ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Positive Role of UV Radiation in Enhancing Secondary Metabolites Production in Fenugreek Leaves

El-Shora H.M.[1], El-Farrash A.H.[2], Kamal H.[2], and Abdelrazek Aya[2]*

1. Botany Department, Faculty of Science, Mansoura University, Egypt

2. Physics Department, Faculty of Science, Mansoura University, Egypt

Manuscript Info

Manuscript History:

Received: 11 March 2015
Final Accepted: 28 April 2015
Published Online: May 2015

Key words:

UV radiation, secondary metabolites, spectrophotometer, fenugreek

Corresponding Author

Abdelrazek Aya

Abstract

Ultraviolet rays are detrimental to all types of living cells. There are several compounds can absorb UV and protect the genetic material of plants called secondary metabolites as alkaloids, flavonoids and phenols. Moreover, these compounds are energetic for herbal drugs synthesis, and have more pharmacological activities. The self-protective secondary metabolism system can be activated by UV irradiation. Therefore, present study aimed to explore the changes of secondary metabolites in response to enhanced UV radiation in freshly collected leaves of fenugreek (*Trigonella foenum graecum*). The impact of two UV illumination doses (15 and 30 watt) was examined at different time intervals (1, 2, 3, 4 hr.). After UV radiation, the content of these compounds dramatically increased. The higher dose 30 watt was more effective than 15 watt. The increase in phenols, flavonoids, alkaloids, tannins, saponins and anthocyanins were investigated by phytochemical screening and UV-spectrophotometer.

INTRODUCTION

Metabolism is defined as the chemical reactions takes place in most living cells. Through these reactions, abundant of organic components inclusive sugars, nucleotides, fatty acids, RNA, polysaccharides, amino acids, proteins, lipids, DNA, etc. are generated. These processes are considered as fundamental and joint to living creature**s** and called as primary metabolism and the attached components are described as primary metabolites.

Above these primary metabolic pathways, other metabolic pathways are generated under confirmed conditions and the created compounds are known as secondary metabolites.

Due to the power action of secondary metabolites in manufacture of more pharmaceutical components, the generation of these compounds by plants has become an active field of study (**jaleel et al., 2007**).

Secondary metabolites play important role in protection of plants versus herbivores, fungi, viruses and bacteria. Also, for protection against UV light and other physical stress secondary metabolites utilized for engage pollinators as well as seed dispersers, as signals **(Wink, 2003 and 2008)**.

Medicinal plants can be affected directly or indirectly by UV radiation such as DNA damage, membranes blighter, breaking of proteins and alterations in transpiration as well as photosynthesis in addition to changes in morphology, growth, and development.

Phenols that absorb light are group of phenylalanine-derived aromatic secondary products that used in protecting plants from harmful effects of UV irradiation **(Li and Oulee, 1993)**. The free radicals produced by UV radiation can be combated by antioxidant ability of phenols **(Strid, 1993 ; Yamamoto et al., 1996)**.

According to increase solar ultraviolet radiation, many biological and biophysical investigators concentrated on UV stress physiology.

There are a lot of active secondary metabolites in fenugreek leaves like alkaloids, flavonoids, and saponins. Present investigation used UV enhancing method to catalyze the secondary metabolism in leaves of fenugreek.

Secondary metabolites in fenugreek have hypo-glycemic effects as they initiate the secretion of insulin from pancreatic beta cells **(Ajabnoor, 1988)**, and hinder alpha-amylase and sucrose activities **(Amin, 1987).**

They also lower total cholesterol, low-density lipoprotein cholesterol (LDL-C) and serum triglycerides **(Valette, 1988; Stark, 1993; Petit, 1993; Al-Habori, 1998; Al-Habori and Raman, 1998)**. These effects may be due to sapogenins, which increase excretion of biliary cholesterol, in turn leading to lowered serum cholesterol levels **(Varshney, 1966; Sidhu, 1986; Sauvaire, 1991; Stark, 1993)**. Most of substances that absorb UV-light are active compounds formed in plants by secondary metabolism.

In the present investigation, the positive health effects of plant secondary metabolites have led to search potential ways for enhancement the contents & properties of these compounds by UV irradiation.

Materials and Methods

Plant material

The experimental plant involved in this investigation was *Trigonella foenum graecum* (Fenugreek, family Fabaceae). Pure seeds were bringed from Egyptian Ministry of Agriculture.

Seeds radiation

Seeds of *Trigonella foenum graecum* were exposed to two various doses of UV radiation (15, 30 watt) at various time intervals (1, 2, 3, 4 hr.) using UV system in Nuclear & Radiation Lab., Physics Department, Faculty of Science, Mansoura University.

Growth of Plant

Seeds were germinated according to **El-Shora (2001)**. They were cleaned by keeping in 10 % sodium hypochlorite for 24 hr. The seeds were then matured between paper towels, moistened with distilled water in sterilized plastic trays and were covered and incubated in dark at 25^{o}C.

Preparation of extract

The aqueous extract was prepared from dried powder of fenugreek leaves. Five grams of powder was soaked in 100ml of distilled H_2O for 24 hr. at room temperature, and filtered through filter paper to obtain 5% w/v.

Then, we centrifuge the filtrate at 3500 rpm for 25 min and use the resultant supernatant for chemical tests.

Phytochemical screening of some secondary metabolites

Chemical investigations were performed on the powdered materials of plant and on the aqueous extract using standard methods to know the various chemical components.

Estimation of total phenols

Total phenolic content of the leaf extract was estimated using Folin-Ciocalteau assay (**Waterhouse, 2001**) procedure with some modifications. Plant extract (100 µl) was added to the 300 µl Folin-Ciocalteau reagent and the mixture was incubated for 10 min at room temperature.

Sodium carbonate solution (800 µl) was then added, mixed and incubated for 3 hrs. at room temperature. The absorbance was taken spectrophotomtrically at 765 nm. The standard curve was prepared using gallic acid. The above steps were repeated by using gallic acid instead of leaf extracts. The content of phenols in the leaf extract was measured based on the equation from the standard curve and expressed as gallic acid equivalent (GAE g/100 g dry weight).

Estimation of flavonoids

Flavonoids were determined by **Woisky and Salatino (1998)** with some modifications. 0.5 ml of the aqueous filtrate of plant extract was added to 0.1 ml of 10% aluminum chloride ($AlCl_3$) followed by addition of 0.1 ml of 1 M of sodium acetate. Then add 2.8 ml of distilled H_2O, and leave the content at room temperature for 25 minutes. Then observe a yellow color that indicates flavonoids presence. Absorbance was read spectrophotometrically at 415 nm.

Estimation of alkaloids

Total alkaloids were estimated quantitatively according to **Harborne (1973)**. One gram of powdered specimen was added to 4:1 of 80 % ethanol and glacial acetic acid and mixed well. The mixture was left to stand for at least 5 hours followed by filtration. The alkaloid in the supernatant was precipitated by a drop wise addition of concentrated ammonia solution. The precipitated alkaloids were filtered on pre-weighted filter paper and were dried in an oven at 80 ^{o}C to a constant weight. The content of alkaloids was measured and expressed as mg/g dry mass of the plant sample.

Estimation of tannins

Estimation of tannins was carried out by **Trease and Evans (1989)**. A sample of 0.5 g of fenugreek dried powder was boiled in 20 ml distilled H_2O and then filtered. Small drops of 0.1 % ferric chloride were added. A blue black color or a brownish green color was observed indicating tannins presence. Absorbance was read spectrophotometrically at 525 nm.

Estimation of saponins

Saponins Presence was indicated by **Trease and Evans (1989)**. About two grams of dried powdered specimen was boiled in 20 ml distilled H_2O and then filtered. Five ml of distilled H_2O were added to Ten ml of filtrate and shaken well for a stable persistent froth. Three drops of olive oil was mixed with this frothing mixture and shaken vigorously.

Then emulsion formation which indicates saponins presence was observed, and absorbance was read spectrophotometrically at 350 nm.

Estimation of anthocyanin content

The content of anthocyanin was estimated by **Hoagland (1980)**. One gram of fresh shoot was grinded in 10ml acidic methanol (HCL, 1% v/v) for 5 min using a homogenizer. The resulting homogenate was centrifuged for 20 min at 5000 rpm. Anthocyanin content was determined by the variance in absorbance (A).

$$\text{Anthocyanin content} = (A_{525}-A_{585nm})/g^{-1}\text{fresh mass in 10ml extract}$$

Results and Discussion

Elicitors enforcement whether biotic or abiotic stress considered as one of the most operative methods used to improvement the amount of various beneficial plants' secondary metabolites (**Vasconsuelo and Boland, 2007; Zhao et al., 2005**). The extremely used external stresses are pathogens, herbivores, insects, UV-light, Gamma rays, and chemical compounds. This research concerned with studying the impact of the most effective abiotic stress UV-light with two illumination doses (15 and 30 watt).

The power of UV irradiation in the accumulation of these secondary metabolites is dependent on the exposure time and the type of UV light.

In the present investigation, the content of phenolic compounds, flavonoids, alkaloids, tannins, saponins and anthocyanin increased (Figs. 1, 2, 3, 4, 5 and 6 respectively). The change in the amount of these compounds is more apparent by using 30 watt UV irradiation.

The total phenols in fenugreek were increased in response to irradiation (Fig. 1) depending on the dose and the time. Phenols in plants may perform useful effects through free radicals scavenging **(Chun et al., 2003)**. Therefore, phenols can protect cells from the oxidative damage caused by free radicals **(Wada and Ou, 2002)**.

UV light utilized to catalyze secondary metabolites realization in fresh medicinal plant leaves and ameliorate their pharmacological efficiency. The synthesis and the accumulation of phenolic compounds in plants were catalyzed by biotic and abiotic stress (**Naczk and Shalidi, 2004**).

The chemical structures of poly phenols give them the capability to act as free radical scavengers. The antioxidant activity of poly phenols depend on the degree of methoxylation and the number of hydroxyl groups (**Bravo, 1998**).

Flavonoids content in fenugreek increased according to irradiation treatment (Fig. 2) and was depend on the dose and the time. This increase expressed the greatest antioxidant activity.

The alkaloid content of fenugreek increased in response to irradiation (Fig. 3), and this increase also dependent on the dose and the time. Alkaloids have a broad range of bioactivities. Alkaloids represent the important constitute of about 80% of antibacterial drugs and 90 % of antimalarial drugs available today **(Wangchuk, 2004)**.

The tannin content increased due to UV treatment (Fig. 4) depending on the dose and the time. Tannins have a key role in protecting plants from herbivores. Also, these compounds can be act as therapeutic agent against microbial infections **(Wink, 2003)**.

Saponin content increased under the effect of UV radiation (Fig. 5), and this increase was also dependent on the dose and the time. Saponins are consisting of a sterol ring with attached sugars **(Price, 1987)**, which have sensitive role in decreasing the amount of cholesterol in hypercholesterolemia organisms.

The results show that the anthocyanin content in fenugreek increased in response to irradiation (Fig. 6), and this increase was dependent on the dose and the time. Anthocyanins represent a group of phenolic compounds responsible for appearance of the red–blue color of several fruits and vegetables, and offer useful effects to human health **(Garcia-Alonso et al., 2004)**. The antioxidant properties in plants are contributed by the presence of phytochemicals such as phenolics, anthocyanins and other flavonoid contents (**Cao et al., 1997**).

Anthocyanins also considered as an important class of flavonoids (**Guo et al., 2008**). Anthocyanin accumulation was stimulated by influence of UV-B radiation in maize (**Sharma et al., 1999**), rice (**Reddy et al.,**

1994) and apple flowers (**Dong et al., 1998**). UV-B increased the accumulation of anthocyanin via stimulating the expression of genes encoding enzymes in anthocyanin biosynthetic pathway.

The same results were carried out by **Gibney et al. (1982)** when mercantile saponins given to hamsters and mice.

In conclusion, as irradiation with UV could be applied for improvement the medicinal plants quality, this study can supply an invention process for pharmaceutical technology and food by enhancing the production of secondary metabolites in fenugreek leaves.

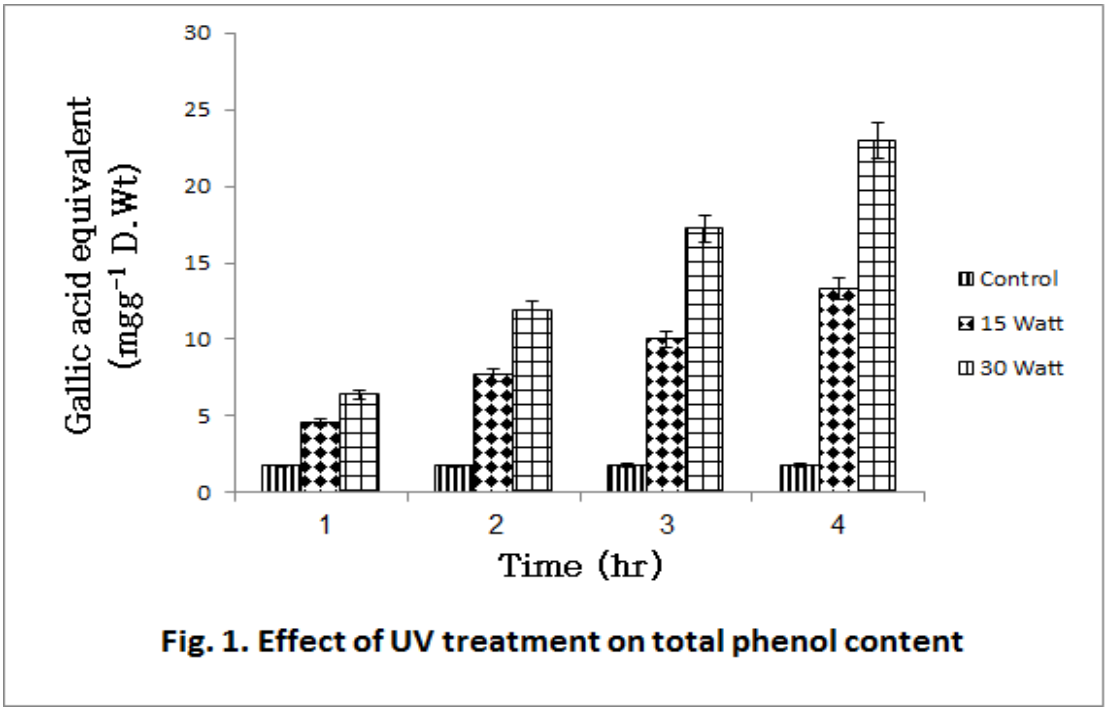


Fig. 1. Effect of UV treatment on total phenol content

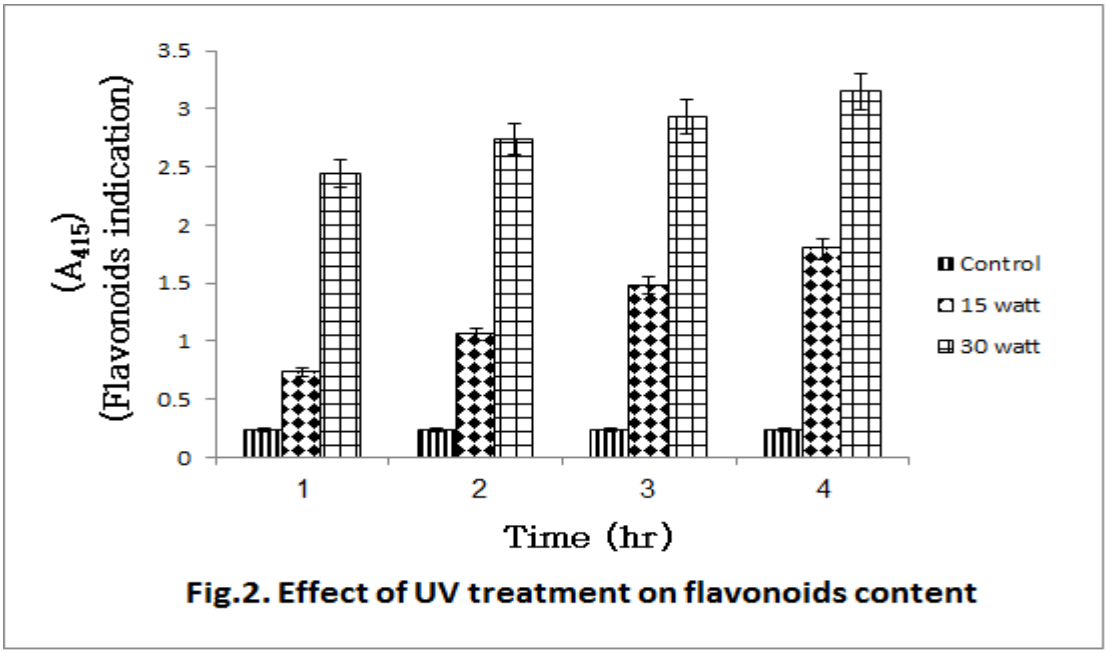


Fig.2. Effect of UV treatment on flavonoids content

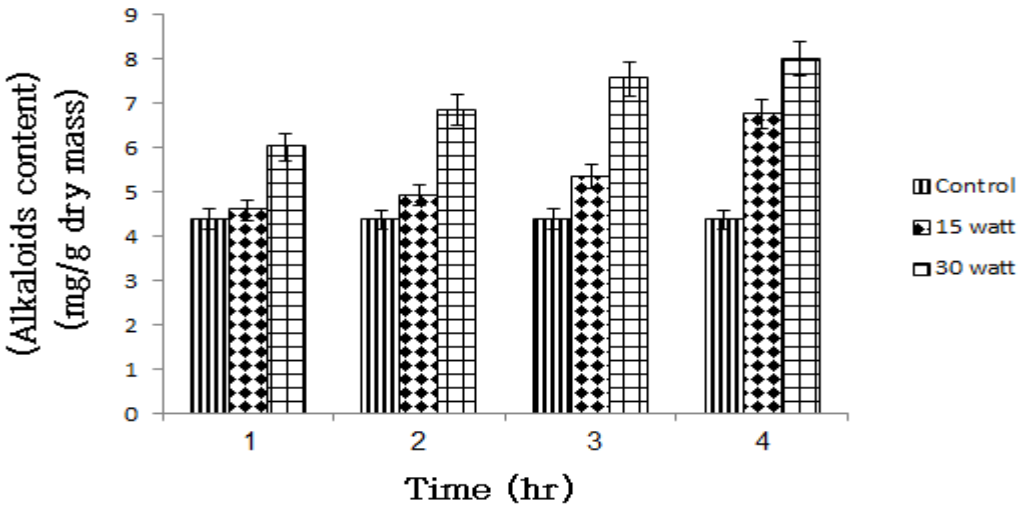
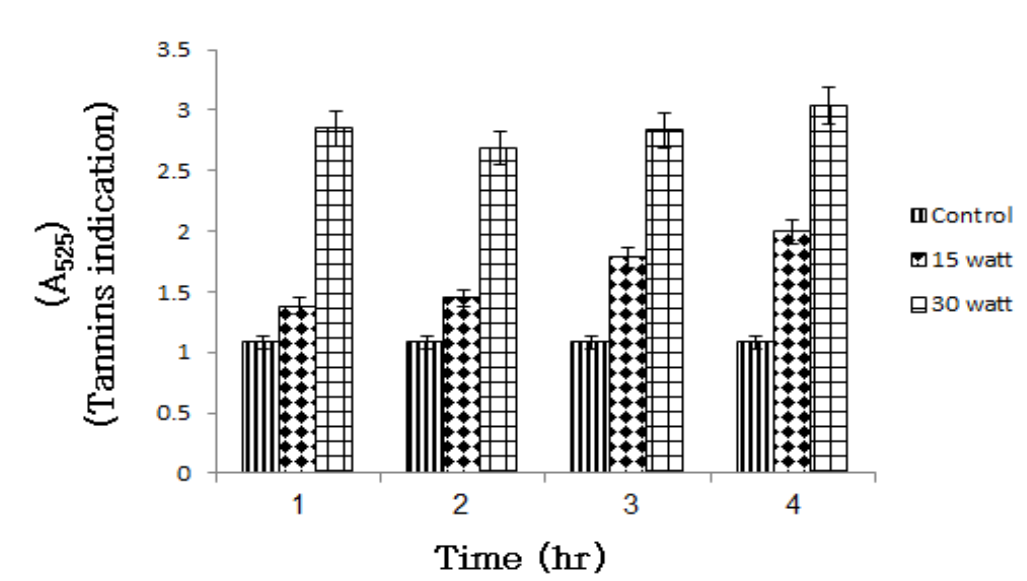


Fig.3. Effect of UV treatment on alkaloids content



Figf.4. Effect of UV treatment on tannins content

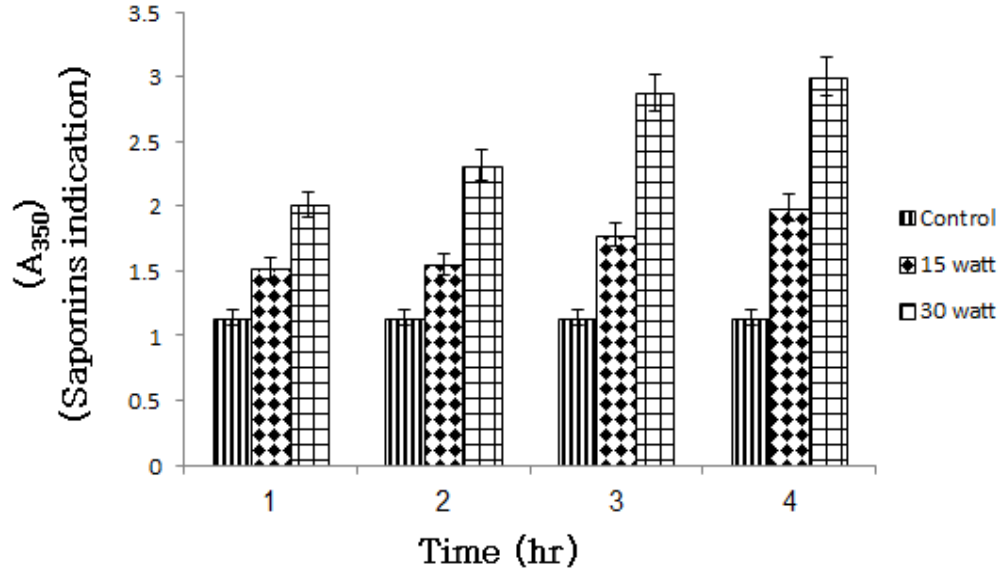


Fig.5. Effect of UV treatment on saponins content

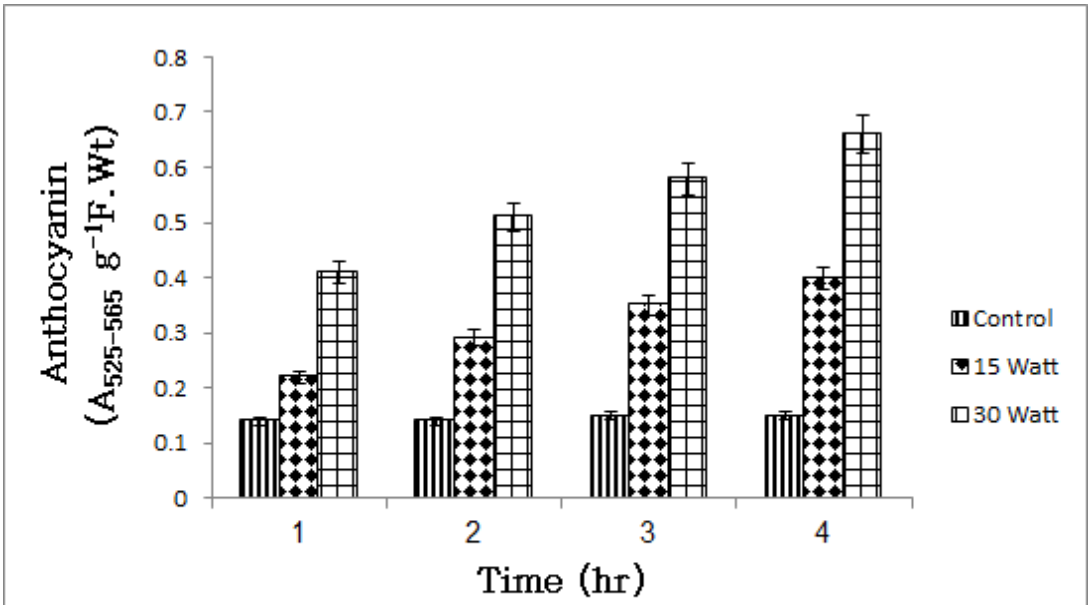


Fig. 6. Effect of UV treatment on anthocyanin content

References

Ajabnoor, M.A., and Tilmisany, A.K.: Effect of Trigonella foenum graceum on blood glucose levels in normal and alloxan-diabetic mice. J Ethnopharmacol 22: 45-49 (1988).

Al-Habori, M., Al-Aghbari, A.M., and Al-Mamary, M.: Effects of fenugreek seeds and its extracts on plasma lipid profile: a study on rabbits. Phytother Res. 12: 572-575 (1998).

Al-Habori, M., and Raman, A.: Antidiabetic and hypocholesterolaemic effects of fenugreek. Phytother Res. 12: 233-242 (1998).

Amin, R., Abdul-Ghani, A.S., and Suleiman, M.S.: Effect of Trigonella feonum graecum on intestinal absorption. Proc. of the 47th Annual Meeting of the American Diabetes Association (Indianapolis U.S.A.). 36: 211a (1987).

Bravo, L.: Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 56: 317-333 (1998).

Cao, G., Sofic, E. and Prior, R. L.: Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radic Biol Med. 22: 749–760 (1997).

Chun, O.K., Kim, D.O., and Lee, C.Y.: Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem. 51: 8067–8072 (2003).

Dong, J., Hung, L.H., Strome, R. and Krause, H.M.: A phosphorylation site in the ftz homeodomain is required for activity. EMPO J. 17: 2308-2318 (1998).

El-Shora, H. and Abo-Kassem, E.: kinetic characterization of glutamate dehydrogenase of marrow cotyledons. Plant Sci. 161: 1047-1053 (2001).

Garcia-Alonso, M., Rimbach, G., Rivas-Gonzalo, J.C., Pascual-Teresa, S.: Antioxidant and cellular activities of anthocyanins and their corresponding vitisins a studies in platelets, monocytes, and human endothelial cells. J. Agric. Food Chem. 52: 3378–3384 (2004).

Gibney, M. J., Pathirana, C., and Smith, L.: Saponins and fibre. Lack of interactive effects on serum and liver cholesterol in rats and hamsters. Atherosclerosis 45: 365-367 (1982).

Guo, J., Han, W. and Wang, M.: Ultraviolet and Enviromental stresses involved in the induction and regulation of anthocyanin biosynthesis: A review. African Journal of Biotechnology. 7: 4966-4972 (2008).

Harborne, J. B.: Phytochemical methods, London. Chapman and Hall, Ltd. 1: 49-188 (1973).

Hoagland, R.E.: Effects of glyphosate on metabolism of phenolic compounds: VI. Effects of glyphosine and glyphosate metabolites on phenylalanine ammonia-lyase activity, growth and protein, chlorophyll and anthocyanin levels in soybean (*Glycine max*) seedlings. Weed Sci. 28: 393–400 (1980).

Jaleel, C.A., Gopi, R., Manivannan, P. and Panneerselvam, R.: Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish Journal of Botany. 31: 245–251 (2007).

Li, J.Y., Oulee, T.M., Raba, R., Amundson, R.G., and Last, R.L.: Arabidopsis Flavonoid Mutants Are Hypersensitive to UV-B Irradiation. Plant Cell. 5: 171-179 (1993).

Naczk, M. and Shalidi, F.: Extraction and analysis of phenolics in food. Chromatogr,J. 1054:95-111 (2004).

Petit, P., Sauvaire, Y., and Ponsin, G., et al.: Effects of a fenugreek seed extract on feeding behaviour in the rat: metabolic-endocrine correlates. Pharmacol Biochem Behav. 45: 369-374 (1993).

Price, K. R., Johnson, I. T., and Fenwick, G. R.: The chemistry and biological significance of saponins in foods and feedingstuffs. Crit Rev Food Sci Nutr. 26: 27-135 (1987).

Reddy, Y. R. et.al: Comparative study of utilization of sunflower and maize straw in sheep and goats. Int. J. Anim. Sci. 9: 337-342 (1994).

Sauvaire, Y., Ribes, G., and Baccou, J. C., et al.: Implication of steroid saponins and sapogenins in the hypocholesterolemic effect of fenugreek. Lipids. 26: 191-197 (1991).

Sharma, R.S., Panigrahi, N. and Kaul. S.M.: Aedes aegypti prevalence in hospitals and schools, the priority sites for DHF transmission in Delhi. Dengue Bull. 23: 109-112 (1999).

Sidhu, G.S., and Oakenfull, D.G.A.: Mechanism for the hypocholesterolaemic activity of saponins. Br J Nutr. 55: 643-649 (1986).

Stark, A., and Madar, Z.: The effect of an ethanol extract derived from fenugreek (Trigonella foenum-graecum) on bile acid absorption and cholesterol levels in rats. Br J Nutr. 69: 277-287 (1993).

Strid, A.: Alteration in Expression of Defense Genes in Pisum-Sativum after Exposure to Supplementary Ultraviolet-B Radiation. Plant Cell Physiol. 34: 949-953 (1993).

Trease, G.E., and Evans, W.C.: Pharmacognosy (13th Ed.). English Language Book Society, Bailliere Tindall, Britain. 378: 386-480 (1989).

Valette, G., Sauvaire, Y., Baccou, J. C., and Ribes, G.: Hypocholesterolaemic effect of fenugreek seeds in dogs. Atherosclerosis. 50: 105-111 (1984).

Varshney, I.P., and Sharma, S.C.: Saponins and sapogenins: part XXXII. Studies on Trigonella foenum-graecum Linn. seeds. J Indian Chem Soc. 43: 564-567 (1966).
Vasconsuelo, A., and Boland, R.: Molecular aspects of the early stages of elicitation of secondary metabolites in plants. Plant Science. 172: 861- 875 (2007).
Wada, L., and Ou, B.: Antioxidant activity and phenolid content of Oregon caneberries. J. Agric. Food Chem. 50: 3495–3500 (2002).
Wangchuk, P.: Bioactive alkaloids from medicinal plants of Bhutan. University of Wollongong Thesis Collections. 315: 1-166 (2004).
Waterhouse, A.L.: Folin-Ciocalteau micro method for total phenol in wine. Current Protocols in Food Analytical Chemistry, I1.1.1-I1.1.8, Wrolstad, R.E., Wiley (2001).
Wink, M.: Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry. 64: 3–19 (2003).
Wink, M.: Plant secondary metabolism: Diversity, function and its evolution. Natural Products Communications. 3: 1205-1216 (2008).
Woisky, R., and Salatino, A.: Analysis of propolis: some parameters and procedures for chemical quality control. Journal of Agricultural Research. 37: 99-105 (1998).
Yamamoto, H.Y., and Bassi. R.: Carotenoids: Localization and Function. Adv. Photosynth. 4: 539-563 (1996).
Zhao, J., Davis, LC., and Verpoorte, R.: Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology Advances. 23: 283-333 (2005).