The Pharma Innovation Journal 2020; 9(9): 52-57

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 52-57

www.thepharmajournal.com
Received: 28-07-2020
Accepted: 30-08-2020

Shesharao
Assistant Professor (SS), IAH & VB, KVAFSU, Kalaburagi, Karnataka, India

Sugunarao
Professor, Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore, Karnataka, India

Sathyanarayana Ml
Professor and head, Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore, Karnataka, India

Shridhar N
Department of Veterinary Pharmacology and Toxicology, Veterinary College, Hebbal, KVAFSU, Bangalore, Karnataka, India

Corresponding Author:
Shesharao
Assistant Professor (SS), IAH & VB, KVAFSU, Kalaburagi, Karnataka, India

Phytochemical analysis of *Trigonella foenum graecum* and *Coccinia indica* for their different components by HPTLC

Shesharao, Sugunarao, Sathyanarayana ML and Shridhar N

Abstract
Trigonella foenum graecum (fenugreek, locally as methi, Fabaceae, and *Coccinia indica* (Bimba, kanduri, Cucurbitaceae) are known for their hypoglycemic and antidiabetic properties in Ayurvedic system of medicine. The traditional medicine involves the uses of different plant extracts or the phytochemical bioactive constituents, which provides the health application at an affordable cost. Secondary metabolites are responsible for medicinal activity of these plants. Qualitative phytochemical analysis of these plants confirm the presence of various phytochemicals like mainly alkaloids, Antherecene derivatives, glycosides, flavonoids, Bitter principles, coumarins, and Saponin etc. The result suggest that the phytochemical properties of these plants which having bioactive constituents are responsible for their hypoglycemic and antidiabetic effects in steptozotocin induced diabetes in rats.

Keywords: Diabetes, hypoglycemic, antidiabetic, streptozotocin

Introduction
Herbal medication has been used for the treatment of variety of ailments and a huge number of populations in the world are still entirely dependent upon traditional medicines. A number of medicinal plants and their formulations are being used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices (Pareek *et al*. 2009) [10]. Since the time of Charaka and Sushrutha indigenous remedies have been used in the treatment of diabetes. From the ethnobotanical information, more than 1200 species of plants have been screened for antidiabetic activity on the basis of ethnomedicinal uses (Singh *et al*., 2011) and about 800 plants with antidiabetic potential have been reported (Venkatesh *et al*., 2010; Singh *et al*., 2011 and Patel *et al*., 2012) [17, 11]. Several plants have been used as dietary adjuvant and in treating a number of diseases even without any knowledge of their proper function and constituents mainly because of their fewer side effects compared to synthetic hypoglycaemic agents and also because of their safety, effectiveness and availability. There are about 200 pure compounds from plant sources reported to reduce blood glucose level. The compounds are mainly alkaloids, Antherecene derivatives, glycosides, flavonoids, Bitter principles, coumarins, and Saponin. The families of plants with most potent hypoglycaemic effects include leguminoseae, lamiaceae, liliaceae, cucurbitaceae, asteraceae, moraceae, rosaceae, euphorbiaceae and araliaceae (Bnouham *et al*., 2006) [2].
Although hundreds of traditional plants with phytochemical compounds have been identified, only a small number of them have been evaluated scientifically for their efficacy. Hence, the present study was conducted with *Trigonella foenum graecum* and *Coccinia indica* indusially and in combination commonly known as methi and Little gourd (kovai) respectively which are reported to possess different compounds (Raju *et al*., 2001; Srinivasan, 2006; Khalki *et al*., 2010) [12, 14, 7] which is helpful in treating diabetes in human being.

Materials and Methods
Phytochemical analysis by HPTLC
Phytochemical analysis of the alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* was carried out using HPTLC technique (Wagner *et al.*, 1984) [18].

Procedure of TLC
Pre-coated silica gel 60F 254 TLC aluminium 10x10 cm (Merck, India) type of plates were used for HPTLC.

From each alcoholic extract, 5 µl samples were spotted on a TLC silica gel plate (CAMAG Linomat 5, Germany). Chromatography was performed using solvent systems. This procedure was followed for the analysis of alkaloids, anthracene derivatives, flavonoids, bitter principles, coumarins, saponins and glycosides.

Preparation of extracts for Thin Layer Chromatography

Alkaloids: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (0.5 g each) were weighed and mixed with 10 ml of 0.5 N HCl. The contents were vortexed and pellets were discarded. To the supernatant 30 per cent Na_2CO_3 (pH 10) was added and centrifuged at 2000 rpm for 5 minutes and supernatant was discarded. The precipitate was washed with chloroform and chloroform extract was collected. Once again the residue was washed with methanol and methanol extract was collected. The chloroform and methanol extract were concentrated to one ml and used for chromatography. The Toluene: Ethyl acetate: Diethylamine: (70:20:10) Chromatography solvent was used.

Detection: Dragendorff reagent

The ready to use reagent (SD Fine-Chem Limited, Mumbai) was used. One ml of Dragendorff reagent was diluted with four ml of acetic acid and 20 ml of water. The plate was immersed in the reagent for one second and examined under white light.

Anthracene derivatives: The finely ground alchholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (0.5 g each) were weighed and extracted by warming for five minutes on the water bath with five ml of methanol. The clear filtrates were used directly for HPTLC. A mixture of ethyl acetate: methanol: water: (100:17:13) was used as solvent system for the detection of anthracene derivatives.

Detection: Natural products-polyethylene glycol

The plate was heated to 100 °C for three minutes, then dipped in solution A (1 g diphenylboronic acid aminoether ester dissolved in 200 ml ethyl acetate), dried and dipped in solution B (10 g polyethylene glycol 400, dissolved in 200 ml dichloromethane).

Bitter principles: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately for 10 minutes with 10 mL methanol at 60 °C on the water bath. The mixtures were filtered and the filtrates were evaporated to a volume of about two ml. Ethyl acetate: methanol: water: (77:15:08) was used as solvent system for the detection of bitter principles.

Detection: Vanillin-sulphuric acid

The reagent consisted of 5 per cent ethanolic sulphuric acid (Solution I) one per cent ethanolic vanillin (Solution II). The plate was sprayed vigorously with 10 ml of solution I, followed immediately by five to 10 ml of solution II after heating the TLC plate at 100 °C for five to10 minutes. The plate was examined under white light and UV 366 nm.

Coumarins: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC. Toluene: ether (1:1, saturated with 10% acetic acid), was used as solvent system for the detection of coumarins

Detection: Potassium hydroxide

Ethanolic KOH (5%) was used as spray reagent. The plate was immersed in the reagent for one second and was observed at UV-366 nm.

Flavonoids: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* were extracted separately with 10 mL methanol for five minutes in a water bath at about 60 °C. The clear filtrates were used for chromatography. Ethyl acetate: formic acid: glacial acetic acid: water: (100:11:11:27) Chromatography solvent was used.

Detection: Fast blue salt B

The spray reagent was prepared by dissolving 0.5 g fast blue salt in 100 mL water. The plate was sprayed and dried. The plate was examined under white light and UV 366 nm.

Glycosides: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (1 g) were extracted separately by shaking with 10 ml methanol for 30 min on the water bath. The clear filtrates were evaporated to about one ml and 20 µl was applied to TLC plates. Toluene: ether (1:1, saturated with 10% acetic acid), was used as solvent system for the detection of glycosides.

Detection: Aniline-diphenylamine phosphoric acid

The spray reagent was prepared using four grams of diphenylamine and four ml aniline which were dissolved in 160 ml acetone. To this 30 ml of O-phosphoric acid was carefully added. The plate was immersed in the reagent for one second and then heated at 120 °C. The plate was examined under white light. This method was used to detect the presence of glycosides.

Saponins: The finely ground alcoholic extracts of *Trigonella foenum graecum* and *Coccinia indica* (2 g) were extracted separately by heating for 10 min under reflux with 10 ml of 70% ethanol and the clear filtrates were evaporated to about five ml for chromatography. Chloroform: methanol: water: (64:50:10) was used as solvent system for the detection of saponins.

Detection: Blood reagent

Ten mL of 3.6% sodium citrate was added to 90 ml of fresh bovine blood. 0.2 ml of this mixture was mixed with 30 ml phosphate buffer pH 7.4. The plate was sprayed in horizontal position and the plate was observed in visible light.

Detection without chemical treatment for all compounds

TLC plates were observed under UV-254 nm and UV-366 nm.

Results

Phytochemical analysis

Phytochemical analysis of *Trigonella foenum graecum* and *Coccinia indica* alcoholic extracts of seed and leaves powder was carried out using High Performance Thin Layer Chromatography Technique (Plates 1-7).

HPTLC profile of alcoholic extract was generated in solvent systems of different polarities in order to ascertain the total

number of chemical moieties which help in designing the method of isolation and characterization of bioactive compounds.

1. Alkaloids
a) Without chemical treatment
There was a pronounced quenching of fluorescence on TLC plates at UV-254 nm, intense blue fluorescence at UV-366 nm for *Trigonella foenum graecum* and *Coccinia indica* extracts on TLC plates at UV-254 nm and UV-366 nm (Plate 1).

b) Dragendorff reagent
There were blue or brown bands on TLC plates at UV-254 nm and on TLC plates at UV-366 nm for *Trigonella foenum graecum* extract (Plate 5).
Intence blue bands appeared on TLC plates for *Coccinia indica* extract at any UV wave lengths (Plate 1).

Inference: The alcoholic *Trigonella foenum graecum* seed extract and *Coccinia indica* leaves extracts are positive for the presence of alkaloids.

2. Anthracene derivatives
a) Without chemical treatment
There was pronounced quenching on TLC plates at UV-254 nm and development of blue bands on at UV-366 nm with brown and light yellow bands at visible light for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extracts (Plate-2).

b) Natural products – polyethylene glycol
Bluish brown bands appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates in visible light and UV - 366 nm. (Plate-2).

Inference: The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract were positive for the presence of anthracene derivatives.

3. Bitter principles
a) Without chemical treatment
There was pronounced quenching of fluorescence for alcoholic *Trigonella foenum graecum* seed extract but there was no pronounced quenching for alcoholic *Coccinia indica* leaves extract on TLC plates at UV-254 nm and UV- 366 nm (Plate 3).

b) Vanillin-sulphuric acid
Greenish fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract but there was no fluorescence appeared for alcoholic *Coccinia indica* leaves extract on TLC plates at UV-366 nm (Plate 3).

Inference: The alcoholic *Trigonella foenu graecum* seed extract consists of Bitter principles, whereas alcoholic *Coccinia indica* leaves extract is negative for the presence of Bitter principles.

4. Coumarins
a) Without chemical treatment
There was distinct fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-254 nm and blue fluorescence appeared on TLC plate at UV-366 nm (Plate 4).

b) Potassium hydroxide
There was appearance of pronounced blue fluorescence for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV-366 nm (Plate 4).

Inference: The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for the presence of coumarins.

5. Flavonoids
a) Without chemical treatment
Fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV-254 nm was observed and light blue fluorescence appeared on TLC plates at UV-366 nm. (Plate 5).

b) Natural products – polyethylene glycol
Intense blue fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-366 nm (Plate 3A) and brown bands appeared in visible light (Plate 5).

c) Fast blue salt B
Blue fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plates at UV-366 nm.

Inference: The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract contain flavonoids.

6. Glycosides
a) Without chemical treatment
There was pronounced fluorescence quenching for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-254 nm (Plate 6).

b) Aniline-diphenylamine phosphoric acid
Dark blue bands appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate at UV-366 nm (Plate 6).

Inference: The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for presence of glycosides.

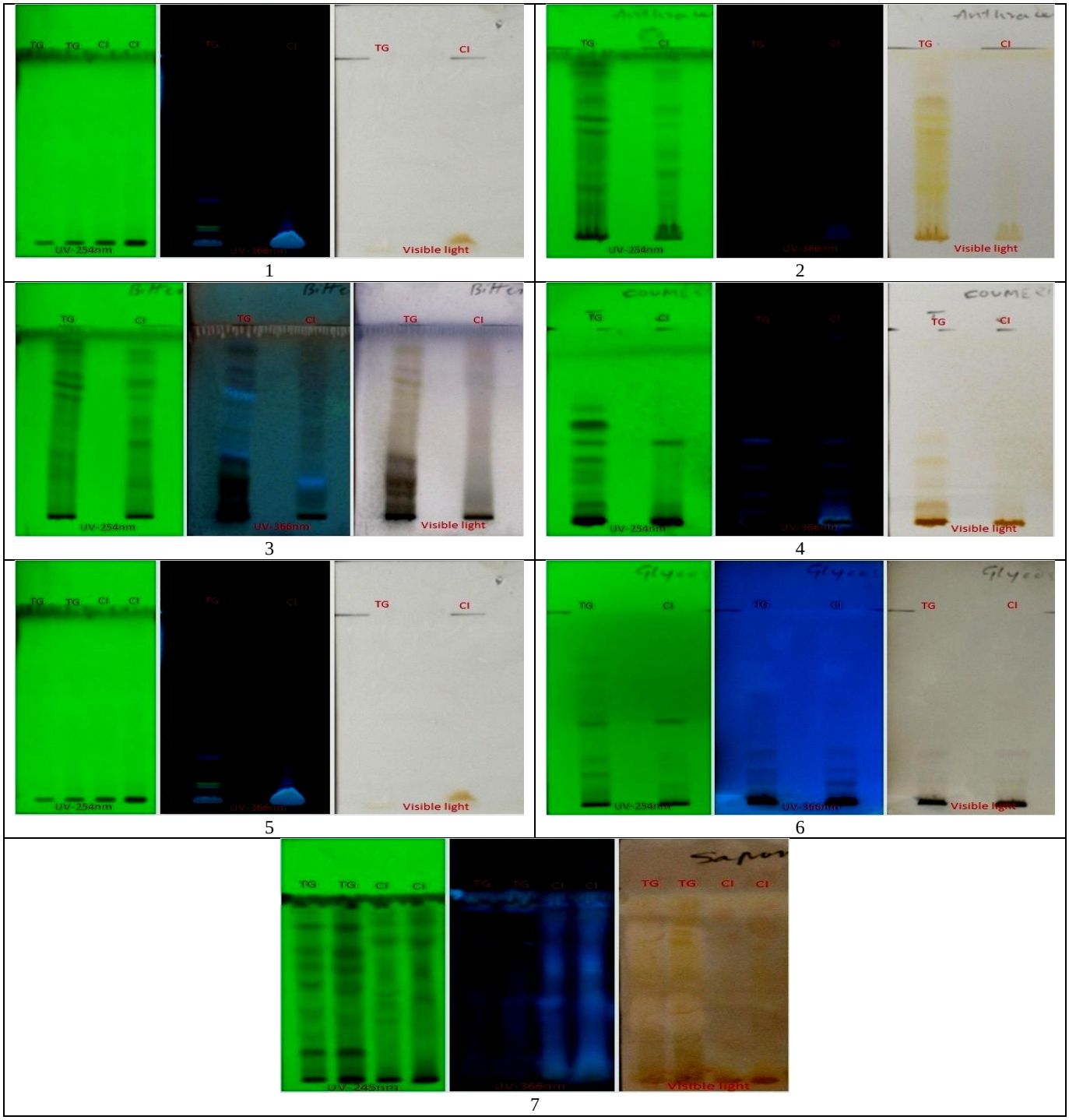
7. Saponins
a) Without chemical treatment
Saponins for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract were detectable by exposure to UV-254 nm or UV-366 nm.

b) Blood reagent
White zones was observed for alcoholic *Trigonella foenum graecum* seed extract whereas, no white zone appeared for alcoholic *Coccinia indica* leaves extract on TLC plate in the visible light (Plate 7).

c) Vanillin-sulphuric acid

Blue zones were observed for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Cocciniai indica* leaves extract on TLC plate at UV-366 nm (Plate 7).

Inference: The alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract are positive for presence of saponins.



Plates 1: Evaluating of fluorescence appeared for alcoholic *Trigonella foenum graecum* seed extract and alcoholic *Coccinia indica* leaves extract on TLC plate in different light pattern

Table 1: Showing analysis of bioactive compounds from *Trigonella foenum graecum* and *Coccinia indica* by HPTLC

Sl No.	Bioactive compounds	*Trigonella foenum graecum*	*Coccinia indica*
1.	alkaloids	Presence	Presence
2.	Antherecene derivatives	Presence	Presence
3.	Bitter principles	Presence	Absence
4.	coumarins	Presence	Presence
5.	flavonoids	Presence	Presence
6.	glycosides	Presence	Presence
7	Saponins	Presence	Presence

Discussion

Phytochemical analysis

Phytochemical analysis of the *Trigonella foenum graecum* and *Coccinia indica* was carried out using High Performance Thin Layer Chromatography (HPTLC) Technique in the present study before conduct of the experiment.

Medicinal plants are extensively used for management of health since a long time and are becoming popular not only in village folk but also in urban people. The medicinal value of plants mainly depends upon their active ingradients or the bioactive substances such as alkaloids, flavanoid, saponins, glycosides and other related active metabolites which are extensively used in the drug and pharmaceutical industry. Thus the preliminary phytochemical tests are helpful in finding chemical constituents of a plant material to which the biological effect obtained could be correlated effectively (Chauhan *et al.*, 2011 and Kalaiselvi *et al*., 2012) [3, 6].

In the present study, the alcoholic extract of both *Trigonella foenum graecum* and *Coccinia indica* were found positive for alkoloid, flavonoid, bitter principle, coumarin, anthracene, saponin and glycoside derivatives.

Several earlier workers have subjected *Trigonella foenum graecum* for their phytochemical analysis (Mowla *et al*., 2009; Ahirwar *et al.*, 2010; Yadav *et al*., 2010; Dande *et al*., 2012; Sumayya *et al.*, 2012; and Sheikh *et al*., 2012) [9, 1, 19, 4, 15, 13] and have observed presence of alkaloid, saponin, flavonoids, bitter principles, coumarins, anthracence derivatives and glycosides similar to the results of the present study. Ahirwar *et al.* (2010) [1] while subjecting petroleum extract, ethalonolic extract and aqueous extract for phytochemical analysis obtained steroids in trigonella seeds only in petroleum extract and absent in aqueous and alcoholic extracts. Dande *et al*. (2012) [4] on phytochemical analysis observed that the steroidal saponins were in large quantity in trigonella seed extract. Mowla *et al*. (2009) [9] subjected crude ethanol extract of *T. foenum graecum* seeds for phytochemical analysis to check the presence of alkaloid, steroid, flavonoid, carbohydrate, glycoside and glucosides in it and observed the presence of alkaloid, steroid and carbohydrate but no flavonoid, glycoside and glucosides in the crude seed extract. Yadav *et al.* (2010) [19] showed the presence of alkaloid, flavonoids, amino acid, tannins, protein, starch, mucilage and saponins in the methonolic and aqueous extracts of Trigonella.

Amino acids like isoleucine, 4-hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, argenine; saponins like graecunins, fenugrin B, fenugreekine, trigofoenosides A-G; Steroidal sapinogens like yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin, gitogenin, neogitogenin, yuccagenin,saponaretin; Fibers like gum, neutral detergent fiber and others like coumarin, lipids, vitamins, minerals, 28 per cent mucilage; 22 per cent proteins; 5 per cent of a stronger-swelling, bitter fixed oil components were reported by (Yadav *et al*., 2010) [19].

Sheikh *et al*. (2013) also observed presence of glucosides, phenol, flavonols, amino acid, alkaloides, steroids, tannin, polysaccharide, pectin and hemicelluloses, fats volatile oil in the ethanolic extract of fenugreek. Sumayya *et al.* (2012) [15] indicated that fenugreek (*Trigonella foenum graecum*) helps in balancing cholesterol, lowering sugar level, curing skin inflammation (wounds, rashes, boils), treating arthritis, asthma, sore throat, due to the phytoconstituents such as flavonoids, alkaloids, terpenoids, steroids, saponins, anthocyanin, tannin etc.

Glucose-lowering and antidiabetic effects of fenugreek have been attributed to the galactomannan rich soluble fiber fraction of fenugreek. Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4-hydroxyisoleucine that occurs in fenugreek at a concentration of about 0.55 per cent. *In vitro* studies have indicated that this amino acid causes direct pancreatic β-cell stimulation. Delayed gastric emptying and inhibition of glucose transport also have been postulated and improve intraperitoneal glucose tolerance in normal mice.

Flavonoids are the natural substances with phenolic structures present in fruits, vegetables, grains, bark, roots, flowers, tea, and wine. Flavonoids have been reported to have antiatheroscelerotic effects, anti-inflammatory effects, anticancerous effects, antithrombogenic effect, antiviral effect and anti osteoporotic effects. The main mechanism by which the flavonoids function is through their antioxidant activity which combines with free radicals and make then unavailable for their action.

The alcoholic leaf extract of *Coccinia indica* was reported (Tamilselvan *et al*., 2011) [16] to possess tannins, saponins, alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides etc on phytochemical analysis. Similar observations have also been reported by Deokate and Khadabadi, 2011; Sivaraj *et al*., 2011 and Kumar *et al*., 2012 [5, 8, 11]. They have reported varying effects of the extract and attributed to antioxidant activity and alkaloids to be responsible for antidiabetic property (Yadav *et al*., 2010 and Deokate and Khadabadi, 2011) [12, 5].

Conclusion

Plants that are rich in secondary metabolites, called medicinal plants are widely used in traditional medicine to combat and cure various ailments. Several plants studied are used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The different extracts of leaves and seeds of medicinal plants contained many bioactive chemical constituents including anthocyanins, steroids, terpenoids, coumarins, fatty acids, tannins, saponins, leucoanthocyanins and emodins. The anti-inflammatory, antispasmodic, antianalgesic and diuretic effects can be attributed to the high steroids, tannins, terpenoids, saponins and glycosides present in medicinal plants. It has been used as an aphrodisiac, neuroprotective, liver tonic, astringent, and to treat bronchitis, asthma, ulcers, emaciation, insomnia, senile dementia and diabetes. While medicinal plants has been used successfully in Ayurvedic medicine for centuries, more clinical trials should be conducted to support its therapeutic use, on the basis of presence of phytochemicals in the leaf extract for secondary metabolites. The alcholic extracts *Trigonella foenum graecum* and *Coccinia indica* were found to be positive for flavonoids, saponins, alkaloid, bitter principles, coumarines, anthracene and glycosides, and *Coccinia indica* was negative for the bitter principle.

Reference

1. Ahirwar D, Ahirwar B. Evaluation of antifertility activity of *Trigonella foenum graecum* seeds. Der. Pharmacia. Sinica., 2010; 1(3):33-39
2. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity - A review of ten yearsof herbal medicine research (1990-2000). Int. J Diabetes. Metab. 2006; 14:1-25.

3. Chauhan, Sharma M, Kharkwal H, Varma A. An international journal of pharmaceutical sciences pharmacognostic, preliminary phytochemical studies and anticancerous potential of *Trigonella foenum-graecum*. J Indian Chem. Soc., 2011; 64(12):776-777
4. Dande, Payal, Suraj Patil. Kvaluation of saponins from *Trigonella foenum graecum* seeds for its antifertility activity. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(3):0974-81
5. Deokate, Khadabadi SS. Pharmacology and phytochemistry of *Coccinia indica.* Journal of Pharmacognosy and Phytotherapy. 2011; 3:155-159
6. Kalaiselvi D, Gomathi, UMA C. Occurrence of Bioactive compounds in *Ananus comosus* (L.): A quality Standardization by HPTLC Mvsc thesis, 2012.
7. Khalki L, M'hamed SB, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from *Trigonella foenum graecum* (L.) in mice. J Ethnopharmacol. 2010; 15:321–325
8. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: its chemistry, traditional and medicinal uses, and pharmacological activities. Pharm. Rev. 2012; 4(7):58-61
9. Mowla A, Alauddin M, Rahman MA, Ahmed K. Antihyperglycemic effect of *Trigonella foenum graecum* (fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: A brief qualitative phytochemical and acute toxicity test on the extract. Afr. J Tradit. Complement. Altern. Med. 2009; 6:255-26
10. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti hyperglycemic potential of *Tridax procumbens* (Linn.). bmc. Complement. Altern. Med. 2009; 9:48.
11. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: An overview on its pharmacological aspects andreported medicinal plants having antidiabetic activity. Asian. Pac. J. 2012; 6(12):607-17
12. Raju J, Gupta D, Rao AR, Yadava PK, Baquer NZ. TSP *Trigonella foenum graecum* (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. Mol. Cell. Biochem., 2001; 22:45-51
13. Sheikh SV, Kolhe DT, Tayade. Pphytochemical analysis of leaves of *Trigonella foenum graecuml* from anjanga on, amravati region of maharashtra state. International Journal of Pharmacy and Pharmaceutical Science. 2012; 3(3):94-96.
14. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): A review of health beneficial physiological effects. Food Rev. Int. 2006; 22:203-224
15. Sumayya AR, Sivagami Srinivasan, Nabeelah Amatullah. Screening and Biochemical Quantification of Phytochemicals in Fenugreek (*Trigonella foenum graecum*). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2012; 3(1):165
16. Tamilselvan N, Thirumalai T, Elumalai EK, Balaji R, David E. Pharmacognosy of Cocciniagrandis: a review. Asian Pacific Journal of Tropical Biomedicine. 2011, S299-S302.
17. Venkatesh S, Reddy BM, Reddy DG, Mullangi R, Lakshman M. Antihyperglycemic and hypolipidemic effects of *Helicteresisora* roots in alloxan-induced diabetic rats: A possible mechanism of action. J Nat. Med. 2010; 64:295-304
18. Wagner H, Bladt S, Zgainski EM. Plant drug analysis; A thin layer chromatography atlas. 2nd Edn., Berlin Heidelberg, New York Tokyo. 1984; 50:244
19. Yadav Rashmi, Richa Tiwari, Partha Chowdhary, Chandan Kumar Pradhan. Pharmacognostical monograph of *Trigonella foenum graecum* seeds. International Journal of Pharmacy and Pharmaceutical Sciences. 2010; 3(5):0975-1491.