**Journal of Plant Sciences**
2015; 3(1-1): 40-44
Published online December 30, 2014 (http://www.sciencepublishinggroup.com/j/jps)
doi: 10.11648/j.jps.s.2015030101.16
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

# *In vitro* regulation of bioactive compounds in *Trigonella* species by mutagenic treatments

**Agarwal Madhu[1], Agarwal Mala[2], Jain S. C.[1]**

[1]Department of Botany, B.B.D. Government P.G. College, Chimanpura (Shahpura), Jaipur, Rajasthan, India
[2]Department of Botany, University of Rajasthan, Jaipur, Rajasthan

**Email address:**
agarwal.mala@yahoo.co.in (A. Mala)

**To cite this article:**
Agarwal Madhu, Agarwal Mala, Jain S. C.. *In Vitro* Regulation of Bioactive Compounds in *Trigonella* Species by Mutagenic Treatments. *Journal of Plant Sciences.* Special Issue: Medicinal Plants. Vol. 3, No. 1-1, 2015, pp. 40-44. doi: 10.11648/j.jps.s.2015030101.16

**Abstract:** Seeds of Trigonella *foenum-graecum* and *Trigonella corniculata* were treated with different concentrations of EMS, MMS and $NaN_3$ to study the effect on steroidal sapogenins production. Enhanced level of both the steroidal sapogenins was observed with all the three chemical mutagens with maximum augmentation in EMS at 0.1 M.

**Keywords:** *Trigonella Corniculata, Trigonella Foenum-Graecum*, Ethyal Methane Sulphonate (EMS), Diosgenin, Tigogenin, Methyl Methane Sulphonate (MMS), Sodium Azide ($NaN_3$)

## 1. Introduction

Plants are the tremendous source for the discovery of new products with medicinal importance in drug development [1]. Today several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world. Secondary metabolites are economically important as drugs, and many drugs of today are simple synthetic modifications or copies of the naturally obtained substances. The evolving commercial importance of secondary metabolites has in recent years resulted in a great interest in secondary metabolism, partially in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology [2-8].Amount of drugs, which are artificially produced in 8 week old callus can be compared with naturally occurring in plant [9].

According to Staba,1980 [10],steroids are divided into four different types(1)sterols are alcoholic and 'ol' suffix(*B*-sitosterol, stigma sterol(2)Steroline are glycosides of sterol and insoluble in water (Cholesterol, estrones(3)Steroidal saponins have 'nin'suffix(diosgenin)(4)nitrogen containing steroidal saponins. Saponins may be either triterpenoids gltcosides or glycosides of steroids with spiroketal side chain. It is of four types (1) Triterpenoid saponins (panaxadiol, panaxatriol and oleonlic acid (2) Steroidal saponins (diosgenin, tigogenin, prototokoronin, tokorogenin, yamogenin, gitogenin, manogenin) (3) Nittrogen containing saponins (Steroidal alkaloids and solasodine) (4) Cardenolide saponins. Approximately two third of the raw material for chemical synthesis of the steroid hormones produced has depend on diosgenin obtained from the plant Dioscorea root [11-13].

Saponins, widely distributed in the plant kingdom, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional and industrial applications [14]. Food and nonfood sources of saponins have come into renewed focus in recent years due to increasing evidence of their health benefits. Saponins can impact the immune system through their adjuvant activity, their ability to improve effectiveness of orally administered vaccines by facilitating the absorption of large molecules, and their immune-stimulatory effects. The ability of saponins to act as immunological adjuvant by enhancing the immune response to antigens has been recognized since 1940's [15-16].

Cholesterol-lowering activity of saponins, which has been demonstrated in animal and human trials, has been attributed to inhibition of the absorption of cholesterol from the small intestine, or the reabsorption of bile acids [17-19]. The cholesterol lowering effect of dietary saponins in humans is also supported by ecological studies [20]. Anticancer activity has been reported for a number of triterpene and steroid saponins [21-22] and diosgenin [23].

These compounds are very useful in pharmaceutical

industries as a natural source of steroidal hormones. Since its discovery, diosgenin is the single main precursor in the manufacture of synthetic steroids in the pharmaceutical industry. To date, diosgenin and related steroidal saponins were commercially obtained from the tubers of *Dioscorea* species; however, it is crucial to discover new and alternative sources of these compounds due to decreasing plant resources as well as increasing demand. One such alternative is fenugreek.

Considering the high market potential of steroidal saponins and vivid reports of steroidal saponins content in fenugreek seeds, the present study was carried out to identify the effects of mutagens on fenugreek. The high levels of steroidal saponins might help to make these plant species economically, utilized as a source of steroidal saponins used for the synthesis of steroid drugs in pharmaceutical industries.

T*rigonella foenum-graecum*, commonly known as fenugreek, is a small seeded annual dicotyledonous legume belonging to the subfamily Papilionaceae, family Fabacecae. *Trigonella* L. includes about 135 species worldwide and is native to southeastern Europe and western Asia [24]. *Trigonella cornicculata* known as cultivated fenugreek/kasuri methi is another economically important species of the genus Trigonella. Fenugreek is widely cultivated in India, Argentina, Egypt, and Mediterranean countries. In India, it is grown extensively in Rajasthan, Gujarat, Madhya Pradesh, Uttar Pradesh, Maharashtra, Punjab, Tamil Nadu, Andhra Pradesh, Himachal Pradesh, and Haryana. About 200 distinguished varieties of fenugreek are distributed in India belonging to Deshi and Champa elite varieties. Improved varieties of fenugreek are Gujarat Methi-1, Rmt-1 (Rajasthan Methi), Prabha (NLM), Methi No. 47, Methi No. 14, Rajendra Kranti (RM-16), Co-1, UM-34, UM-35, Kasuri, and Kasuri Selection. The karyological study of *Trigonella* taxa, somatic chromosome numbers were observed as $2n$ = 14, 16, 30, and 46 and B chromosome was also observed in somatic cells of some taxa [24]. The genome size of *Trigonella foenum-graecum* is approximately 685 Mbp (c-value of approximately 0.7), which is approximately 1.5-fold larger than the model legumes, *Lotus corniculatus* L. var. *japonicus* Regel [syn. *Lotus japonicus* (Regel) K. Larsen] and barrel (*Medicago truncatula* Gaertn.), both of which have compact genomes of approximately 470 Mbp [25].

Different parts of the plant such as leaves and seeds are consumed in India. Fenugreek is regarded as the oldest known medicinal plant in recorded history and has been used to reduce blood sugar and lower blood cholesterol in humans and animals[26-27]. It contains three important chemical constituents with medicinal value, that is, (i) steroidal sapogenins, (ii) galactomannans, and (iii) isoleucine. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products[28]. Beneficial effects of fenugreek can be attributed to its bioactive molecules, which include saponins, alkaloids, flavonoids, mucilaginous fiber, lysine-rich proteins, and volatile oils. It has been reported that fenugreek contains 81 phyconutrients and diosgenin, a steroid saponin found in fenugreek seeds, is the most bioactive component[27]. Diosgenin is often used as a raw precursor for the production of steroidal drugs and hormones such as testosterone, glucocorticoids, and progesterone. Studies reveal that a maximum level of diosgenin [(25R)-5-spirosten-3h-ol] is found to be in young leaves (20 mg g–1 dry weight) and in mature seeds the percentage ranges from 0.28 to 0.92%. Mcanuff[29] reported that steroidal sapogenins were effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes. At present natural diosgenin is procured economically from the tubers of certain wild species of Mexican yam (*Dioscorea* spp.). However, this process is both time consuming and costly, requiring several years before the yam tubers grow to a size where they possess a sufficient concentration of diosgenin to be used as source of commercial and pharmaceutical reagent[30-32]. Fenugreek may be a viable alternative for production of diosgenin because of its shorter growing cycle, lower production costs, and consistent yield and quality.

*Trigonella* species are known to contain steroidal sapogenins [33-38], especially diosgenin, and thus, they are of great significance to the pharmaceutical industry. Mutagens have been used to improve the quality and quantity of the chemical produced by crops[39],but very little work has been carried out on the effect of chemical mutagens on steroidal sapogenins in tissue cultures in *Trigonella* species[40-42].Although the effect of chemical mutagens on steroidal sapogenins has been investigated in *Trigonella* species [43].The combined effect of irradiations and incubation with sodium azide on diosgenin content were similarly studied in *T.foenum-graecum*[44].Therefore, in the present investigation we have studied the individual effect of ethyl methane sulphonate(EMS),methyl methane sulphonate(MMS)and sodium azide($NaN_3$) on bioactive compounds diosgenin and tigogenin level in *T. foenum-graecum* and *T.corniculata* with the aim to regulate their biosynthesis in these plant species.

## 2. Materials and Methods

The surface sterilized seeds of both *T. foenum-graecum* and *T.corniculata* were individually treated with different concentrations of EMS, MMS and $NaN_3$, washed to remove trace(s) of mutagens used and inoculated on RT medium to obtain callus. The resultant callus maintained by periodic subcultures (6-8 weeks) in each case onto fresh medium.

In both the plant species, the calli of each sample were harvested after 8 weeks of the transfer age (being the maximum callus growth), dried and growth indices (GI) were calculated (final dry weight-initial dry weight).Each of the treated sample was dried, powdered, defatted (petrol40-60$^0$) and hydrolyzed with 15% ethanolic HCL (w/v).Each hydrolysate was processed further [45] using ethyl acetate to extract the steroidal sapogenins. Later such samples were reconstituted in chloroform, filtered, dried again and weighed.

The steroid extract after analytical TLC (Silica gel; chloroform-hexane-acetone, 23:5:2; spray-anisaldehyde

reagent) were subjected to preparative TLC and the bands coinciding to diosgenin (Rf0.59) and tigogenin (Rf0.65), eluted and crystallized. The purity of the isolated compounds was checked by 2D-TLC(Silica gel:ID:dichloromethane-methanol-formamide,93:6:1; 2D: cyclohexane-ethylacetate-water,600:400:1).Both compounds were subjected for mp, mmp and spectral studies and the data compared with those of standards [46-47].For quantification of the two sapogenins, the spectrophotometric method[48] was followed after TLC on silica gel G.

An increase in growth indices with low concentration of all the three chemical mutagens were recorded in both the plant species, where the maximum enhancement was observed in 0.2M EMS(~59%),0.0125M MMS(91%)and 0.0001M $NaN_3$ treatments as compared to control(Table1).However, the increased doses of mutagenic treatments resulted in decline in the GI. Increased growth indices at 0.02 M MMS and 0.0001M $NaN_3$ have also been investigated by Jain and Agarwal(1993)[42]in *Trigonella foenum-graecum* cell cultures.

# 3. Results and Discussion

***Table 1.*** *In vitro effect of chemical mutagens of growth index (GI) and bioactive principles (Mg/GDW) in Trigonella species*

| Treatment (M) | *T. foenum-graceum* | | | *T. corniculate* | | |
|---|---|---|---|---|---|---|
| | Bioactive Principles | | | | | |
| | GI* | Diosgenin | Tigogenin | GI* | Diosgenin | Tigogenin |
| EMS-Control | 4.45 | 7.40 | 2.05 | 4.95 | 1.50 | 0.75 |
| 0.1 | 6.28 | 18.45 | 8.15 | 6.93 | 2.15 | 0.95 |
| 0.2 | 6.75 | 9.25 | 4.25 | 8.44 | 2.40 | 1.10 |
| 0.3 | 4.83 | 4.25 | 1.20 | 5.23 | 1.12 | 2.94 |
| 0.4 | 3.85 | 3.20 | 0.95 | 3.24 | 0.95 | 0.43 |
| MMS-Control | 4.45 | 7.35 | 2.10 | 5.12 | 1.45 | 0.70 |
| 0.0125 | 8.25 | 9.40 | 3.15 | 4.23 | 2.40 | 0.94 |
| 0.025 | 6.28 | 18.95 | 4.25 | 6.29 | 1.20 | 0.79 |
| 0.05 | 4.12 | 9.95 | 1.90 | 4.28 | 0.92 | 0.64 |
| $NaN_3$- Control | 4.22 | 7.45 | 2.15 | 5.43 | 1.45 | 0.83 |
| 0.00005 | 5.92 | 9.20 | 3.10 | 4.49 | 2.94 | 0.95 |
| 0.0001 | 6.14 | 10.15 | 4.25 | 8.23 | 2.15 | 1.10 |
| 0.0005 | 5.63 | 12.50 | 3.20 | 5.29 | 1.10 | 0.84 |
| 0.001 | 4.59 | 6.40 | 2.05 | 4.24 | 0.95 | 0.62 |

*Dry weight basis

An increase in the level of both the steroidal sapogenins were observed at low concentration of all the three chemical mutagens in both *T. foenum-graecum* and *T.corniculata* .The maximum augmentation in both diosgenin and tigogenin were observed at 0.1 M EMS(~2-3fold),0.025 M MMS(~2-2.5fold)and 0.0001 M $NaN_3$(~2fold)in *T. foenum-graecum* ,while in *T.corniculata* the maximum augmentation were observed at 0.2 M EMS(~65%),0.0125 M MMS (~75%) and 0.0001 M $NaN_3$ (~2fold)treatments .However ,their higher concentrations proved to be inhibitory for the enhancement of their bioactive compounds .Jain and Agarwal (1993)[42]also observed the 2.5 fold increase in diosgenin level in *T. foenum-graecum* by chemical mutagenic treatments. Similarly, the regulatory effect of this chemical mutagen in *T. foenum-graecum* and *T.corniculata.* Were observed [43] *in vivo*.

Enhanced yield of diosgenin and other steroids in the cell cultures of *T. foenum-graecum* by colchicines (0.04 to0.1%),EMS(0.05 to 0.4%) and maleic hydrazide treatments has been investigated by earlier workers [40-41].from the above findings, it is established that the chemical mutagens may regulate the callus growth as also the steroidal sapogenins in the plants.

# 4. Conclusions and Future Prospects

Steroidal sapogenins present in trigonella species are a group of plant secondary metabolites and are valuable source of drugs(49-50). It is possible to increase the amount of these compounds, by using tissue culture technique. One of the effective methods to increase the steroidal sapogenins quantitatively is the mutagenic treatments in the form of mutagens. In the present work seeds of *T. foenum-graecum* and *T.corniculata* were treated with different concentrations of EMS,MMS and $NaN_3$,and inoculated on RT medium to obtain callus.

An increase in the level of the steroidal sapogenins, diosgenin and tigogenin was observed at low concentration of all the three chemical mutagens in both *T. foenum-graecum* and *T.corniculata* .In future we can enhance the concentration of bioactive compounds in medicinal plants by using different mutagens.

# Acknowledgment

Financial assistance from the UGC, New Delhi, is gratefully acknowledged.

# References


[1] G. Hansen, M.S Wright, " Recent advances in the transformation of plants. Trends Plant Sci.,"vol4, pp 226–31,1999.

[2] F. Di Cosmo, M. Misawa, "Plant cell and tissue culture: alternatives for metabolite production. Biotechnol Adv." Vol 13,pp425–53,1995.

[3] G. Hansen, M.S Wright, "Recent advances in the transformation of plants. Trends Plant Sci.,"Vol4, pp 226–31,1999.

[4] A. Giri, M.L Narasu , " Transgenic hairy roots. Recent trends and applications. Biotechnol Adv.,"Vol18pp1–22,2000.

[5] L.D.Sajc, G, Grubisic and, N. Vunjak, " Bioreactors for plant engineering: an outlook for further research," Biochem Eng J.,Vol 4,pp89–99,2004.

[6] S.R.Rao, and G.A. Ravishankar , " Plant cell cultures: Chemical factories of secondary metabolites," Biotechnol Adv. ,Vol20,pp101–53,2002.

[7] P.H.Canter, H. Thomas and E.Ernst ,"Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology," Trends Biotechnol ,Vol23,pp180–5,2005.

[8] M.Z. Abdin, " Enhancing bioactive molecules in medicinal plants," in Natural Products-Essential resources for human ,Zhu Y, Tan B, Bay B, Liu C, Eds. Singapore: World Scientific Publishing Co. Pvt. Ltd, 2007, pp. 45–57.

[9] M. Christian, "Steroids-Chemical Constituents of *Phyllanthus fraternus* webester through TLC & HPTLC .International research Journal of Chemistry,". http/ijrc.petsd.org,pp40-49,2013.

[10] E.J. Staba, "1980.Plant tissue culture as a source of biochemicals, "CRC Press, Florida,1980.

[11] E. Capsi ,J.P Moreau and P.J. Raman, " Journal of American Chemical Society, "Vol.96, pp8107,1974.

[12] M.E.Curtin,"1983.Harvesting profitable products from plant tissue culture , Biotechnology,"Vol1,pp649-657.

[13] N.P.Prasad, and E..K.J Amnal, "*Cortus matorticanus* H. Wedl-a new source for diosgenin,"curr.Sci.,Vol52(170)pp825-826,1983

[14] K. Hostettmann, A. Martson, "Saponins", Cambridge, New York, Cambridge University Press,pp310-25,2005

[15] R.Bomford,M.Stapleton,S.Windsor,J.E.Beesley,E.A.Jessup,K. R.Price, "Adjuvanticoity and ISCOM formation by structurally diverse saponins"Vaccine,Vol10(572-577),1992

[16] G.Francis, Z.Kerem, H.P.Makkar and K.Becker, "The biological action of saponins in animal systems: A review" Br J Nutr,Vol88pp587-605,2002.

[17] D.Oakenfull,G.S.Sidhu, "Could saponins be a useful treatment for hypercholesterolemia" Eur J Clin Nutr,Vol44pp79-88.

[18] H.Matsuura, "Saponins in garlic as modifiers of the risk of cardiovascular disease" J NutrVol131pp1000-1005,2001.

[19] S.W.Kim,S.K.Park,S I Kang,H.C.Kang,H.J.Oh,C.Y.Bae, "Hypocholestromic property of *Yucca schidigera* and *Quillaja saponaria* extracts in human body. Arch Pharm Res,Vol26pp1042-1046,2003.

[20] L. Chapman ,T.Johns, R.L.Mahunnah, "Saponin-like in vitro characteristics of extracts from selected non-nutrient wild plant food additives used by Masaai in meat and milk based soups"Ecol Food Nutr,Vol36pp1-22,1997.

[21] M.A.Berhow, E.D.Wagner, S.F.Vaughn and M.J.Plewa, "Characterization and antimutagenic activity of soyabean saponins" Mutat Res,Vol448pp11-22,2000.

[22] S.M.Kerwin, "Soy saponins and the anticancer effects of soyabeans and soy-based foods. Curr Med Chem Anticancer Agents,Vol4pp263-272,2004.

[23] J.Raju, J.M.Patlolla, M.V.Swamy and C.V. Rao, "Diosgenin ,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,Vol13pp1392-1398,2004

[24] E.Martin, H. Akan, M. Ekici, and Z. Aytac, "New chromosome numbers in the genus *Trigonella* L. (Fabaceae) from Turkey" Afr. J. Biotechnol. Vol 10pp116–125,2011.

[25] N. D.Young, J. Mudge, and T.H. Ellisz, "Legume genomes: More than peas in a pod" Curr. Opin. Plant Biol.Vol 6pp199–204.,2003

[26] V.Mathur and N.K. Mathur, "Fenugreek and other lesser known legume galactomannan-polysaccharides: Scope for development" J. Sci. Ind. Res.. (India) Vol64:475–481,2005.

[27] M.Aasim, N. Hussain, E.M. Umer, M. Zubair, B.S. Hussain, S. Saeed, T.S. Rafique, and C. Sancak, " *In vitro* shoot regeneration of fenugreek (*Trigonella foenum-graecum* L.) using different cytokinins" Afr. J. Biotechnol., Vol. 9 pp7174–7179,2010.

[28] S.Acharya, A. Srichamroen, S. Basu, B. Ooraikul, and T. Basu, " Improvement in the nutraceutical properties of fenugreek (*Trigonella foenum-graecum* L.)" Songklanakarin J. Sci. Technol.,Vol 28pp1–9,2006.

[29] M.A.McAnuff, F.O. Omoruyi, E.Y. Morrison, and H.N. Asemota, "Plasma and liver lipid distributions in streptozotocin-induced diabetic rats fed sapogenin extract of the Jamaican bitter yam (*Dioscorea polygonoides*)" Nutr. Res.Vol 22pp1427–1434,2002.

[30] S.N. Acharya, S.N., J.E. Thomas, and S.K. Basu, " Fenugreek, an alternative crop for semiarid regions of North America" Crop Sci. Vol 48pp841–853,2008.

[31] A Rosser, "The day of the yam" Nurs. Times Vol 81:47–48,1985.

[32] D. R. Zerbino, and E. Birney, " Velvet: Algorithm for de novo short read assembly using de Bruijn graphs" Genome Res.Vol 18pp821–829,2008.

[33] R.E.Marker ,R.B. WagnerP. R., Ulshafer,E.L. Wittecker , " Goldsmith DPJ & Ruof CH . New Sources of sapogenins, J Amer Chem Soc Vol69,pp2242-2244,1947.

[34] F.R.Y.Fazli and Hardman, "The spice, fenugreek (*Trigonella foenum-graecum* )its commercial var. of seed as a source of diosgenin," Trop SciVol 10pp66-78,1968.

[35] I.P.Varshney,and A.R.Sood , "Study of sapogenins from *Trigonella foenum-graecum* Linn. Stem and leaves and *T.corniculata* Linn. leaves and flowers," Indian J Apple ChemVol34,pp208-210,1971.

[36] H.Wagner ,M.A Tyengar, and L H'orthammner, ".Vicine-1 and 2 in the seeds of *Trigonella foenum-graecum,"* .PhytochemistryVol12,pp2548,1973.

[37] M.B.Bohannon , J.W. Hagemann ,F.R. Earle and A.S.Barclay , "Sceening seed of *Trigonella* and three related genera for diosgenin," PhytochemistryVol13,pp1513-1514,1974.

[38] A.R. Sood, "Chemical components from the leaves of *Trigonella foenum-graecum* Linn .," Indian J Pharm Vol37,pp100-101,1975.

[39] R.Robson, B. Sigurbjornsson,and A.Micke , "Plant character to improve by mutation breeding, In Manual of mutation breeding,"pp188 IAEA, Vienna,1977.

[40] S.C.Jain , ".Secondary product from some Indian medicinal plants in tissue cultures," In IV Intl. Cong Plant Tissue Cell Cult. Calgry Abstr, pp1421,1978.

[41] J.Guzewoska ,Furmanowa, Beldowka and & MIN Rzedowski, " *Trigonella foenum-graecum* in tissue culture and maleic hydrazide on callus formation and diosgenin production" in VII Cong. Polish Genet. Soc Lodz Poland, 1983,pp114.

[42] S.C.Jain, and M, Agarwal, "Effect of chemical mutagens on steroidal sapogenins in *Trigonella* species, "Phytochemistry,Vol26(8),pp2203-2205,1987.

[43] S.C.Jain, and M. Agarwal, "Effects of chemical mutagens on growth and diosgenin level in cell cultures of *Trigonella foenum-graecum* L.," Indian Drugsvol3pp127-128,1993.

[44] K.P. Bhusari, S.N.Lulay and G.V.Umalkar, "Effect of irradiation and incubation with sodium azide on the yield of diosgenin in *Trigonella foenum-graecum,"* Indian Drugs Vol 19, pp187, 1982.

[45] Y.Tomita ,A. Uomori,and H. Minato , "Steroidal sapogenins and sterols in tissue cultures of *Dioscorea tokoro*," Phytochemistry vol 9,pp111-114,1970.

[46] R.E.Marker ,R.B. Wagner P. R., Ulshafer,E.L. Wittecker , " Goldsmith DPJ & Ruof CH . New Sources of sapogenins, J Amer Chem Soc Vol69,pp2242-2244,1947.

[47] P.Khanna, and S.C.Jain, "Diosgenin,gitogenin and tigogenin from *Trigonella foenum-graecum* L. tissue cultures, "LioydiaVol36pp96-98,1973.

[48] G. L. Sanchez, A. Medina, and R.R.Soto, "Spectrophotometric determination of diosgenin in *Dioscorea composita* following thin layer chromatography ,"Analyst,Vol97,pp973,1972.

[49] K. Vaidya, A. Ghosh, V. Kumar S. Chaudhary, N. Srivastava, K. Katudia, T. Tiwari and S. K. Chikara , "De Novo Transcriptome Sequencing in *Trigonella foenum-graecum* L. to Identify Genes Involved in the Biosynthesis of Diosgenin" The Plant Genome,Vol6(2),2013.

[50] M.Arivalagan, K.K. Gangopadhyay and G. Kumar, "Determination of Steroidal sapogenins and fixed oil content in fenugreek genotypes" Indian J. Pharmaceutical Sciences,Vol75(1)pp110-113,2013