

# Assessment of phytochemicals, antioxidant activity of extracts and various fractions of *Trigonella foenum graecum* leaves, stems and roots

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## Abstract

The present study was aimed to estimate the antioxidant activities of leaves, stems and roots extracts of *Trigonella foenum graecum* which belongs to the family Fabaceae. The Antioxidant activities of fenugreek has been evaluated by using three different solvents such as double distil water, 80% acetone, methanol in vitro assays and antioxidant compounds like Phenolics, Alkaloids, Tannins, Saponins evaluated in this plant. The antioxidant activity was evaluated by DPPH and hydrogen peroxide methods. The total Antioxidant capacity was determined by spectrophotometrically. Antioxidant activity depends leading concentration and increased with increasing amount of the extracts. The result obtained in the present study indicates that the *Trigonella foenum graecum* extract serve as the potential source of natural antioxidants.

Keywords; *Trigonella foenum graecum*, Alkaloid, Antioxidant, free radical.

## 1. Introduction

*Trigonella foenum-graecum* L. (fenugreek) is an important herbs and therapeutic legume plant used in several parts of the world. It is an annual herb from the family Fabaceae. The producers of fenugreek are Spain, India, Turkey, Iran, Morocco, Pakistan, France, Bangladesh, China, Nepal, Egypt, and Argentina.<sup>[1]</sup> It is also known for treating hypoglycemic and hypocholesterolemic, inflammatory disorders.

Chemical mutagens & Irradiation can be used to produce point mutation in fenugreek. Besides these antioxidants, minerals, optimum level, and vitamins also contribute to prevent aging as well as heart diseases as and cancer<sup>[2]</sup>.

It is also good source of calcium, iron,  $\beta$ -carotene and several vitamins<sup>[3]</sup>, It has been used to increase the colour and flavouring, also modifies the texture of food materials. Fenugreek is beneficial influence on digestion and also has the ability to modify the food<sup>[4]</sup>.

Pharmacological properties of fenugreek have been explored to identify a role for the plant in diabetes management<sup>[5]</sup> and in cardiovascular health<sup>[6,7,8]</sup> indicating the presence of bioactive compound in fenugreek, which may be responsible for its health benefits. Many studies have indicated the medicinal effect of fenugreek leaves and seed. Fenugreek. *foenum-graecum* seeds and its saponin constituents have been revealed to have anticarcinogenic ability.<sup>[9,10]</sup> The health profit of **fenugreek** contain relief from dandruff, respiratory disorders, biliousness, stomach disorders respiratory disorders, mouth ulcers, anemia, sore throat, loss of taste, fever, diabetes.<sup>[11]</sup>

## 2. Method and material

### Material

Wash and clean and dried Leaves, stems and roots of fenugreek were selected for analysis and store in dark place. The mature dry leaves, stems and

roots material was collected from west Nimad region of Madhya Pradesh, India. store in dark place.

### **2.1 Preparation of fenugreek leaves, stem, root extract in different solvent**

Preparation of the extract 20 g of dried powder of fenugreek leaves, stems, and roots were separately soaked in 60ml of different solvent (methanol, water, acetone + water) for 48 hours on rotator shaker. Than filtered by using Whatman filter paper and obtained filtered material were used as extract. [12]

## **Methods**

### **2.2 Analysis Of Nutritional Factors**

#### **2.2.1 Phenolics**

Phenolics stuffing through the Folin-Ciocalteu colorimetric method. ( Mallick & singh,1980 ) [13.] Defatted section (1 g.) was ground in a mortar grinder with 10 times volume of 80% ethanol (10 ml.). subsequent to that it centrifuged at 10,000 r.p.m. for 20 mints for obtain homogenized form . The remains was extracted with five times the volume of eighty percents ethanol. The supernatant was evaporate up to aridness. The remains was after that, dissolved in 5 ml. distil water. dissimilar aliquots ranging from( 0.2-2.0) ml were put into test tubes. In every tube, whole volume was made up to 3 ml. with distil water followed by adding of Folin-Ciocalteu reagent (0.5ml.). following 3min, 2ml. of 20 %  $\text{Na}_2\text{CO}_3$  solution was added to every tube and mix properly. The tube were kept in steaming water for 1 mint also only then cooled and brought to room temperature after that absorbance was calculated at 650 nanometre against a reagent blank. To measure the amount of total phenol content in the sample absorbance was compared with standard catechol solution (0.1 mg./ml. catechol).

#### **2.2.2 Phytic Acid**

Dried fenugreek leaves, stem and root were crushed into a fine meal, then powdered 50mg leave samples were extracted overnight in 0.4mM HCl follow by centrifugation for 20 mint at 10,000 rpm. at room temperature. Supernatant was collected and was used as a font for the testing of phytic acid. 10 $\mu$ l of sample was taken in a microtiter plate, diluted with 90  $\mu$ l distilled water, followed by addition of 100microliter colorimetric reagent(3M  $\text{H}_2\text{SO}_4$ , 2.5% ammonium molybdate ,10% (w/v) ascorbic acid & distil water in 1:1:1:2 ratio. The contents were incubate for 60 mint at room temperature and absorbance was taken at 650 nm with Systronic UV-vis spectrophotometer. [14]

#### **2.2.3 Tannins**

400mg of finely powdered defatted meal of methanol ,water and acetone was mixed with 40ml distilled water. The deferment was then boil for 30 min cooled and subsequently centrifuged at 2000 rpm. for 10mint and used as a so.urce for tannin assessment. Tannins were estimated using Folin-Denis reagent which was prepared by adding 100g of sodium tungstate and 20g of phosphomolybdic acid in 750 ml of water followed by addition of phosphoric acid (50 ml). The contents were refluxed for 2hrs and subsequently the volume was made up to 1 litre. The reagent so prepared was stored in a dark bottle. Later than extraction, 1ml of the clear supernatant was used because a starting place of tannins and this 5ml of Folin-Denis reagent 10ml of sodium carbonate solution were added followed by dilution to 100ml with water. The tubes were incubated for 30min and the colour thus developed was read at 700nm using Systronics UV-vis spectrophotometer. [15]

#### **2.2.4. Saponin**

The measure of oil emulsion formation of saponin was used for the selection of saponin [16]. Briefly, extract and a variety of fractions (20mg) suspended in 20ml of distil water and boiled for 5 min. In

10ml of the on top of filtrate 5ml. of distil water was additional and mixed well to develop the froth. Development of emulsion following mixing the forth with olive oil confirmed the existence of saponin.

## 2.3 Evaluation of Antioxidant Properties

### 2.3.1 DPPH Activity

DPPH radical scavenging activity was resolute according to the methods described by Hitoshi *et al.* 2004 . Methanolic extracted as per the procedures described by Tomoyuki *et al.*, 2002. The powdered sample (2gm) was extracted by vigorous shaking with 20ml of 60% methanol containing 0.1% HCl for 4 hours at room temperature. The suspension thus was centrifuged at 10000rpm for 15 min at 4<sup>0</sup>C and the supernatant was filtered through Whatmann no1 filter paper and the filtrate was stored at -20<sup>0</sup>C. Aliquots of 100ul sample extract were added to 2.9 ml of DPPH reagent (in 0.1 mM methanol) and subjected to vigorous vortices. Incubation was ready in dark for 30mint at room temperature & the discoloration of DPPH was measured against blank at 517nm<sup>[17]</sup>.

$$\text{Percentage inhibition} = \frac{[(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance})] \times 100}$$

### 2.3.2 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide (2Mm) working solution was made by mixing with 50mM phosphate buffer (pH 7.4). Reaction mixture was prepared by the addition of 0.1 ml of extract and each fraction with 0.4ml of 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6ml of 50mM H<sub>2</sub>O<sub>2</sub> and allowed to stand for 10min. The absorbance of the mixture was record at 230 nm . Following equation was used to determine the capacity to scavenge H<sub>2</sub>O<sub>2</sub>.

$$\text{Hydrogen peroxide scavenging activity (\%)} = \frac{(1 - \text{Absorbance of sample} / \text{absorbance of control}) \times 100.}$$

### 2.3.3 Total Antioxidant Capacity

Phosphomolybdate assay scheme was used to determine the total antioxidant activity of the methanol extract and various fractions<sup>[18]</sup>. To a reagent solution; sulphuric acid (0.6mM), sodium phosphate (28mM) and ammonium molybdate (4 mM); 100μl of each sample was added and incubated at 95<sup>0</sup>C in a water bath for 90min. behind cooling to room temperature, the absorbance was record at 765 nm against reagent blank. The total antioxidant capacity was determined by use following formula:

$$\text{Total Antioxidant capacity (\%)} = \frac{[(\text{control absorbance} - \text{sample absorbance}) / (\text{control absorbance})] \times 100}$$

## 3. Result and Discussion

Bioactive compounds from plants are being used as food additives, insecticides cosmetics, perfumes, pigments, fine chemicals, and dyes<sup>[19]</sup>. There is strong correlation established linking phytochemical constituents of a plant with its pharmacological activity<sup>[20]</sup>. nowadays, it is required that a system of standardization is established for every plant medicine in the market because the scope for variation in dissimilar batches of medicine is enormous<sup>[21]</sup>. Present study showed the presence of phytochemicals in *Trigonella foenum graecum* leaves, stems, and roots extract. The extraction was done using different solvents like water, methanolic and acetone + water extracts. The *Trigonella foenum graecum* leaves, stems, roots extracts contains a large number of phytochemicals such as tannin, phytic acid, phenolics and saponins.

### 3.1. Qualitative screening of bioactive compounds (tannins, phenolics and phytic acid, saponin) presents in the fenugreek leaf, stem, and root in a different extract.

Table 1. Phytochemicals of *Trigonella foenum graecum* leaves, stems, and roots extract in Methanolic solvent.

Extract	Methanol		
	Leaves	Stems	Roots
1.tanin	1.86±0.05	0.55±0.04	0.29±0.05
2.Phenolics	0.88±0.03	0.16±0.04	0.18±0.05
3.Phytic	2.86±0.04	1.08±0.05	0.16±0.05
4.Saponins	1.26±0.05	0.29±0.05	0.39±0.04

**Tanins:-** The highest concentration (1.86±0.05mg/gm) of tannin found in leaves extract and lowest concentration (0.29±0.05mg/gm) was found in roots extract.

**Phenolics:-** The highest concentration (0.88±0.03mg/gm) of phenolics was found in leaves extract and lowest concentration (0.16±0.04mg/gm) in stems extracts.

**Phytic:-** the highest concentration was again found in phytic acid was found in the leaves (2.86±0.04mg/gm) and lowest concentration (0.16±0.05mg/gm) was present in roots extracts.

**Saponins:-** the highest concentration (1.26±0.05mg/gm) was present in leaves and lowest concentration (0.29±0.05mg/gm) was found in stems extract.

Table 2. Phytochemicals of *Trigonella foenum graecum* leaves, stems, and roots extract in water solvent.

Extract	Water		
	Leaves	Stems	Roots
1.tanin	0.79±0.03	0.08±0.05	0.26±0.05
2.Phenolics	0.51±0.05	0.04±0.04	0.12±0.04
3.Phytic	2.98±0.05	0.88±0.05	0.04±0.03
4.Saponins	0.76±0.04	0.18±0.05	0.26±0.03

**Tanins:-** The highest concentration (0.79±0.03mg/gm) of tannin found in leaves extract and lowest concentration (0.08±0.05mg/gm) was found in stems extracts.

**Phenolics:-** The highest concentration (0.51±0.05mg/gm) of phenolics was found in leaves extract and lowest concentration (0.04±0.04mg/gm) in stems extracts.

**Phytic:-** the highest concentration (2.98±0.05mg/gm) was again found in the leaves and lowest concentration (0.04±0.03mg/gm) was present in roots extracts.

**Saponins:-** the highest concentration (0.76±0.04mg/gm) was present in leaves And lowest concentration (0.18±0.05mg/gm) was found in stems extracts.

**Table 3. Phytochemicals of *Trigonella foenum graecum* leaves, stems, and roots extract in Acetonic solvent.**

Extract	Acetone + Water		
	Leaves	Stems	Roots
1.tanin	0.46±0.03	0.03±0.05	0.09±0.05
2.Phenolics	0.11±0.04	0.06±0.03	0.01±0.05
3.Phytic	1.39±0.04	0.36±0.04	0.002±0.05
4.Saponins	0.23±0.04	0.02±0.05	0.25±0.04

**Tanins:-** The highest concentration (0.46±0.03mg/gm) of tannin found in leaves extract and lowest concentration(0.03±0.05mg/gm) was found in stems extracts.

**Phenolics:-** The highest concentration (0.11±0.04mg/gm) of phenolics was found in leaves extract and lowest concentration (0.01±0.05mg/gm) in roots extracts.

**Phytic:-** the highest concentration (1.39±0.04mg/gm) was again found in the leaves and lowest concentration (0.002±0.05mg/gm) was present in roots extracts.

**Saponins:-** the highest concentration (0.25±0.04mg/gm) was present in roots And lowest concentration (0.02±0.05mg/gm) was found in stems extracts.

Significant At(P≤0.05). All Value Are The Three Dates

Abbreviation -Mean ± Standard Deviation (Sd)

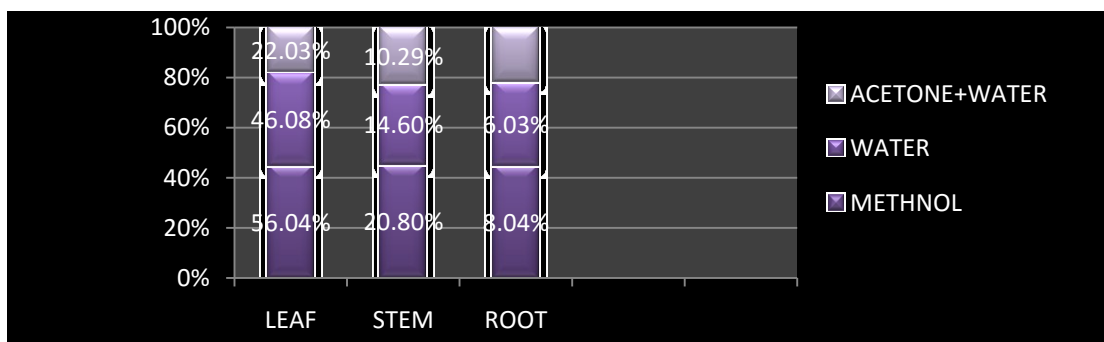
Presence of saponins and flavonoids as the major compounds in *T.foenum-graecum*<sup>[22, 23]</sup> can around explain anti-inflammatory activity of the plant . From generally results, we can include that fenugreek extract possess an antioxidant effect.

Regarding the presence of different secondary metabolites in this plant, separation and identification of biological compounds of the plant is valuable for finding new agents with antioxidant activity. It has been report that the fenugreek leaves exert antiulcer activity during the flavonoids since flavonoids are report to protect the mucosa by preventing the formation of lesions by various necrotic agents <sup>[24]</sup> the latter experiments are running.

### 3.2 Evaluation of anti-oxidant properties of these phytochemicals of fenugreek leaves, stem and root.

#### 3.2.1. DPPH Scavenging Activity:

The antioxidant activities of methane extract of fenugreek were expressed as percent DPPH radical scavenging activities with higher values indicating greater antioxidant activity.





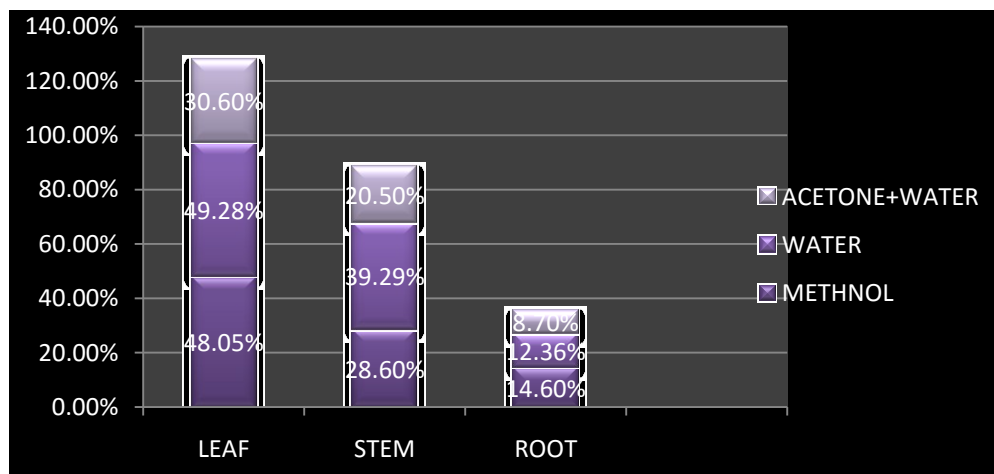
**Graph 1: Graphical representation of antioxidant activities of DPPH.**

The antioxidant activity of phytochemicals range from 56.04 %. with the highest activity exhibited by methane leaf extract and the lowest activity exhibited by water extract.(Graph 1)The DPPH radical scavenging activity of *T. foenum graecum* leaf, stem & root in different extracts. Among all the solvent extracts tested, methanol, acetone, and water leaf extract had better scavenging activity (56.04%), (46.08%), (22.03%) followed by stem (20.80), (14.60%), (10.29) and root (8.04%), (6.03%)(3.85%) in different three extracts.

**3.2.2. Hydrogen Peroxide Scavenging Activity:**

Our results are in agreement with our earlier publication that the radial scavenging capacity of

fenugreek might be mostly related to their awareness of phenolic hydroxyl group (Singh et al(2015)). The scavenging abilities of methane extract of fenugreek showed hydroxyl radical – scavenging activities (40.6%) at a level of 200µl in the reaction mixture with the highest activity exhibited by fenugreek variety methane extract of fenugreek and the lowest concentration activity exhibit by acetone+ water fenugreek remove. The anti-oxidative action of *T. foenum graecum* leaf, stem & root extracts in dissimilar solvents. Leaf’s extract in methane, water and acetone shows inhibition 48.05%, 49.28% and 30.60%. As with other extracts (stem and root), less inhibition. Stem in methane-,\_water and acetone according 28.60%, 39.29% and 20.50% and in root inhibition as compare stem in different solvent 14.60%, 12.36%, 8.70%. Higher inhibition found in methane extract for leaf, stem and root and other extract have less inhibition of hydrogen peroxidation or could not be achieved even at higher concentrations. (Graph 2)



**Graph 2: Graphical representation of antioxidant activity hydrogen peroxide inhibition.**

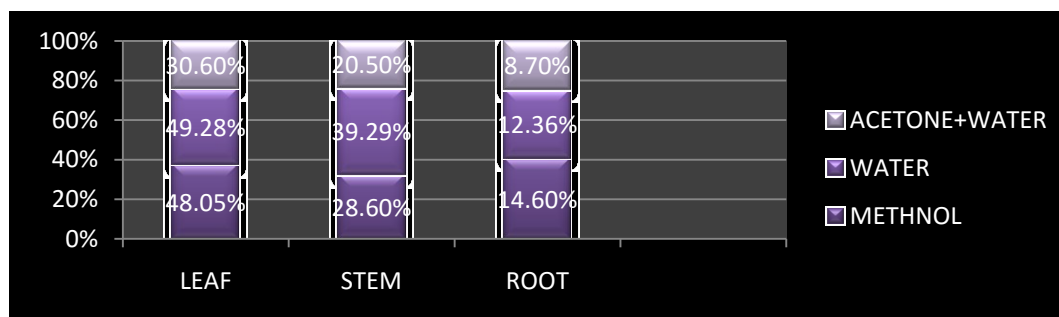
**3.2.3 Total Antioxidant**

The total antioxidant capacity was base on the decrease of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex, at acid pH [ 18 ]. It was observed that the highest

activity exhibited by fenugreek methane-leaf extract (48.05%), water-leaf extract (49.28%), acetone-leaf extract (30.60%). And the lowest activity exhibited by stem (28.60%, 39.29%, 20.50%) and root

(14.60%,12.36%,\_8.70%) in the three

different solvents respectively. (Graph 3)



Graph 3: Graphical representation of total antioxidant capacity.

#### 4. Conclusion

Strong antioxidant capacity of the leaves, root, stem extract of fenugreek and its derived fractions for different in vitro antioxidant assays may be related with the antioxidant constituents. It contains diverse chemical compounds which have shown therapeutic activities, for example, tannins, phytic acid, saponin which are reported as antidiabetics, hypoglycaemic, anti-inflammatory & immunomodulatory activity. Our research concludes presence of many phytoconstituents in *Trigonella foenum graecum* roots, stems, and leaves extracts which can provide various useful biological activities to this medicinal plant. This re-evaluate will be helpful to carry out more scientific investigation to prove the medicinal properties of fenugreek in human being volunteers.

#### 5. References

- [1] Morton J.F., (1990). Mucilaginous plants and their uses in medicine. *J. Ethnopharmacol.*, 29: 245-266.
- [2] Hemlambe S. S., Dande R Payal, (2001). Fenugreek (*Trigonella foenum-graecum* L.): An overview. *International Journal of current Pharmaceutical Review and research*,2(4), ISSN:0976-822X.
- [3] Kochhar, A., Nagi M. and Sachdeva R., (2006). Proximate composition, available carbohydrates, dietary fibre and anti nutritional

factors of selected traditional medicinal plants. *J. Hum. Ecol.*, 19: 195-199.

- [4] Meghwal M., Goswami T.K., (2012). A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. *J. Food Process Technol.*3, 9.

- [5] Puri D., Prabhu K.M., Murthy P.S., (2002). Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. *Indian Journal of Physiology and Phamacology* 46:457-462.

- [6] Petit P.R., Sauvaire Y.D., Hillairebuys D.M., Leconte O.M., Baissac Y.G., Ponsin G.R., Ribes G.R., (1995b) Steroid saponins from fenugreek seeds - extraction, purification and pharmacological investigation on feeding behaviour and plasma cholesterol. *Steroids* 60:674-680.

- [7] Sauvaire Y., Baissac Y., Leconte O., Petit P., Ribes G., (1996). Steroid saponins from fenugreek and some of their biological properties. *Advances in Experimental Medicine and Biology* 405:37-46.

- [8] Hannan J.M.A., Rokeya B., Faruque O., Nahar N., Mosihuzzaman M., Khan A.K.A., Ali L. (2003). Effect of soluble dietary fiber fraction of *Trigonella foenum-graecum* on glycemic, insulinemic, lipidemic and platelet aggregation status of Type 2 diabetic model rats. *Journal of Ethnopharmacology* 88:73-77.

- [9] **Sur P.**, Das M., Gomes A., Vedasiromoni J.R. and Sahu N.P., (2001). *Trigonella foenum graecum* (fenugreek) seed extract as an antineoplastic agent. *Phytother. Res.*, 15: 257-259.
- [10] **Hibasami G.**, Moteki H., Ishikawa K., Katsuzaki H., Imai K., Yoshioka K., Ishii Y. and Komiya T., (2003). Protodioscin isolated from fenugreek (*Trigonella foenumgraecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int. J. Mol. Med.*, 11: 23-26.
- [11] **Duke J.A.** and E.S. Ayensu, (1985). Medicinal Plants of China. Vol. 1, Reference Publications, *Algonac, MI., USA.*, ISBN: 9780917256264, Pages: 705.
- [12] **Alo M.N.**, Anyim C., Igwe J.C., Elom M., and Uchenna D.S., (2012). Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amygdalina* and *Aframomum melegueta* Pelagia Research Library Advance in Applied Science Research. 3(2):844-848.
- [13] **Mallick C.P.** & Singh M.B., (1980). In: plant Enzymology and Histo Enzymology, *Kalyani publisher* New Delhi. 286.
- [14] **Wilcox J.K.**, Premchandra G.S., Young K.A. & Raboy V., (2000). Isolation of High seed inorganic P, low-phytate mutants, *Crop Science. Crop Science.* 40:1601-1605.
- [15] **Schanderi S.H.**, (1970). Methods in Food Analysis. New York. p-709.
- [16] **Harborne J.B.**, (1973). Phytochemical methods: London; *London Chapman and Hall, Ltd.*;49-188.
- [17] **Hitoshi S.**, Alexson T., Tropepe V., Donoviel D., Elia A.J., Nye J. S., Conlon R. A., Mak T.W., Bernstein A., and Vander K.D.,(2002). Notch pathway molecules are essential for the maintenance, but not the generation of mammalian neural stem cells. *Genes & Dev.*16:846-858.
- [18] **Umamaheshwari M.**, Asokkumar K., Somasundaram T., Subhadra Devi V., Ravi T.K., (2007). Xanthine oxidase inhibitory activity of some Indian medical plants. *J. Ethnopharmacol.* 109:547-551.
- [19] **Balandrin M.J.** and Klocke J.A., (1988). Medicinal Aromatic and industrial materials from plants. Bajaj, Y.P.S. editor, *Biotechnology in Agriculture and Forestry Medicina and Aromatic Plant. Springer Verlag, Berlin, Heidelberg.* 1-36.
- [20] **Gupta S.S.**, (1994). Prospects and perspectives of natural plant products in medicine. *Indian Journal of Pharmacology.* 26: 1-12.
- [21] **Ekka N.R.**, Namdeo K.P, Samal P.K., (2008). Standardization strategies for herbal drugs – An overview. *Research Journal of Pharmacy and Technology.* 1: 310-312.
- [22] **Shang M.**, Cai S, Han J, Li J, Zhao Y, Zheng J, Namba T, Kadota S, Tezuka Y, Fan W., (1998). Studies on flavonoids from Fenugreek (*Trigonella foenum graecum*). *Zhongguo Zhong Yao Za Zhi*; 23: 614-6.
- [23] **Raju J.**, Patlolla JMR, Swamy MV, Rao CV. Diosgenin, (2004). A steroid saponin of *Trigonella foenum graecum* (Fenugreek, inhibits azoxymethane induced aberrant crypt foci formation in F344 rats and induces apoptosis in HT-29 human colon cancer cells. *Cancer Epidemiol Biomarkers Prev* 13: 1392-8.
- [24] **Pandian B. R.**, Anuradha CV, Viswanathan P., (2002). Gastroprotective effect of fenugreek seeds (*Trigonella foenum-graecum*) on experimental gastric ulcer in rats. *J Ethnopharmacol* 81: 393-7.