131.46±10.13de	83.81±9.35fg	297.25±23.11f	524.29±52.3ab	2854.63±175.42h	510.36±50.25e	NA	0.4651±0.00457e	0.0458±0.0042a	0.0036±0.033d
NN-120	117.57±8.81cd	74.22±8.32ef	359.53±32.3g	206.31±21.24c	NA	457.97±45.11cde	2.82±0.12ij	0.5630±0.00558b	0.0223±0.0021hij	NA

Results are expressed as Mean ± SEM of triplicate determinations. Values with different letters (a, b, c, etc.) differ significantly ($p \leq 0.05$) by Duncan's Multiple Range Test (DMRT).

Table 4: Pearson Correlation coefficients between various phytochemical attributes and antioxidant activity of fenugreek sprouts of different developmental phase.

	DPPH	TPC	TFC	MC	FRAP	ABTS	NOS	TCC	ALP
TPC	0.692**								
TFC	0.510**	0.842**							
MC	0.339*	0.461**	0.322*						
FRAP	-0.351*	-0.263	-0.176	-0.240					
ABTS	0.096	-0.054	-0.139	0.161	-0.659**				
NOS	0.090	-0.007	-0.014	-0.092	0.348*	-0.343*			
TCC	-0.071	-0.252	-0.240	-0.219	0.108	-0.192	0.168		
ALP	0.186	0.104	-0.023	-0.155	-0.051	0.161	-0.025	-0.061	
BCB	-0.207	-0.334*	-0.370**	-0.341*	0.413**	-0.341*	0.337*	0.481**	0.137

Figures in bold indicate significant correlation; (*, **) = significant at $p < 0.05$ or 0.01, respectively.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Maisarah *et al.*,[54] have also claimed a strong correlation between carotene content and β- carotene protective activity. Though a significant correlation was established between phenolics and antioxidant activities such as DPPH scavenging, metal chelating and β- carotene protective activity, on the other hand, insignificant correlation was also found between phenolics and other antiradical activity. This might be due to the variation in the stoichiometry of the reactions between the antiradical components in the sprout extracts and the various radicals, which may be considered as a probable reason for the difference in their scavenging potential of these free radicals.[55]

CONCLUSION

In conclusion, the present study has illustrated significant increase of antioxidant activity along with phenolics content by elicited sprouting in fenugreek with sodium nitroprusside and potassium ferricyanide. When analyzing the time course of germination the stimulatory effect of nitric oxide donors was most pronounced at the early phases of germination i.e. from 24h to 48h and after that the action was declined. The work supports the hypothesis that nitric oxide donors offer significant role in enhancement of phenolics and antioxidant activity during the germination phase of fenugreek. This knowledge can be used to design the sprouting techniques of fenugreek for 48h stage for improving the nutritional and health-relevant functional value of legume sprouts with these elicitors.

Acknowledgement: The authors are grateful to the Department of Science and Technology (DST) for providing financial support for this research work under INSPIRE Scheme.

REFERENCES

1. Petropoulos GA, Fenugreek - The genus *Trigonella*, Taylor and Francis, London and New York, 2002.
2. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M, Therapeutic applications of fenugreek, Alternative Medicine Review, 8, 2003, 20-27.
3. Xue WL, Xuan-She L, Zhang J, Yong-Hui L, Zhi-Lun W, Rui-Juan Z, Effect of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats, Asia Pacific Journal of Clinical Nutrition, 16 (1), 2007, 422-426.
4. Karim A, Nouman M, Munir S, Sattar S, Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes, International Journal of Pharmacology, 7, 2011, 419-439.
5. Shabbeer S, Sobolewski M, Anchoori RK, Kachhap S, Davidson N, Hidalgo M, Fenugreek: a naturally occurring edible spice as an anticancer agent, Cancer Biology & Therapy, 18, 2009, 272-8.
6. Subhashini N, Nagarajan G, Kavimani S, Anti-inflammatory and *in vitro* antioxidant property of *Trigonella foneum graecum* seeds, J Pharmacological and Toxicological methods, 6(4), 2011, 371-380.
7. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI, *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds, Food Chemistry, 103, 2007, 31–37.
8. Yasmin H, Kaisar MA, Sarker Md, Moklesur R, Rahman MS, Rashid MA, Preliminary Anti-bacterial Activity of Some Indigenous Plants of Bangladesh, Journal of Pharmaceutical Sciences, 8(1), 2009, 61-65.
9. Mitra A, Bhattacharya D, Dose-dependent effects of Fenugreek composite in Diabetes with dislipidaemia, Internet Journal of Food Safety, 8, 2006, 49-55.
10. Gowda TSS, Dinesha R, Harsha R, Srinivasa L, Free radical scavenging activity of lutein – isolated from methi leaves(*Trigonella foenum graecum*), International Journal of Pharmacy and Pharmaceutical Science, 2, 2010, 2.
11. Randhir R, Lin YT, Shetty K, Phenolics their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors, Asia Pacific Journal of Clinical Nutrition, 13(3), 2004, 295-307.
12. Zhang LG, Wang YD, Zhao LQ, Shi SY, Zhang LX, Involvement of nitric oxide in light-mediated greening of barley seedlings, Journal of Plant Physiology, 163, 2006a, 818–826.
13. Kopyra M, Gwozdz EA, Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*, Plant Physiology and Biochemistry, 41, 2003, 1011-1017.
14. Ruan HH, Shen WB, Ye MB, Xu LL, Protective effects of nitric oxide on salt stress -induced oxidative damages to wheat (*Triticum aestivum*) leaves, Chinese Science Bulletin, 47, 2002, 677–681.
15. Delledonne M, Zeier J, Marocco A, Lamb C, Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response, proceeding of the National Academy of Sciences, USA, 98, 2001, 13454-13459.
16. Sarath G, Bethke PC, Jones R, Baird LM, Hou G, Mitchell RB, Nitric oxide accelerates seed germination in warm-season grasses, Planta, 223, 2006, 1154-1164.
17. Pagnussat GC, Simontacchi M, Puntarulo S, Lamattina L, Nitric oxide is required for root organogenesis, Plant Physiology, 129, 2002, 954–956.
18. Zhao LQ, Zhang F, Guo JK, Yang YL, Li BB, Zhang LX, Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed, Plant Physiology, 134, 2004, 849–857.
19. Wang SY, Antioxidant capacity of berry crops, culinary herbs and medicinal herbs, Acta Horticulturae, 620, 2003, 461–473.
20. Wiseman H, Halliwell B, Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Journal of Biochemistry, 313, 1996, 17–29.
21. Hassimotto NMA, Genovese MI, Lajolo FM, Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps, Journal of Agricultural Food Chemistry, 53, 2005, 2928–2935.
22. Mandal P, Ghosal M, Antioxidant activities of different parts of tree tomato fruit, International Journal of Pharmaceutical Sciences Review and Research, 13(2), 2012, 39-47.
23. Andarwulan N, Batari R, Sandrasari DA, Bolling B, Wijaya N, Flavonoid content and antioxidant activity of vegetables from Indonesia, Food Chemistry, 121, 2010, 1231–1235.
24. Mansour EH, El-Adawy TA, Nutritional potential and functional properties of heat-treated and germinated fenugreek seeds, Lebensmittel-Wissenschaft & Technology, 27(6), 1994, 578-572.
25. Blois MS, Antioxidant determinations by the use of a stable free radical, Nature, 181, 1958, 199-1200.
26. Oyaizu M, Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucoamine, Japanese Journal of Nutrition, 4, 1986, 307-315.
27. Dinis TCP, Madeira VMC, Almeidam LM, Action of phenolic derivates (acetaminophen, salycilate, and 5-aminosalycilate) as

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

inhibitors of membrane lipid peroxidation and peroxyl radical's scavengers, Archives of Biochemistry and Biophysics, 315, 1994, 161.

28. Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A, Gardes-Albert M, Antioxidant action of Ginkgo biloba extracts EGb 761. Methods in Enzymology, 234, 1994, 462-475.

29. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Biology Medicine, 26, 1999, 1231.

30. Bauchet N, Barrier L, Famconneu B, Radical scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae), Phytotherapy Research, 12, 1998, 159-162.

31. Velioglu YS, Mazza G, Gao L, Oomah BD, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, Journal of Agricultural Food Chemistry, 46, 1998, 4113–4117.

32. Al-Saikhan MS, Howard LR, Miller JCJr, Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L.), Journal of Food Science, 60, 1995, 341–343.

33. Chandler SF, Dodds JH, The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*, Plant Cell Reports, 2 , 1993, 105–10.

34. Sultana B, Anwar F, Ashraf M, Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts, Molecules, 14, 2009, 2167-2180.

35. Thimmaiah SK, Standard Methods of Biochemical Analysis, Kalyani Publishers: New Delhi, India, 2004, 304–307.

36. Newmark HL, Plant phenolics as potential cancer prevention agents. In: Dietary phytochemicals in cancer prevention and treatment, Plenum, Press; New York, 1996, 25–34.

37. Cervato G, Carabelli M, Gervasio S, Cittera A, Cazzola R, Cestaro B, Antioxidant properties of oregano (*Origanum vulgare*) leaf extracts, Journal of Food Biochemistry, 2000, 24, 453–465.

38. Rice-Evans CA, Miller JN, Paganga G, Structure antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology Medicine, 20 , 1996, 933–956.

39. Moein MR, Moein S, Ahmadizadeh S, Radical Scavenging and Reducing Power of *Salvia mirzayanii* Subfractions, Molecules, 13, 2008, 2804-2813.

40. Ademiluyi AO, Oboh G, Antioxidant properties of methanolic extracts of mistletoes (Viscum album) from cocoa and cashew trees in Nigeria, African Journal of Biotechnology, 7, 2008, 3138-42.

41. Shinar E, Rachmilewitz EA, Oxidation denaturation of red blood cells in thalassemia, Seminars in Hematology, 27, 1990, 70-91.

42. Hebbel RP, Leung A, Mohandas N, Oxidation-induced changes in microheological properties of the red cell membrane, Blood, 76, 1990, 1015-1022.

43. Rund D, Rachmilewitz E, β-thalassemia, New England Journal of Medicine, 2005, 353,1135-1146.

44. Loukopoulos D, Combined therapy with deferiprone and desferrioxamine in thalassemia major, Hematology Journal, 90, 2005, 1305-1305.

45. Siddhuraju P, Becker K, Studies on antioxidant activities of mucuna seed (Mucuna pruriens var utilis) extract and various nonprotein amino/imino acids through *in vitro* models, Journal of the Science of Food and Agriculture, 83, 2003, 1517–1524.

46. Yang J, Guo J, Yuan J, *In vitro* antioxidant properties of rutin, LWT–Food Science and Technology, 41 (6), 2008, 1060–1066.

47. Kagan VE, Tyurina YY, Recycling and Redox Cycling of phenolic antioxidants, Annal of New York Academic Science, 854, 1998, 425-434.

48. Randhir R, Lin YT, Shetty K, Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors, Journal of Process Biochemistry, 39, 2004, 637–646.

49. Randhir R, Shetty K, Developmental stimulation of total phenolics and related antioxidant activity in light- and dark-germinated corn by natural elicitors, Journal of Process Biochemistry, 40, 2005, 1721–1732.

50. Lai HY, Lim YY, Evaluation of Antioxidant Activities of the Methanolic Extracts of Selected Ferns in Malaysia, International Journal Environmental Science Development, 2(6), 2011.

51. Sultana B, Anwar F, Pirzybyiski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Euginea jambolana* Lam. Trees, Food Chemistry, 104, 2007, 1106-1114.

52. Kumar KS, Ganesan K, Rao PVS, Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) – An edible seaweed, Food Chemistry, 107, 2008, 289-295.

53. Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY, Lee MH, Free radical scavenging activity of Taiwanese native plants, Phytomedicine, 10, 2003, 170-175.

54. Maisarah AM, Nurul AB, Asmah R, Fauziah O, Antioxidant analysis of different parts of *Carica papaya,* International Food Research Journal, 20(3), 2013, 1043-1048.

55. Khan RA, Khan MR, Sahreen S, Ahmed M, Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill, Chemistry Central Journal, 6, 2012, 12.

Source of Support: Nil, **Conflict of Interest:** None.

Corresponding Author's Biography: Mr. Palash Mandal

Mr. Palash Mandal was graduated from University of Kalyani, Kalyani, West Bengal, India and post graduated also from same University. He was the recipients of gold medal at post graduation and achieved CSIR-NET fellowship from HRDG, Govt. of India. At post graduation, he has taken specialization in Plant Physiology and Biochemistry. He is having 12 years of teaching experience at North Bengal University, Siliguri, India. Currently he is working with bioactivities of plant peptides and *in vitro* antioxidant and anti-diabetic activities of medicinal and aromatic plants. At North Bengal University, he is guiding many post graduate and doctoral students.

© Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.