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Review Article

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Review on Fenugreek (*Trigonella foenum-graecum* L.) and its Important Secondary Metabolite Diosgenin

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is a medicinal plant used worldwide since ancient times. Its use as smelling agent and spice was documented since 15th century. The genus *Trigonella* includes around 260 species diffused worldwide and belonging to Fabaceae family. In the last decades, a number of studies highlighted the biological activities and therapeutic properties of this species mainly attributed to bioactive secondary metabolites such as alkaloids, flavonoids, steroids and saponins. In particular, diosgenin, a steroidal saponin, has been investigated for its medicinal uses and fenugreek has been reported as source of raw material for the production of steroidal hormones. This review article focuses on the cultivation, genetics, ecophysiology and traditional uses of fenugreek, as well as on its medicinal properties, phytochemical and nutrient contents. Extraction procedures and pharmacological activities of diosgenin are also reviewed, as well as methods for its chemical analyses. This review focuses on the medicinal importance of Fenugreek and its important secondary metabolite diosgenin. The review article complies the results of pre-clinical studies conducted to establish the various medicinal applications of diosgenin. This will help researcher to discover fundamental role of diosgenin as a potential product for drug manufacturers and use of fenugreek as a source of diosgenin.

Keywords: ethnobotany, ethnopharmacology, Fabaceae, saponins, sex hormones, steroids

Introduction

Trigonella foenum-graecum L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. Around 260 species of *Trigonella* are diffused worldwide (Acharya, 2006). The genus name *Trigonella* means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay' (Petropoulos, 2002). It is an annual crop and dicotyledonous plant belonging to the subfamily Papilionaceae, family Fabaceae. The plant is erect or branched and, generally, grows to a height of about 30-60 cm, depending on the variety. It has trifoliate, pinnate leaves; roots bearing nodules; white to yellow flowers, flowering 30-40 days after sowing; 3-15 cm long, thin pointed, hoop-like pods; golden yellow seeds (Petropoulos, 2006; Basch *et al.*, 2003; Acharya, 2008; Moradi and Moradi, 2013).

It is believed that fenugreek was native from Europe, though De Candolle mentioned that it could be a plant from Indian origin. If fenugreek was from European origin, then, it should be common in Europe, but this is not the case. Fazli and Hardman reported that fenugreek was native from Punjab and Kashmir, Mesopotamia desert, Persia and some European countries as Greece, Italy and Spain (Linnaeus, 1753; De Candolle, 1886; Fazli and Hardman, 1968). The fenugreek leaves were used to produce the Egyptian incense *Kuphi*, a holy smoke used in fumigation and embalming rites (Rosengarten, 1969). Well documented fenugreek uses date back to 15th century. Melius compiled the details of this plant species in the well-known Kolozsvar Herbarium, in 1578 (Petropoulos, 2003). Hidvegi mentioned some uses of fenugreek, in 16th century (Hidvegi, 1984). Howard reported that the fenugreek seed powder was used, in 17th century, to expel placenta after child birth (Howard, 1987). Despite its uses since centuries,

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fenugreek was not studied in detail in the past, even if, since the last decades, its biological activities are seeking attention of the scientific community.

This review article focuses on the genetics of fenugreek, as well as on its medicinal properties, phytochemical and nutrient contents. Extraction procedures and pharmacological activities of diosgenin are also reviewed, as well as methods for its chemical analyses.

Fenugreek Plant

Fenugreek cultivation, varieties and uses in the world

Fenugreek has good adaptability to diverse atmospheric conditions, temperatures and soils which makes it cultivable in different habitats and more than 20 countries of Asia, Europe, Africa, America and in some areas of Australia (Fig. 1), where it is used as food ingredient, flavoring agent, herb and spice (Rouk and Mangesha, 1963; Fazli and Hardman, 1968; Rosengarten, 1969). In India, fenugreek is cultivated mainly in Rajasthan (around 80% of the national production), Gujarat, Uttaranchal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab.

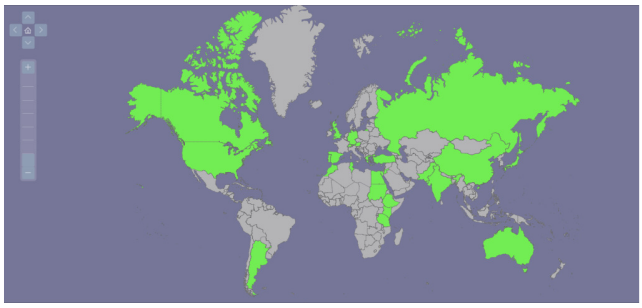

Fig. 1. Cultivation of *Trigonella foenum-graecum* L. in the world; in green, countries were fenugreek is cultivated

Diverse fenugreek genotypes are present in the world, differing in growth habits, morphology, seed quality and crop yield. Since fenugreek is a self-pollinating plant, breeders successfully developed varieties by using breeding techniques. Some of the most important fenugreek varieties diffused throughout the world are summarized in Table 1.

Nowadays, fenugreek is used in various countries: as spice in Iran, for preparing flavor cheese in Switzerland, seed powder along with flour for making flat bread in Egypt, seedlings as vegetable in India and Pakistan (Moradi and Moradi, 2013). In Africa, people use fenugreek seeds as coffee-substitute, to control insect infestations in grain storages, in cosmetic industries and so on.

Fenugreek genetics

The few data available on fenugreek kariotype and genome have identified the somatic chromosome numbers of *Trigonella* taxa as $2n$ = 14, 16, 30 and 46 along with B chromosomes (Martin *et al*., 2011; Vaidya *et al*., 2013). The C-value of fenugreek was found to be 0.7, which is 1.5 fold higher than the values of model legumes *Lotus corniculatus* L. var. *japonicus* Regel [syn. *Lotus japonicus* (Regel) K. Larsen] and barrel (*Medicago truncatula* Gaertn.); genome size of both these species is around 470Mbp, whereas the genome size of fenugreek is around 685Mbp (Young *et al*., 2003; Vaidya *et al*., 2013). Despite fenugreek is an important medicinal plant, its whole genome sequencing has not been carried out yet, and only transcriptome sequencing data were published earlier by our research group. The transcriptome contains 42 million high quality reads, with 18,333 transcripts functionally annoted and 6,775 transcripts related to metabolic pathways including diosgenin biosynthesis (Vaidya *et al*., 2013). In particular, targeting transcriptome sequencing of fenugreek has been

Table 1. List of fenugreek varieties available worldwide

Fenugreek Variety	Country	References
CO-1, Rajendra Kanti, RMt-1, LamSel 1, Pusa Early Bunching	India	Edison (1995)
UM-9, UM-17, UM-18, UM-23, UM-25, UM-26, UM-27, UM-32, UM-33, UM-36, UM-50, UM-52, UM-58, UM-67, UM-70, UM-75, UM-77, UM-79, UM-83, UM-84, M-105, UM-112, UM-113, UM-114, UM-115, CVT UM-5, CVT UM-17, CVT UM-32, CVT UM-34, CVT UM-35, CVT UM TC 2336, CVT TG 1084, CVT GF 1, CVT CC, CVT NLM, NLM, CO 1, Local check, CT Lam Sel 1	India	Kamal *et al.* (1987)
RG-07, TG-3, TG-13, TG-18, TG-24, TG-34, UM-5, UM-6, UM-17, UM-20, UM-34, UM-35, UM-38, NI-01, MP-14, IC-99, LamSel 1, Local Bobes, Pusa Earlier, Bangalore-Local	India	Prasad and Hiremath (1985)
T-8	India	Paroda and Karwasra (1975)
HM-46	India	Singh *et al.* (1994)
IC-74	India	Singh and Singh (1974)
'Gharbin-6'	Egypt	Bunting (1972)
'Ali Lunghe', 'Ali Corte'	Italy	Del' Gaudio (1953)
Ionia	Greece	Vaitsis (1985); Anonymous (1996)
Gouta	France	Haefele *et al.* (1997)
Fluorescent, Ethiopian	England	Petropoulos (1973)
Barbara, Margaret, Paul	England	Hardman (1980); Evans (1991)
Fluorescent, Ethiopian, Kenyan, Moroccan	England	Petropoulos (1973)

studied by Vaidya *et al*. in order to identify the genes of diosgenin pathway (Vaidya *et al*., 2013). Studies on fenugreek genome should be improved, since they could provide information about the bioactive phytochemicals of this important medicinal plant.

Ecophysiology of fenugreek plants

As previously introduced, fenugreek is widely distributed in most parts of the world because of its adaptation to different climatic conditions and environments (Fig. 1). This plant is tolerant to drought and able to grow well in areas with moderate to low rainfall; its temperature requirement is as low as 10-15 °C, though it can neither grow well in extreme high nor in extreme low temperatures (Talelis, 1967; Duke, 1986). For instance, in Greece, fenugreek is cultivated both in summer and winter, because of its adaptive nature (Talelis, 1967). It has been reported that the maximum fenugreek yield occurs in areas where dry season with low temperatures follows rainy season, which are the typical subtropical and Mediterranean conditions. Ethiopian fenugreek is cultivated in areas having climatic conditions similar to the Mediterranean region ones (Rouk and Mangesha, 1963). In India and Egypt, fenugreek is grown in winter (Pareek and Gupta, 1981), though in Germany it is cultivated at the beginning of spring. For Indian weather, early sowing around the middle of October gave higher yields than late sowing around the middle of November (Rathore and Manohar, 1989). Crop improvement can play significant role in increasing yield for any plant. Many studies demonstrated work for fenugreek crop improvement (Petropoulos, 2002; Acharya *et al*., 2008). Different crop improvement techniques are popular from which majorly used methods are: consequent selection from all available accessions, artificial crossing and mutational breeding. In case of fenugreek, artificial crossing is difficult because it is self-pollinated plant. Mutational breeding and consequent selection among world accessions are better ways to improve the crop (Petropoulos, 2002), and much of the breeding with fenugreek has utilized these two approaches.

Fenugreek phytochemicals and nutrients

The three main classes of fenugreek secondary metabolites include saponins, flavonoids and alkaloids. This plant seeds were reported to contain 35% alkaloids, 10% flavonoids (100 mg per g of fenugreek seeds), 4.8% saponins and 0.2-0.9% diosgenin (Jani, 2009; Meghwal, 2012; Vaidya, 2013). Alkaloids, along with some other volatile compounds, are mainly responsible for the bitter taste and typical aroma of fenugreek (Kumar, 2012, Faeste, 2009). Additionally, it is a significant source of vitamin B_1, iron, silicon, sodium, protein, amino acids, fatty acids and dietary fiber (Sharma *et al*., 1990). In particular, the plant is rich in soluble fiber, mucilage and galactomannan which decrease the uptake of bile salts and starch absorption (Madar, 1990). Fenugreek seeds contain the amino acid 4-hydroxyisoleucine which increases insulin secretion (Haefelé *et al*., 1997). The plant also represents a significant source of antioxidants (Naidu, 2011). In obese subjects, short-term beneficial effects of fenugreek fiber were reported on satiety, blood glucose, insulin response and energy intake (Mathern *et al*., 2009). In addition, fenugreek fiber can bind to and remove carcinogens in the intestine (Meghwal, 2012).

Fenugreek traditional uses

As previously introduced, fenugreek use as herbal remedy in traditional healing systems dates back to 15th century. Dried seeds and leaves, in different forms, were used for curing many symptoms and ailments. For instance, a paste prepared with ground fenugreek seeds was used to treat eczema, rash or inflammatory conditions. Ethnoveterinary uses of fenugreek were also documented. Traditional uses of fenugreek are summarized in Table 2.

Therapeutic properties

A number of pharmacological activities have been investigated in order to clarify the therapeutic properties of fenugreek and its main metabolites (Mehrafarin *et al*., 2010). Some studies have been carried out on extracts from different plant parts (seeds, leaves) or pure phytochemicals (saponins, steroids, alkaloids) to validate traditional uses of fenugreek as herbal remedy. These studies, reported in Table 3, investigate, in some cases, the pharmacological mechanisms by different experimental models.

Need of improvement in fenugreek cultivation

In India, fenugreek was grown in 94,760 and 93,605 hectares, in the years 2010-2011 and 2011-2012, respectively, with a production of 12,785 and 11,529 million tons, respectively. The production of fenugreek was 1.34 and 1.23 million tons per hectare, in the years 2010-2011 and 2011-2012, respectively (Vidyashankar, 2014). In India, eight research and development projects were planned for spices including fenugreek along with research institutions and state universities under the All India Coordinated Research Project[13]. However, researchers found difficulties in growing fenugreek due to the lack of information regarding this plant (Petropoulos, 2002). Therefore, varieties and techniques to improve fenugreek quality and yield from poor lands are urgently needed in such scenario.

Diosgenin

As previously reported, fenugreek is a rich source of saponins such as yamogenin, tigogenin and diosgenin. Diosgenin is the most important out of all four mentioned secondary metabolites. It has a molecular weight of 414.621 g mol-1 and is made up of 27 carbon, 42 hydrogen and three oxygen atoms. Its scientific name is (3β,25R)-spirost-5-en-3-ol, $C_{27}H_{42}O_3$ and its chemical structure is illustrated in Fig. 2. It has estrogenic activity and represents the most important bioactive phytochemical in fenugreek, used as raw precursor for the industrial, large scale synthesis of steroidal drugs and hormones such as testosterone, norethisterone, glucocorticoids and progesterone. Diosgenin also exhibited anticancer and antiaging activities, as well as cardioprotective and contraceptive properties (Aradhna *et al*., 1992; Liu *et al*., 1993; Qin *et al*., 1997; Dias *et al*., 2007; Lee *et al*., 2007; Tada *et al*., 2009; Yan *et al*., 2009; Gong *et al*., 2010; Agarwal *et al*., 2015). Russell Marker and colleagues developed the semisynthesis of

Table 2. Traditional uses of fenugreek

Plant parts and preparation	Symptoms or illnesses	References
Seeds	Chapped lips, stomach irritation, mouth ulcers	Duke (1986)
Soaked seeds	Constipation	Fazli and Hardman (1968); Rosengarten (1969)
Decoction (various parts)	Tuberculosis, sore throat, infection of stomach and intestine, remedy for gonorrhea and vermin	Schauenberg and Paris (1990); Fazli and Hardman (1968)
Poultice of seeds	Gouty pains, neuralgia, sciatica, swollen glands, wounds, furuncles, fistulas, tumours, sores, skin irritation, abscesses and carbuncles	Sharma *et al.* (1996); Pandian *et al.* (2002)
Crushed seeds with powdered charcoal	Bruises, swellings, boils, ulcers, swelling of testicles	Pandian *et al.* (2002); Bunney (1984); Reger (1993)
Seeds	Constipation and diarrhea	Evans (1991); Sharma *et al.* (1996)
Poultice of seeds (in Malaya)	Burns, cough, dropsy, hepatomegaly, splenomegaly	Duke (1986); Bhatti (1996); Sharma *et al.* (1996)
Seeds (in China)	Abdominal and hypogastric pain, chilblains, cholecystitis, fever, hernia, impotence, nephrosis and rheumatism	Duke (1986)
Fenugreek tea	Soothing to the gastrointestinal tract	Pandian *et al.* (2002)
Alcoholic fenugreek extracts	To expel poisons and unwanted materials from the human body	Howard (1987)
Seeds	To prepare the base of medicinal preparation called 'Luddoo'	Rouk and Mangesha (1963)
Seed powder	Inflammations and suppurations	Fluck (1988)
Seeds (aqueous extract)	Antibacterial activity	Bhatti *et al.* (1996)
Decoction of the seeds	Remedy for diabetes	Evans (1991); Khosla *et al.* (1995); Sharma *et al.* (1996)
Decoction of the seeds	Substitute of insulin	Oliver-Bever (1986)
Seeds	Anti-diabetic activity	Daoud (1932); Valette *et al.* (1984); Oliver-Bever (1986); Evans (1991);
Seeds	To reduce serum cholesterol in animals	Sharma *et al.* (1991)
Seeds	To increase milk production in animals	Bunney (1984)
Seeds	Anticancer activity, fibromas	Singhal *et al.* (1982); Evans (1991)
Seeds	To induce labour during child birth	Yoshikawa *et al.* (1997)
Various parts	For treating weakness and oedema	Basch *et al.* (2003)

Table 3. Pharmacological activities of fenugreek

Plant parts/ phytochemicals	Pharmacological activities	References
Seed powder	Decrease of glycaemia, improvement of symptoms in type-2 diabetes patients	Mitra and Bhattacharya (2006)
Seeds	Decrease of glycaemia in type 2 diabetes patients	Madar *et al.* (1988); Jain *et al.* (1995); Sharma *et al.* (1996)
Seeds	Diabetes control in type 1 diabetes patients	Sharma *et al.* (1990)
4-Hydroxyisoleucine extracted from seeds	Increase of insulin secretion	Sauvaire *et al.* (1998)
Various parts	Increased number of insulin receptors	Raghuram *et al.* (1994)
Various parts	Increase of insulin secretion in animals	Ethan *et al.* (2003)
Alkaloids, flavonoids, saponins	Decrease of lipaemia, glycaemia and cholagogic for treating diabetes mellitus	Izzo *et al.* (2005)
Extract rich in saponins	Decrease of hypercholesterolemia in rats	Petit *et al.* (1995)
Seeds	Management of long-term diabetes complications	Ribes *et al.* (1986)
Seeds	Decrease of glycemia in diabetic patients	Sowmya and Rajyalakhsmi (1999)
Various parts	Treatment of hyperglycemia in type-1 diabetic rats	Basch *et al.* (2003)
Aqueous extract (various parts)	Ulcer protective	Srinivasan (2006)
Diet with 30% fenugreek seeds	In female rabbits: antifertility and reduction of developing foetuses In male rabbits: decrease of testis weight, plasma concentration of androgens and sperm production	Bin-Hafeez *et al.* (2003)
Leaves and seeds	Reduction of perspiration, fever, allergies, bronchitis and congestion	Faeste *et al.* (2009)
Seeds	Treatment of sinus and lung congestion	Singhal *et al.* (1982)

Leaves and seeds	Antimicrobial	Sowmya and Rajyalakhsmi (1999)
Leaves and seeds	Treatment of hypertension and hypercholesterolemia	Sowmya and Rajyalakhsmi (1999); Roberts (2012)
Leaves and seeds	Prevention of gas formation in digestive tract	Mathern (2009)
Steroids, flavonoids, alkaloids	Raw material for hormones and pharmaceuticals	Blank (1996)
Leaves and seeds	Antibacterial, anticancer, antiulcer, antinociceptive	Sowmya (1999); Mathern (2009)

Table 4. Pharmacological activities of diosgenin

Pharmacological activities	References
Antifungal activity on *Candida albicans, C. glabrata* and *C. tropicalis*	Sautor (2004)
Inhibition of replication of hepatitis C virus (HCV)	Wang (2011)
Decrease of glycaemia and intestinal disaccharidases *in vivo*	McAnuff (2006)
Decrease of the activity of the ATP-citrate lyase, pyruvate kinase and glucose-6-phosphate dehydrogenase in the liver of diabetic rats	McAnuff (2005)
Inhibition of glucose-uptake and glucagon-induced hepatic glycogen phosphorylase A activity *in vitro*	Al-habori (2001)
Anticoagulant activity, inhibition of platelet aggregation and thrombosis *in vivo*	Gong (2011)
Decrease of plasma total cholesterol and LDL-cholesterol, increase of HDL-cholesterol and total cholesterol ratio in rats	Xu *et al.* (2009)
Enhancement of BK (Ca)-channel activity in human cortical neurons	Wu (2006)
Decrease of blood pressure, oxidative stress and cardiac remodeling in the heart of adenine-induced chronic renal failure rats	Manivannan (2015)
Increase of antioxidant level, decrease of ACE activity, lipid peroxidation level and cardiac fibrosis in chronic renal failure rats	Manivannan (2015)
Inhibition of platelet aggregation and acetylcholinesterase activity	Sautour (2004)
Decrease of serum total cholesterol levels in rats	Ghayur (2011)
Inhibition of accumulation of triglycerides and expression of lipogenic genes in mice	Ma HY *et al.* (2002)
In vivo increased levels of superoxide dismutase in the plasma and liver, glutathione peroxidase and catalases in erythrocytes	Uemura (2011)
Upregulation of antioxidant enzymes in rats	Son *et al.* (2007)
Suppression of inflammation within the atherosclerotic lesion *in vivo*	Son *et al.* (2007)
Anti-inflammatory activity in RAW 264 macrophages induced by the conditioned medium derived from 3T3-L1 adipocytes	Choi *et al.* (2010)
Food allergy, suppression of intestinal inflammation	Hirai *et al.* (2010)
G0/G1 arrest and inhibition of proliferation induced by IGF-1 in human thyroid cells	Huang *et al.* (2009)
Inhibition of migration and invasion of human prostate cancer PC-3 cells by reducing matrix metalloproteinase expression	Cai *et al.* (2014)
Regulation of Jun N-terminal kinase, phosphatidylinositide-3 kinase/Akt signaling pathways and NF-κB activity in human prostate cancer PC-3 cells	Chen *et al.* (2011)
Sensitization of HT-29 cells to TRAIL-induced apoptosis	Chen *et al.* (2011)
Inhibition of tumor growth in MCF-7 and MDA-231 xenografts in nude mice	Lepage *et al.* (2011)
Inhibition of melanogenesis by activating the PI3K pathway	Srinivasan *et al.* (2009)
Apoptosis induction and growth inhibition of HCT-116 human colon carcinoma cells	Lee *et al.* (2007)
Apoptosis induction and downregulation of COX-2 expression and activity in human rheumatoid synovial cells	Raju and Bird (2007)
Inhibition of viability and proliferation of breast cancer cells	Liagre *et al.* (2007)
Osteoclastogenesis suppression by inhibiting NF-kappaB-regulated gene expression	Li *et al.* (2005)
Growth inhibition and apoptosis induction in HT-29 human colon cancer cells	Shishodia and Aggarwal (2006)
p53 activation and cell cycle arrest in the different cell lines	Raju *et al.* (2004)
Anticancer activity on S-180, HepA, U14 transplant mice and L929, HeLa, MCF cells	Corbiere *et al.* (2004)
Normalization of lipid levels by excretion through faeces in diabetic rats	Wang *et al.* (2002)

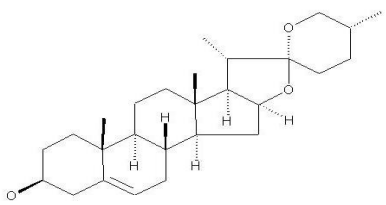

Fig. 2. Chemical structure of Diosgenin (3β,25*R*)-spirost-5-*en*-3-ol) [$C_{27}H_{42}O_3$, 414.621 g mol^{-1}]

progesterone from diosgenin at Parke-Davis Pharmaceutical Company, in 1940. Progesterone is produced in various formulations by many pharmaceutical companies for hormone replacement therapy during menopause. Noteworthy, in US, the overall market for this therapy is above $3.7 billion. Medicinal uses of diosgenin are summarized in Table 4.

Diosgenin extraction methods

Different methods have been reported for the extraction

of crude saponins and pure diosgenin from fenugreek tissues. Separation and extraction of diosgenin can be carried out by thin-layer chromatography (TLC) or high-performance thin-layer chromatography (HPTLC), whereas, in other methods, it can be extracted directly in pure form. In the method described by Li *et al*. (2012), diosgenin was extracted from cultured cells of *Dioscorea zingiberensis*. Cells were ground to a fine powder which was immersed in 95% ethanol, subjected to sonication, for 2 h, followed by hydrolysis at 121 °C, for 2 h, with 1 mol/L sulfuric acid. Then, the hydrolysate was extracted thrice with petroleum ether followed by washing with 1 mol/L NaOH and distilled water until it became colorless. The petroleum ether fraction was concentrated to dryness and, finally, dissolved in acetonitrile followed by filtration using 0.22 μm filter for HPLC analysis (Li *et al*., 2012). In the diosgenin extraction method by Trivedi *et al*. (2007) powder from fenugreek stem, leaf and seed tissues was refluxed with 2.5 M ethanolic sulfuric acid at 80 °C, for 4 h, cooled and filtered through Whatman filter No. 1. The filtrate was diluted with water and extracted with chloroform which was, then, evaporated on water bath and further eluted in chloroform, before separating on TLC plates (Trivedi *et al*., 2007). In this method, *n*-hexane can also be used instead of chloroform to extract diosgenin, which can be dried and dissolved in acetonitrile followed by filtration through 0.22 μm filter. This method yields pure diosgenin without extraction through TLC or other methods.

Diosgenin determination and measurement

Some methods for determination of diosgenin extracted from various plant tissues have been developed, including spectrophotometry, TLC, HPTLC, high-performance liquid chromatography (HPLC) and gas chromatography (GC). In the spectrophotometric method described by Baccou *et al*. (1977) and Uematsu *et al*. (2000) and modified by Chapagain and Wiesman (2005), plant extracts were treated with anisaldehyde, sulfuric acid and ethyl acetate to be measured at 430 nm for diosgenin determination. Trivedi *et al*. (2007) used calibration curve method for quantifying the extracted diosgenin: they found the green color spot on the TLC plate corresponding to diosgenin at Rf = 0.30 ± 0.04 and visualized the same in scanner III at 428nm (Trivedi *et al*., 2007). Li *et al*. (2012) developed a spectrophotometric method to quantify diosgenin. They dissolved the compound in acetonitrile and evaporated it till dryness at room temperature followed by dissolving diosgenin in perchloric acid and measuring at 410 nm. They also developed a HPLC method for diosgenin analysis and reported a retention time (RT) = 18.064 ± 0.096 for this compound.

Conclusions

Fenugreek is a medicinal plant used, since centuries, as herbal remedy in many traditional healing systems. This plant also represents a significant source of diosgenin, a saponin used as precursor for the synthesis of steroid hormones. Preclinical studies investigated the pharmacological activities of fenugreek and its main metabolite diosgenin, focusing on their anti-diabetes, cardioprotective, anticancer and antimicrobial properties. Noteworthy, fenugreek can be a more suitable alternative for diosgenin production than the conventional method using yams, due to ease of production, short life cycle and less cost. However, the main limitation is the natural occurrence of diosgenin present in fenugreek, which is around 0.2-0.9%. Therefore, this plant can be targeted to increase the yield both in terms of biomass and secondary metabolite production by different strategies, including genetic engineering, classic breeding, cultivation techniques and agricultural practices such as the use of fertilizers (Basu *et al*., 2009; Ahmed *et al*., 2010). Intriguingly, the use of elicitors, such as methyl jasmonate, was shown to significantly increase the yield of diosgenin from fenugreek plants, upregulating genes involved in its biosynthetic pathway (Chaudhary *et al*., 2015).

In conclusion, despite the topics focused in this review article, various issues related to fenugreek biology, phytochemistry and pharmacology still remain to be investigated, as well as the therapeutic properties of diosgenin need to be substantiated by randomized clinical trials. In the future, all these aspects could further improve the economic, industrial and medical relevance of these natural resources.

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