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Antioxidant activity of *Trigonella foenumgraecum* L. for prevention of various diseases

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Abstract

The present study aims on antioxidant potential in *Trigonella foenumgraecum* L. (Ajmer methi variety). Leaves have been used for the determination and quantification of antioxidant constituents and free radical scavenging activities for treatment and prevention of various diseases like diabetes mellitus, atherosclerosis, cataract, rheumatism, cancer and other auto immune diseases like ageing and antioxidants like phenols, flavonoids, flavonols, proanthocyanin, anthocyanin, total carotenoid, β-carotene, antioxidant enzyme systems like catalase, peroxidase, polyphenol oxidase, glutathione reductase activities and free radical scavenging assays like FRAP, ABTS, DPPH were evaluated. The results showed highest phenolic content 38.3 ± 0.5 mg/g dry wt and FRAP free radical scavenging was 10 ± 0.05 %, recorded maximum than the other assays.

Key words: Antioxidants, *Trigonella foenumgraecum*, free radical scavenging activity

Introduction

Antioxidant is a "substance that delays, prevents and removes the oxidative damage to a target molecule", like DNA, lipids and proteins are usually being damaged, due to the consequences by the action of free radicals (Halliwell and Gutteridge, 2010), and leading to prevention, protection of various diseases like cancer, diabetes, cataract, ageing, *etc*, and as an oxygen scavengers (Halliwell, 2011). Majority of the diseases and the disorders are mainly linked due to the oxidative stress by the free radicals. Medicinal plants having various phytochemicals, are involved in enhancing long-term good health (Scalbert and Williamson, 2000), and for maintaining good health in ancient times, the use of plant constituents was the only source for curing and preventing of various diseases (Higdon and Frei, 2003).

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Trigonella foenumgraecum L. belongs to the family, Leguminosae and is commonly known as (English: fenugreek, Hindi:methi). The plant consists of antioxidants which protects the organisms from the damage caused by free radical-induced oxidative stress (Goyal *et al*., 2013). The plant is used for curing diabetics, inducing low levels of cholesterol, digestive diseases, high fever, blood pressure, *etc*., and many other phytochemical compounds are present for curing various diseases (Bhatt *et al.*, 2006). The leaves are consumed widely in India. According to the National Research Council, it is a green leafy vegetable which is rich in calcium, iron, and other vitamins (Ahmadiani *et al*., 2007). *Trigonella foenumgraecum* L. leaves posseses an potential antiulcer agent, antidiabetic, hypoglysemic activity, antifungal, analgesic, anti-inflammatory, antipyretic, antistress, antihyperglycemic activity, antileukemic (Anand *et al* 2012; Acharya, 2010) and various other medicinal properties (Rakesh *et al*., 2012). The plant produces a diverse range of bioactive molecules, making it as a rich source of different types of medicines to protect against cellular damage, due to the reactive oxygen species (ROS) (Singh and Mohan Prasad, 2013; Nair *et al*., 2005.; Nadkarni, 1994.; Agarwal and Prabhakaran , 2005; Chaurasia *et al*., 1995) .

Material and Methods

Chemicals required

TPTZ (**2, 4, 6-Tri (2 - pyridyl) - *s* - triazine**), NADP (Nicotinamide adenine dinulceotide phosphate), ABTS (2,2' - azinobis3-ethylbenzthiazoline-6sulphonic acid), DPPH (2,2 -Diphenyl - 1-picryl hydrazyl) obtained from Sigma - Aldrich and E Merk.

Plant material

The *Trigonella foenumgraecum* L. plant material was collected from Agricultural Research Station, Rajendernagar, RR District, Andhra Pradesh, India.

Preparation of plant material

The leaves were picked and washed with water to remove all unwanted plant materials and sand, air dried under light exposure (27° - 30 °C for 7days), pulverised in mill, and sieved and stored in an airtight container for further use.

Preparation of extract

Ten grams of leaf powder was extracted by maceration in 100ml of methanol at 30° C for overnight , followed by extracting and stirring with 100ml of distilled water at 30° C for overnight, and centrifuged at 5000rpm for 20min and the supernatant were pooled and made up to 100ml.

Determination of phenols

Phenolic contents in the extracts were determined by the modified Folin Ciocalteau Method (Wolfek , 2003). 2ml of the leaf extract was mixed with 5ml folin-ciocalteau reagent (diluted with water 1:10 v/v) and 4ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30min at 40°C for colour development. Absorbance was then measured at 765 nm, using Shimadzu 160A UV-VIS double beam Spectrophotometer . Total phenolic content was expressed as mg/g dry wt., gallic acid equivalent.

Determination of flavonoids

Aluminium chloride colorimetric method was used for the determination of flavonoids (Chang and Win, 2002). 0.5ml of leaf extraction methanol was separately, mixed with 1.5 ml of methanol ,0.1 ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415nm with a Shimadzu 160A UV/VIS double beam Spectrophotometer.

Determination of flavonol

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakarn (2007). To 2 ml of the sample, 2 ml of 2% AlCl , ethanol and 3 ml (50g/l) sodium acetate solutions were added .The absorption was read at 440 nm by Shimadzu 160A UV-VIS double beam Spectrophotometer and then was read after 2.5h at 20°C. Extract samples were evaluated at a final concentration of 0.1mg /ml. The total flavonols content was calculated as quercetin(mg/g), using the following equation based on the calibration curve: $y = 0.0255 x$, $R^2 = 0.9812$, where x is the absorbance and y is the quercetin equivalent.

Determination of proanthocyanidins

Determination of proanthocyanidins was based on the procedure reported by Sun *et al*.(1998). A volume of 0.5 ml of the extract solution was mixed with 3ml of 4% vanillin-methanol solution and 1.5ml of HCl. The mixture was allowed to stand for 15min, the absorbance was measured with Shimadzu 160A UV-VIS double beam Spectrophotometer at 500nm.Total proanthocyanidins content were expressed as catechin equivalents (mg/g) .

Determination of anthocyanins

The total anthocyanins content was determined according to pH differential method by Giusti *et al*. (2001). The leaf extract was dissolved in potassium Chloride-hydrochloric acid buffer solution at pH 1.0 and Sodium acetate trihydrate (CH_3 COONa.$3H_2O$) buffer solution at pH 4.5. Methanolic extracts were mixed with 3.6ml of the corresponding buffers and read against water as a blank at 510 and 700nm. Absorbance was calculated, using the the formula A = [(A510-A700)-(A510-A700)]. Results are expressed in mg/g dry wt.

Extraction and determination of total carotenoids

Carotenoids were estimated by the method of Jensen (1978), One-gram fresh sample was crushed with methanol and centrifuged. Residue was discarded and supernatant was concentrated to dryness. The dried extract was dissolved in 10 ml of ether, 5 ml of 10% KOH and the mixture was kept for one hour at room temperature in dark. The ether layer was washed with 1 ml of 3% NaCl (in distilled water) for 3 times to remove alkaline methanol and dried over sodium sulphate for one hour. The absorbance of ether extract was measured at 450 nm by using Shimadzu 160A UV-VIS double beam Spectrophotometer, and was expressed as mg/g fresh wt.

Extraction of β-carotene

10g of leaf sample was rinsed with distilled water to remove sand and then cut into pieces and lyophilized to remove the moisture content. Resulting dried samples were powdered using grinder. These ground samples were extracted twice with a total volume of 100 ml of 70% aqueous methanol. The mixture was shaken on an orbital shaker for 75 min at 2500rpm and then filtered through Whatman No 1 filter paper. The combined methanolic extract was then evaporated at 55°C , using waterbath and dried to powder in a lyophilizer.

Determination of β- carotene

β- carotene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100mg) was vigorously shaken with 10ml of acetone - hexane mixture (4:6) for 1min. The absorbance of the filtrate was measured at λ = 453, 505, 645 and 663 nm by Shimadzu 160 A UV-VIS Spectrophotometer. Contents of β-carotene were calculated according to the following equations:

$$\beta\text{-carotene} = 0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}.$$

The values are expressed as mg/g of extract, where, A= Absorbance recorded at specific wavelengths.

Extraction of antioxidant enzymes

(Catalase, peroxidase and polyphenol oxidase

1 g of fresh plant material was taken and placed in a pre -cooled mortar and ground with 10ml of cold 0.05M Tris-HCl buffer (pH7.0).The extract was passed through cheese cloth and centrifuged at 1000rpm for 20 min. The supernatant was used as crude enzyme for the activities of catalase, peroxidase, and polyphenol oxidase.

Determination of polyphenoloxidase

Polyphenol oxidase was estimated as per the method of Kar and Mishra (1975). The reaction mixture consists of 2ml of Tris - HCl buffer 0.1M (pH7.0), 1ml of pyrogallol (0.01M) and 1ml of enzyme extract .The assay mixture was incubated for 5min. at 25°C. The reaction was stopped by adding 1ml of 2.5 N H_2SO_4. The absorbance at 425nm was measured by Shimadzu 160A UV-VIS double beam Spectrophotometer and the enzyme activity was expressed in absorbance units/g fresh wt.

Determination of peroxidase activity

Peroxidase activity was estimated as per the method of Kar and Mishra (1975). The reaction mixture consisted of 2ml of 0.1 M Tris -HCl buffer (pH 7.0), 1ml of pyrogallol (0.01M), 1ml of H_2O_2 (0.05M) and 1ml enzyme, 1ml of 0.05M H_2O_2 and 1ml of enzyme extract. The reaction mixture was incubated at 25°C for 5 min. The reaction was stopped by adding 1ml of 2.5 N H_2SO_4. The amount of purpurogallin formed, was estimated by measuring the absorbance at 425nm by Shimadzu 160A UV-Vis double beam Spectrophotometer.The enzyme activity was expressed in units/g fresh wt.

Determination of catalase

Catalase activity was estimated as per the method of Barber (1980). The reaction mixture consists of 1ml of enzyme, 2ml of hydrogen peroxide and 3ml of 0.05M Tris-HCl buffer (pH7.0). The reaction was stopped by 1ml of 2.5N H_2SO_4. After 5 min of incubation at 20°C, the residual H_2SO_4 was titrated with 0.05 M, $KMnO_4$. A blank was prepared by adding 1ml of 2.5N H_2SO_4 initially to the reaction mixture at zero time. Catalase enzyme activity was expressed as units / g fresh wt.

The enzyme units were calculated by using the following formula:

$$\text{Catalase activity} = 25/2 \times 0.85 \times V/W$$

where,v =difference in the titer value between control and treatment,w =Fresh weight of the sample in grams, 0.85mg of H_2O_2 = 1ml of $KMnO_4$, (0.05M).

Extraction of glutathione reductase

The leaf material was weighed separately and ground in water at a concentration of lg /5 ml. The extracts were centrifuged at 10,000 rpm for 10 min and the supernatants were kept under refrigerated conditions and used for enzyme estimations.

Determination of glutathione reductase

Glutathione reductase activity was determined according to the method of Beutler (1984). 0.2 ml of sample, 1.5ml of 0.3 M phosphate buffer (pH 6.8.). 0.5ml of 25 mM EDTA, 0.2 ml of 12.5 mM oxidized glutathione and 0.1 ml of 3 mM NADPH was added. Decrease in absorbance was measured against that of blank at 340 nm. The enzyme activity is calculated in terms of units/ mg fresh wt.

Extraction for FRAP assay

Soxhlet extraction method was employed, for the preparation of 50% alcoholic extracts of the leaf powered sample, was extracted for 6 hours. The collected solvent extract was evaporated, dried and stored at 4°C.

Ferric reducing antioxidant property (FRAP) assay

The FRAP reagent was prepared from 300 mM of sodium acetate buffer (pH 3.6), 10 mM of TPTZ solution, 40 mM HCl as a solvent, 20 mM iron (Fe^3) chloride solution in a volume ratio of 10:1 :1, respectively (Ren -You *et al*., 2009). The FRAP reagent was prepared freshly and warmed to 37°C in a waterbath before use. 100 µL of the diluted sample was added to 3 ml of the FRAP reagent, the absorbance of the reaction mixture was then detected at 593nm by using Shimadzu 160A UV-VIS double beam Spectrophotometer , after 4 min in room temperature .The standard curve was constructed by using $FeSo_4$ solution and the results were expressed in percentage.

Extraction and determination of radical scavenging by ABTS and DPPH assay

The leaf material was collected, dehydrated (in a chamber below 40°C for 48 h), powdered with a mechanical grinder and stored in an air-tight container.

The methanolic leaf extracts were prepared by adding 1 g of dry powder of the leaf materials in 100 ml methanol, further stirring at 150 rpm (steelmet incubator shaker, India) at ambient temperature for 3 h. Insoluble residues from the solutions were removed by centrifugation at 8,000 g for 10 min (cooling centrifuge) and the clear supernatants were used for analysis. The extracts were stored at 4°C in plastic vials, till further use. All the estimations were performed in triplicates.

DPPH radical scavenging activity

The DPPH radical scavenging activity was estimated by measuring the decrease in the absorbance of methanolic solution of DPPH (Brand-Willams *et al.*, 1995). In brief, 5 ml DPPH solution (3.3 mg of DPPH in 100 ml methanol), 1ml of each plant extracts were added, incubated for 30 min in the dark and the absorbance (A1) was measured at 517 nm. The absorbance (Ao) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Ascorbic acid (5-50 µg/ml) was used as a standard. Results were expressed of percentage as inhibition of DPPH radical. Scavenging ability (%) was calculated by using the formula:

DPPH radical scavenging activity (%) = $[(A0 - A1)/A0] \times 100$, or where, A0 was the absorbance of reaction control and A1 was the absorbance of extracts or standards.

ABTS radical scavenging activity

The $ABTS^{+}$ cation radical scavenging activity of the extracts was determined according to the modified method of Re *et. al*. (1999). A stock solution of ABTS was produced by mixing 7 mM aqueous solution of ABTS with Potassium persulfate (2.45 mM) in the dark at ambient temperature for 12-16 h before use. The radical cation solution was further diluted until the initial absorbance value of 0.7± 0.005 at 734 nm was reached. For assaying test samples, 0.98 ml of ABTS solution was mixed with 0.02 mL of the plant extracts. The decrease in absorbance was recorded at zero min and after 6 min. Scavenging ability relative to the reaction control (without plant extract as 100%) was calculated by using the formula.

ABTS radical scavenging activity (%) = [(Initial reading-final reading)/Initial reading] x 100, where initial reading is absorbance at zero min and final reading is absorbance for 6 min.

Statistical analysis

All results are expressed as Mean ± Standard deviation. All results are means of three replicates. The data were correlated, using Pearson correlation coefficient at $p<0.05$. SPSS 15 Version was used for the statistical analysis.

Results and Discussion

The phenol, flavonoid, flavonol, proanthocyanin, anthocyanin, total carotenoid and β-carotene content in *Trigonella foenumgraecum* ranged from 38.3±0.5 mg/g gallic acid of equivalent of dry wt., 22±0.5 mg/g dry wt., 11±0.57 mg/g dry wt., 0.9±0.3mg/g dry wt., 0.495±0.004 mg/g dry wt., 20±0.5 mg/g Fr wt ., 0.03±0.01. mg/g Fr wt (Figure 1). Among the various antioxidant secondary metabolites, phenolic content recorded highest, compare to other compounds and has high level of hydrophobic phenolic forms. Antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (Nagulender *et al.*, 2007). These phenolics appear to be effective quenchers of free radicals and they protect the tissues against elevated oxidative stress, generated during consumption of water, which contain H_2O_2. Reduction of phenoxyl radicals by reductants can recycle phenolic antioxidants, thus enhancing the antioxidant activity further.

The present study showed various methods employed and the ability of the synthesis of secondary metabolites depends on the direction of plant ontogeny. Each plant species enters its specificity into metabolic processes which results in the synthesis of various metabolites. Variations in the concentration of secondary metabolites are the result of both biotic and abiotic factors. Quantitative and qualitative analysis of secondary metabolites in plants (Stankovic *et al.*, 2010; Stankovic *et al*., 2011). Phenols are the large class of secondary metabolites (including flavonoids) are among most active antioxidants, as well as most important stabilization factors of the oxidative processes and have many pharmacological and biological properties. The catalase,peroxidase, polyphenol oxidase and glutathione reductase enzyme activity in *Trigonella foenumgraecum* ranged from 8.8±0.35 U/g fresh wt., 7.2±0.02 U/g fresh wt., 1.3±0.2 U/g fresh wt., 0.24±0.002 U/g fresh wt (Figure 2). Statistically catalase activity has shown maximum activity than the reports given by Saujanya *et al.* (2012). The FRAP, ABTS , DPPH, scavenging activity in *Trigonella foenumgraecum* ranging from 10±0.09., 0.5±0.01., 0.8±0.04 (%), however, FRAP scavenging activity was recorded the highest (Figure 3). There are many methods that differ in terms of their assay principles and experimental conditions and in particular antioxidants have varying contributions to total antioxidant potential (Cao *et al*., 1998). In this study, we used the FRAP assay because it is quick and simple to perform, to measure the antioxidant capacity of pure compounds according to their reducing ability and antioxidant power (Aneta *et al*., 2007) compared with the earlier results, had much more high FRAP activity. The assay of the plant extracts were carried out according to the protocol, the plant extracts

were determined in the form of bar chart(Acharya *et al*., 2010), showed maximum scavenging activity and was found that, there was an increase in the antioxidant activity which was proportional with the polyphenol content as shown by the increased activity. Antioxidant compounds such as polyphenols are more efficient, reducing agents for ferric iron (Wong *et al*., 2006;Vasim *et al*., 2012) and the results were showing maximum content. Studies on radical scavenging activities and its potential in *Trigonella foenumgraecum* were observed by (Saujanya *et al*., 2012 ; Nahid Sohrevardi and Firouzeh Shorevardi , 2012.; Subhashini *et al*., 2011; Gauiya Bashri *et al.*, 2013) and compararive studies on FRAP in *Trigonella foenumgraecum* (Mohamad Arfan *et al*., 2013), showed maximum radical scavenging activity and has been widely used to evaluate the free radical scavenging effect of natural antioxidants.

Table1: Antioxidant secondary metabolite content in the leaves of *Trigonella foenumgraecum* L.

S. No.	Antioxidant Secondary metabolite	Antioxidant Secondary metabolite content Mean ± SD (mg/g dry wt)
1.	Phenol	38.30 ± 0.50
2.	Flavonoid	22.00 ± 0.50
3.	Flavonol	11.00 ± 0.57
4.	Proanthocyanin	00.90 ± 0.30
5.	Anthocyanin	00.50 ± 0.04
6.	Total carotenoid	00.20 ± 0.05
7.	β-Carotene	00.03 ± 0.01

Note: Total carotenoid and β–Carotene are expressed in mg/g fresh wt.

Table 2:Antioxidant enzyme activity (U/g fresh wt) in the leasves of *Trigonella foenumgraecum* L.

S. No.	Antioxidant enzymes	Antioxidant enzyme content Mean ± SD (mg/g Fr wt)
1.	Catalase	8.80 ± 0.35
2.	Peroxidase	1.30 ± 0.20
3.	Polyphenoloxidase	7.20 ± 0.02
4.	Glutathione reductase	0.24 ± 0.002

Table 3: Antioxidant assay activties in (%) in the leaves of *Trigonella foenumgraecum* L.

S. No.	Antioxidant assays	Antioxidant assays percentage inhibition content
1.	FRAP	10.00 ± 0.09
2.	ABTS	00.50 ± 0.01
3.	DPPH	00.80 ± 0.04

Note: FRAP (Ferric reducing antioxidant property), ABTS (2,2' -azinobis3-ethylbenzthiazoline-6sulphonic acid), DPPH (2,2 -Diphenyl -1-picryl hydrazyl)

Conclusion

The aim of the study in *Trigonella foenumgraecum*, used for the present investigation has been evaluated for the antioxidant potential and radical activities. The antioxidant potential varies considerably among different radical scavenging assay, and the results showed promising source of natural antioxidant, which prevents and protects from various diseases and disorders caused by the free radical damage to the organism.

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Conflict of interest

We declare that we have no conflict of interest.

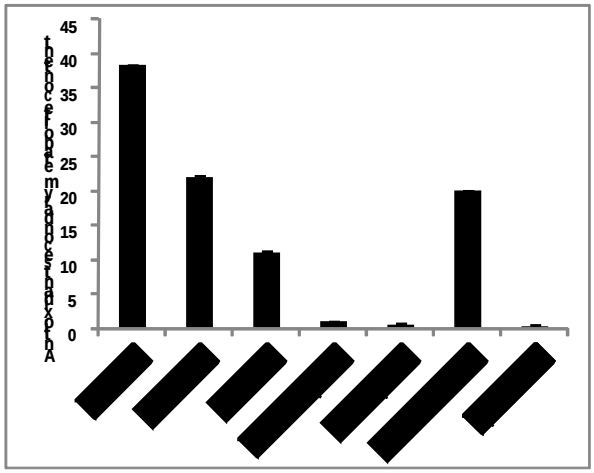

Figure 1: Showing the antioxidant secondary metabolite content expressed in mg/g dry wt, except total carotenoid and β-carotene mg/g fresh wt. in .*Trigonella foenumgraecum* L.

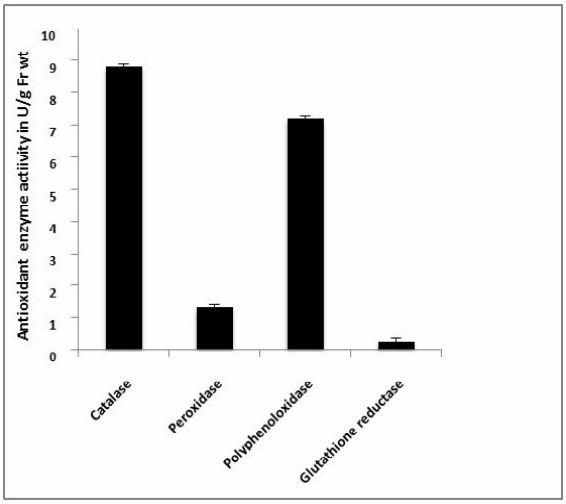

Figure 2: Showing antioxidant enzyme activity (Units/g Fr wt) in *Trigonella foenumgraecum* L.

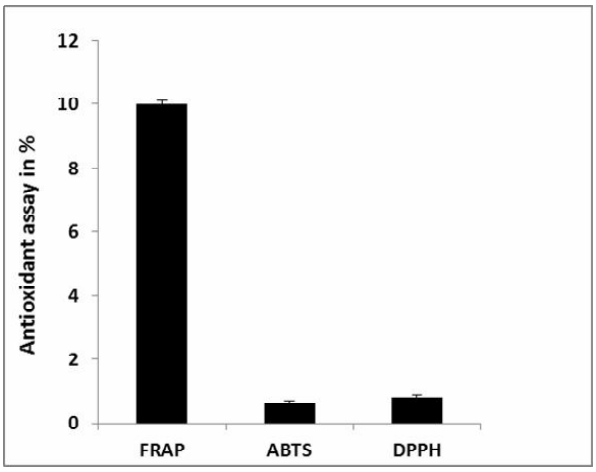

Figure 3: Showing antioxidant assays (%) in *Trigonella foenumgraecum* L.

References

Acharya, S. N.; Acharya, K. S. and Basu, S.K. (2010). Antioxidant and antileukemic properties of selected Fenugreek genotypes grown in western Canada. J. Plant Sci., **91**:99-105.

Agarwal, A. and Prabakaran, S.A. (2005). Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. Indian. J. Exp. Biol., **43**:963-974.

Ahmadiani, A.; Javan, M.J.; Brown, J.; Wright, J.; Scofield, R.H. and Aston, C.E. (2007). Anti-inflammatory and antipyretic effects of *Trigonella foenumgraecum* leaves extract in the rat . J. Ethnopharmacol., **75**:283-286.

Anand, S.C.; Nagaraju. B.; Nazeer.; Audhnasu Ranjan.; Swain.; Faiyaz Ahmed.; Padmavathi, G.V. Narendra.; Sharath Chandra. J. N.; and Shampalatha (2012). Antiulcer activity of *Trigonella foenum graecum* leaves in cold resreaint stress induced ulcer model, molecular and clinical pharmacology. **75**:283-286.

Aneta Wojdy,O.A.; Jan Oszmianski and Renata Czemerys (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chemistry, **105**:940-944.

Barber, J.M. (1980). Catalase and peroxidases in primary leaves during development and senescence. Physiol., **97**:135-144.

Beutler Book, E. (1984). Red cell metabolism. A Manual of Biochemical Methods. 3rd Edn.,Grune and Stratton, New York.

Bhatt, L.R.; Lim, J.A.: Chai, K.Y.; Kang, J.I.; Oh, H.K. and Baek, S.H. (2006). Natural Product Sciences, **4**:226-231.

Brand Willams, W.; Cuvelier, M. and Berset. C. (1995). Use of a free radical method to evaluate antioxidant activity.Lebensm Wiss Technology, **28**:25-30.

Cao, G.; Sofic, E and Prior, R.L. (1998). Antioxidant capacity of tea and common vegetables. J. Agr. Food Chem., **44**:3426-3431.

Chang Yang, M. and Wen, Chern (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analysis, **10**:178-182.

Chaurasia, S.; Tripathi, P. and Tripathi, Y.B. (1995). Antioxidant and anti-inflammatory property of Sandhika: A compound herbal drug. Indian. J. Exp. Bio., **33**:428-432.

Gauiya Bashri.; Vijay Pratap Singh and Mohan Prasad (2013). A review on nutritional and antioxidant values, and medicinal properties of *Trigonella foenumgraecum* L. Biochem. Pharmacol., **2**:118.

Goyal, A.K.; Mishra. T and Sen. A. (2013). Antioxidant profiling of Latkan (*Baccaurea ramiflora* Lour.) wine. Indian J. Biotechnol., **12**:137-139.

Giusti Mónica, M.; Ronald, E. and Wrolstad (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. Current Protocols in Food Analytical Chemistry., **12**:30-34.

Halliwell, B. and Gutteridge, J.M. (2010). Antioxidants: Molecules, Medicines and Myths. Biochem. Biophys. Res. Commun., **393**(4) :561-564 .

Halliwell. B. (2011). Free radcals and antioxidants. Trends Pharmacol. Sci., **32(**3):125-130.

Higdon, J.V. and Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit. Rev. Food Sci. Nutr., **43**(1):89-143.

Jensen, A. (1978). Chlorophyll and carotenoids. ***In***: Hellebust, A and Crargei, J.S. (eds) Handbook of phytological methods. Combridge, Combridge University Press, London. pp:5-7 .

Kar and Mishra (1975). Inorganic pyrophosphatase activity during rice leaf senescence. Can. J. Bot., **53**:503-510.

Kumran, A. and Karunakaran, R.J. (2007). *In vitro* antioxidant activities of methanol extracts of phyllanthus species from India. Lebens-Wiss Technologie, **40**:344-352.

Mohamad Arfan; Saira Gul Rabia Usman; Arshad Khan; Abdur Rauf; Naveed Muhammad; Syed Uzair; Ali Shah; Ajmal Khan and Mumtaz Ali (2013). The Comparative Free Radical Scavenging Effect of *Trigonella foenumgraecum*, *Solanum nigram* and *Spinacia oleracea*. Academic Journal of Plant Sciences**,** **3**:113-116.

Nadkarni, K.M. (1994). The Indian material medica with ayurvedic unani and home remedies. 3rded. Bombay: Popular Prakashan., pp:1240-1243.

Nagata, M and Yamashita, I (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit: Nippon Shokuhin Kogyo Gakkaish., **39**(10):925-928.

Nagulendran, K.R.; Velavan, S,;Mahesh, R and Begum, V.H. (2007). *In vitro* antioxidant activity and total polyphenolic content *of Cyperusrotundus Rhizome*s. Chemistry, **4**(3):440-449.

Nair, R.; Kalariya, T and Sumitra, C. (2005). Antibacterial activity of some selected Indian medicinal flora. Turk. J. Biol., **29**:41-47.

Nahid Sohrevardi and Firouzeh Sohrevardi (2012). Essential oil composition and antioxidant activity of *Trigonella foenumgraecum* L. plant. Intl. J. Agri. Crop Sci., **4**(12):793-797.

Rakesh Kumar Singh; Kanti Bhooshan Pandey and Syed Ibrahim Rizvi (2012).Medicinal properties of some indian spices. Ann. Phytomed., **1**(1):29-33.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radicals in Biology and Medicine, **26**:1231-1237.

Ren-You Gan.; Lei Kuang.; Xiang-Rong Xu.;Yuan Zhang.; En-Qin Xia.; Feng-Lin Song and Hua-Bin Li. (2009). Screening of Natural Antioxidants from traditional Chinese medicinal plants ascociated with treatment of rheumatic disease. Molecules, **15**:5988-5997.

Saujanya Kumar Jha and Shailendera Kumar Srinivastava (2012). Determination of antioxidant activity of *Trigonella foenumgraecum* Linn.in mice. Ann. Phytomed., **1**(1):69-74.

Singh, Vijay Pratap and Mohan Prasad (2013). A review on nutritional and antioxidant values, and medicinal properties of *Trigonella foenumgraecum* L. Biochem. Pharmacol., **2**:118.

Scalbert, A. and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. Source J. Nutr., **130**:2073-2085.

Stankovic, M. S.; Topuzovic, M.; Solujic, S. and Mihailovic, V. (2010). Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. J. Med. Plant Res., **4**:2092-2098.

Stankovic, M.S.; Niciforovic, N.; Topuzovic, M. and Solujic S. (2011).Total phenolic content, flavonoid concentrations and antioxidant activityof the whole plant and plant parts extracts from *Teucrium montanum* L. var. *montnum*,f. *supinum* (L.) Reichenb. Biotechnol. Biotechnol., **25**:2222-2227.

Subhashini, N.; Thangathirupathi1, A. and Lavanya, N. (2011) Antioxidant activity of *Trigonella foenumgraecum* using various *in vitro* and *ex vivo* models .International Journal of Pharmacy and Pharmaceutical Sciences, **3**(2):96-102.

Sun, J.S.; Tsuang,Y.W.; Chen, I.J.; Huang,W.; Hang,Y.S. and Lu (1998). An ulltra a weak chemiluminiscence study on oxidative stress in rabbits following acute thermal injury. Burns, **24**:225-231.

Vasim Khan.; Abdul Kalam Najmi and Pillai, K.(2012). A pharmacological appraisal of medicinal plants with antidiabetic potential. J. Pharm. Bioallied. Sci., **4(**1): 27-42.

Wolfe, K. (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., **51**:609-614.

Wong, S.P.; Leong, L.P. and Koh, J.H.W. (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem., **99**:775-783.