DATA REGARDING THE MORPHOLOGIC AND CHEMICAL CHARACTERIZATION OF *TRIGONELLA FOENUM-GRAECUM* SEEDS

George-Ciprian PRIBAC[1], Aurel ARDELEAN[1], Violeta TURCUȘ[1], Claudia-Crina TOMA[1], Coralia COTORACI[1], Liana MOȘ[1], Constantin CRĂCIUN[2]
[1]"Vasile Goldis" West University Arad, Romania
[2]"Babes-Bolyai" University, Cluj-Napoca, Romania

*** Correspondence:** Pribac George-Ciprian, „Vasile Goldis" West University Arad, Faculty of Medicine, Pharmacy and Dental Medicine, Department of Cell and Molecular Biology, no. 1 Feleacului St., Arad, +40-257-212204, e-mail: gpribac@uvvg.ro
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ABSTRACT. *Trigonella foenum-graecum* is an annual, herbal specie, that grows spontaneously on cereals crops and is also known as fenugreek. As powder, the drogue can be identified through chromatography, in the following experiment conditions. Test solution: 1,0g drogue powder mixed with 0.5 ml methanol for 5 minutes in the water bath, heated at 65^{O}C, afterwards chilled and filtrated. Reference solution: 3,0 mg hydrochloric trigonelin dissolved in 1,0 ml methanol. Load: 20 µl test solution and 10 µl reference solution correspond to 2 cm of GF 254 silica gel tape. Solvent system: water – methanol (30 – 70) and migration distance: 10 cm; detection and evaluation: within UV light, with λ=254 nm wavelength. The fenugreek has in its chemical composition 45-60% carbohydrates, especially mucilages located in the call walls; in the endosperm two types of galactomanans are predominant: 1,4-β-glicosil-manose which alternates with α-glicosil-manoze, both connected with a small proportion of xilose. Also, starch and oligo-sacharide fibres are present. As conclusion, even if at least 11 vegetal products were identified, with hypo-cholesterol potential, the proofs remain limited. Along the cholesterol reduction property, some of these plants may have positive aspects for cardio-vascular disorders, by increasing the HDL level and inhibiting the lipid oxidation. The adverse effects of these cures are almost insignificant. Nevertheless, new researches are necessary to establish the therapeutic value of these plants in the hyper-cholesterolemy treatment.

Keywords: *Trigonella* sp., seeds, morphochemical characterization, diosgenin, trigonellin

INTRODUCTION

Trigonella foenum-graecum (fenugreek) is part of the following classification:

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Subfamily: Faboideae
Tribe: Trifolieae
Genus: Trigonella

Fabaceae family includes a variety of trees to annual herbs. Synapomorphies include indeterminate inflorescences (sometimes reduced to one flower), a short hypanthium, a single carpel with a short gynophore, and fruits that are legumes.

The leaves are usually alternate and compound. Most often they are even- or odd-pinnately compound (e.g. *Caragana* and *Robinia* respectively), often trifoliate (e.g. *Trifolium, Medicago*) and rarely palmately compound (e.g. *Lupinus*), in the *Mimosoideae* and the *Caesalpinioideae* commonly bipinnate (e.g. *Acacia, Mimosa*). They always have stipules, which can be leaf-like (e.g. *Pisum*), thorn-like (e.g. *Robinia*) or be rather inconspicuous. The leaflets have sometimes evolved into tendrils (e.g. *Vicia*). Their margin is entire or, occasionally, serrate. Both the leaves and the leaflets often have wrinkled pulvini to permit nastic movements.

In many species the leaves have structures evolved to attract ants, that, being predatory, protect the plant from herbivore insects (a form of coevolution). Extrafloral nectaries are common among the *Mimosoideae* and the *Caesalpinioideae* and are also found in some *Faboideae* (e.g. *Vicia sativa*). In some *Acacia* the modified hollow stipules are inhabited by ants.

The flowers always have five generally fused sepals and five free petals. They are generally hermaphrodite and have a short hypanthium, usually cup shaped. There are normally ten stamens and one elongated superior ovary, with a curved style. They are usually arranged in indeterminate inflorescences. *Fabaceae* are typically entomophilous plants (i.e. they are pollinated by insects) and the flower are usually showy to attract the pollinators.

In the *Caesalpinioideae* the flowers are often zygomorphic, as in *Cercis*, or nearly symmetrical with five equal petals in *Bauhinia*. The upper petal is the innermost one, unlike in the *Faboideae*. Some species,

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like some in the genus *Senna*, have asymmetric flowers, with one of the lower petals larger than the opposing one and the style bent to one side. The calyx, corolla, or stamens can be showy in this group.

Fig. 1 *Trigonella foenum-graecum* L. (fenugreek)

In the *Mimosoideae* the flowers are actinomorphic and arranged in globose inflorescences. The petals are small and the stamens, which can be more than just ten, have long coloured filaments which are the most showy part of the flower. All of the flowers in an inflorescence open at once.

In the *Faboideae* the flowers are always zygomorphic and have a specialized structure. The upper petal, called the banner, is large and envelops the rest of the petals in bud, often reflexing when the flower blooms. The two adjacent petals, the wings, surround the two bottom petals. The two bottom petals are fused together at the apex (remaining free at the base), forming a boat-like structure called the keel. The stamens are always ten in number and their filaments can be fused in various configurations, often in a group of nine stamens plus one separate stamen.

The ovary of these plants most typically develops into a legume. A legume is a simple dry fruit that usually dehisces (opens along a seam) on two sides. A common name for this type of fruit is a "pod", although that can also applied to a few other fruit types. A few species have evolved samarae, loments, follicles, indehiscent legumes, achenes, drupes, and berries from the basic legume fruit.

Many *Fabaceae* host bacteria in their roots within structures called root nodules. These bacteria, known as rhizobia, have the ability to take nitrogen gas (N_2) out of the air and convert it to a form of nitrogen that is usable to the host plant (NO_3^- or NH_3). This process is called nitrogen fixation. The legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship.

Depending on the type of the male organ of the flower, on the leaves' shape, on the number of folioli pairs and the shape of the fruit, in this family there are 3 groups of species:

- *Fabaceae* with dialistemon stamen (free stamens);
- *Fabaceae* with monoadelf stamen (unified stamens);
- *Fabaceae* with diadelf stamen (two sets of stamens).

Trigonella foenum-graecum pertains to the last of this groups (Hodişan V., 1993).

Synonims of the medical plant product: *semen Trigonellae* (latin); Bockshornsamen, Griechische Hensamen (german); Graine de fenugrec (French), fenugreek (English).

Origins: from the mediteranean region, Ukraine, India and China, where is cultivated on large areas.

The drug is extracted exclusively from cultivated plants and, especially, from existent crops in India, Morocco, China and Turkey.

Trigonella foenum-graecum is an annual, herbal specie, that grows spontaneously on cereals crops and is also known as fenugreek. The plant is erect, 30-60-70 cm tall, without or with ramifications only at the higher level. The root is tap-rooted. The stem is cylindrical, fistulous and with no hairs. The leaves are long with petioli (1-2 cm), with spear-shaped stipels, obovate leaflets, slightly elongated and dentated, with no hairs on the superior face and slightly hairy on the inferior face. The terminal foils have a petiole, 2-3 cm in length and two times longer than wider, and the lateral folioles are sessile. The flowers are grouped in 1-2 pieces at the base of higher leaves, 12-18 mm in length, butterfly-like type, with a yellow colour or slightly violet. The sepals are hairy, with the teeth equal to the tube. The corolla has purplish-blue pleats at the base; inferior petals – that build the bottom hull – are very short. The fruit is an elongated pod, straight and slightly curved, long up to 8-10 cm and wide 0,5-1 cm, terminated through a long rostrum of 2-4 cm. It contains 10-20 compressed seeds, prismatic, 4-5 mm in length and 3-4 mm in width, of a yellowish colou, fine tuberculated and with a scent of cumarin. The plant blossoms in june-july (Crăciun et al., 1976).

MATERIALS AND METHODS

Microscopic description of the vegetal product

The transversal section of the seed is characteristic and comprises epidermal cells with radial disposition, in palisades, with thinned external and lateral walls. The lumen is more or less bottle like. The cuticle can be seen as a lighting line along the superior-terminal part of the cell. Under the epidermis, the hypodermis is made of a characteristic layer of basal cells, which get thinner in their apical part and posses superior ridges. Among these cells, large intercellular spaces can be seen. 3-4 cell lines, frequently compressed, with thin walls and smoothly tangent inclined are following. The endosperm comprises large cells, with thinned walls, laminated and mucilaginous. The embryo is formed of thin walled cells, comprising oil globules and some starch (about 5μm) (Weder JK, Haussner K, 1991).

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As powder, the drogue can be identified through chromatography, in the following experiment conditions:

- the test solution: 1,0g drogue powder mixed with 0.5 ml methanol for 5 minutes in the water bath, heated at 65^{O}C, afterwards chilled and filtrated;
- the reference solution: 3,0 mg hydrochloric trigonelin dissolved in 1,0 ml methanol;
- the load: 20 µl test solution and 10 µl reference solution correspond to 2 cm of GF 254 silica gel tape;
- the solvent system: water – methanol (30 – 70);
- the migration distance: 10 cm;
- detection and evaluation: within UV light, with λ=254 nm wavelength.

The reference solution is showing in the inferior half a trigonelin area strongly marked, and the test solution determined the appearance of a trigonelin area in the same Rf and with the same intensity. A few more additional areas would appear, poorly marked.

Following, the chromatographic plate is sprayed with Dragendorff in 0,1 N sulphuric acid powder reagent. For both solutions, an intense red-orange colouration will appear immediately, on a grey-brown background, characteristic for the trigonelin areas.

In the case of the test solution, in the superior part of the chromatogram a large and sparkly area will appear, of yellow-brown colouration, due to the tri-glycerids presence. In the inferior part, 1-2 white-yellowish areas would appear, due to the phospholipids (not always markedly separated from the tri-glycerids). Among the yellow thin band from the beginning of the line and the triglicerids area, usually a red-orange area will appear.

With Iron chlorine (III), the embryos cotyledons get red, and by potassium hydroxid treatments yellow (the trigonelin reaction).

Originating in the Mediterranean region, the plant has special demands for the soil and air temperatures. Germinates at +8^{O}C and tolerates air temperatures comprised between +30^{O}C and +35^{O}C, or even higher. The other demands are relatively low, regarding the humidity and the soil nature and texture. The environmental factors which correspond to it's ecological demands permit a better development in the southern part of Romania, mostly in Braila and Constanta counties. Despite all of these, it is more rare among crops, as spontaneous form, situation in which it has no economical importance.

After harvesting, the seeds are kept in thin layer, in dry an well aerated chambers, rowing it from time to time, till the humidity level will be at the most 12%.

The technical reception conditions allow maximum 2% impurities (remains from the fruits walls, immature seeds, broken seeds); also, maximum 0.5% external corps are accepted, along the seeds from the quarantine plants, and 0.5% mineral substances.

Raw materials: Semen Foenum - graeci – formed of romb shaped seeds, more or less compressed, 3-5cm long and 4mm wide. The seeds are yellowish or brownish coloured, oblique parallellipipedic and fine tuberculated (Figure 2). The smell is cumarine like, aromatic taste, sour. In contact with the water, they gonflate and become mucilaginous. The colour could vary between breeds, with colours varying from red to dark-brown. In fresh status they are odourless, but the characteristic smell becomes strong after drying. The test is oil like and unpleasant, but also fade and aromatic.

Fig. 2 Seeds of *Trigonella foenum-graecum* L.

RESULTS AND DISCUSSION

The fenugreek has in its chemical composition 45-60% carbohydrates, especially mucilages located in the call walls; in the endosperm two types of galactomanans are predominant: 1,4-β-glicosil-manose which alternates with α-glicosil-manoze, both connected with a small proportion of xilose. Also, starch and oligo-sacharide fibres are present.

Regarding the galactomanans, researches regarding to establish the perspectives of using the plants to obtain a galactomanan full rubber were made. The raw material work is based on modern technologies, appeared in the 70's in China. Along with *Trigonella foenum-graecum* other species as *Cyanopsis tetragonoloba* (L.) Taubert or *Sesbania cannabina* are used. The idea that *Trigonella* has the advantage of a better adaptability, a better productivity, an easier cultivation and a rotation on larger areas was accredited. This is the reason why to obtain galactomanan resources by large *Trigonella* cultures represent a successful method.

Studies referring to galactomanans existence were made also in comparison with other species: *Sesbania marginata* and *Ceratonia siliqua* (Buckeridge MS, Dietrich SMC, 1996). The seeds of the tropical species: *Sesbania marginata* present a live endosperm, of which cells have thick walls, with a high content in galactomanans. The oligo-sacharids and the protein corpuscles of the species in the *Raffinose* family are stocked in cells. Among the cell peel and the endosperm an aleuron layer is located. The *Sesbania* seed anatomy is intermediary between the one determined before the radicule appearance to other seeds which contain galactomanans, as the ones of *Trigonella foenum-graecum* and *Ceratonia siliqua*. These oligo-sacharids are decomposed simultaneously in endosperm and in embryo, mean while the galactomanan (apparently limited at endosperm level) is decomposed after germination. Three hydrolazes of galactomanan-α-galactosidaze, endo-β-manaze şi β-manosidaze were detected in isolated endosperms, establishing that their action reaches a maximum in the exponential faze of the galactomanan decomposition. This observation suggests a strong correlation between the enzymatic activity and the cell wall polysacharids decomposition, due to the germination. The results indicate that, unlike of *Trigonella foenum-graecum* enzymes, the enzyme hydrolases of *Sesbania marginata* seem to have a concrete action because the manose/galactose proportion of the polysacharids has grown significantly before the degradation process has ended. It can be than concluded that the α-galactosidaze attacks the polymers before other hydrolases. The galactomanans degradation products (galactose and manose) do not accumulate nor in the endosperm or in the embryo, they being probably metabolised and used as energy source for the initial development of the plantlet.

Trigonella foenum-graecum still has as chemical component approximately 20-30% proteins, rich in triptofan but poor in sulphur amino-acids and 6-10% fat oil, rich in unsaturated fat acids (in the embryo). The species contains protein inhibitors, which act on the human trypsin and chemotrypsin (Weder JK, Haussner K, 1991). The reaction between the three Bowman-Birk proteinase inhibitors was investigated, isolated from the fenugreek seeds (TFI-B2, TFI-N2 şi TFI-A8), on one part, and the human and cow proteinase, on the other part, by studying the complexes formed and their properties. TFI-B2, the trypsin and chemotrypsin Lys-Leu inhibitor, can link 1,9 mol of human trypsin (HT), 1,3 mol of cow trypsin (BT) and/or 0,4 mol of human (HCT) or cow (BCT) chemotrypsin for 1 mol of inhibitor. HT was tied at the two reaction places, and BT mostly at the place which contain lysine which reacted whit the trypsine, mean while HCT and BCT were tied mostly at the places were leucin existed, which reacted with chemotrypsin. TFI-N2, the Arg-Leu trypsin inhibitor, could tie 1 mol of BT and BCT, but only 1 mol of HT and 1,2 mol of HCT for 1 mol of inhibitor. In addition to the ordinary connection, the human enzymes could be connected to a “wrong” locus. TFI-A8, the trypsin Arg-Arg inhibitor, ties 2 mol of HT or BT for 1 mol of inhibitor to the two trypsin rection locus, mean while HCT and BCT are tied of one of the “wrong” reaction locus. The reaction locus (Weder JK, Haussner K, 1991) of the three trypsin and chemotrypsin inhibitors, isolated from the fenugreek seeds (TFI-B2, TFI-N2, TFI-A8) were investigated through chemical modification and degradation of the native carboxi-pepsidase inhibitors and of the ones enzymatic modified. TFI-B2 contains lysine and leucin in the trypsine and chemotrypsine reaction locus, the C-terminal sequence being (Lys)-Phe-Leu-Ile. TFI-N2 contains arginine and leucin in the trypsine and chemotrypsine reaction loecus, respectively the (Tyr)-Lys-Ile-Leu sequence at the C-terminal end. TFI-A8 contains two arginine, one for each reaction locus. At least one of these locus could tie partly chemotrypsine too. The (Leu)-Phe-Ile-Arg sequence was identified as the C-terminal end of TFI-A8. These results confirmed that all the three inhibitors from the fenugreek seeds belong to the Bowman-Birk proteinase inhibitor family.

The inhibitors were isolated from preparates anionic exchange chromatography, followed by iso-electric focalisation, by using the pH gradients and the channel technique (Weder JK, Haussner K, 1991). After purification, the inhibitors action over different enzymes was tested. TFI-A8 presented a high degree of trypsin inhibition (8,2mg of human trypsin/mg and 8,1mg of cow trypsin/mg) and a very low level of chemotrypsin inhibition (0,8mg of human chemotrpsin/mg and 1,0mg of cow chemotrypsin/mg). TFI-N2 has inhibited similarly the four enzymes (5,0mg/mg HT and 4,1mg/mg BT; 4,9mg/mg HCT and 3,7 BCT). TFI-B2 presented a high degree of trypsin inhibition (7,5mg/mg HT and 5,1mg/mg BT) and a very low level of chemotrypsin inhibition (1,8mg/mg HCT and 1,9mg/mg BCT). As a medium situation, the human enzymes were inhibited in a larger amount than the cow enzymes by the purified inhibitors. They contained important quantities of cystein (5-6 disulphidic bridges/molecule), aspartic acid, treonine, serine and proline. They did not contained valine and metionine, and two of them did not contained tryptofan.

To eliminate the galactomanans which impede the purification, the roasted seedswere separated, on density basis, in two fractions: the seeds peel + endosperm and the cotyledons + embryos (C + E). The inhibitors isolation from fenugreek through extractions and C + E fractioning, followed by nitrogen sulphate fractioning and isolated anhydrous trypsin chromatography, had as result a 700 times enrichment (Weder JK, Haussner K, 1991).

In the fenugreek chemical composition appear several steroidic saponines, under a Δ3-furostene and 5-α-furostani-3,26-bis-glicoside shape. After removal at C26, they transform in spirostanol-glicoside, and after hydrolysis, they set free diosgenine and yamogenine (0,1-2,2%). The furanic glycosides are probably the most sour substances of the drogue. There is also diosgenine ester 3-peptidic. The sterols include cholesterol and sitosterol.

The saponines are, generally, of heterosidic nature, where the glucidic part is fixed of an aglicon through an oxidril. Through hydrolysis, they form a glucidic part (glucose, fructose, ramnose and arabiose), glucuronic acid, galacturonic acid and an aglicon called sapogenin or sapogenol. The hydrolytic separation of the saponines is realized easy in some cases, even in the vegetal product, mostly when this has a high degree of humidity. Other times, they are much more resistant, and the separation is made successively. Through an easier hydrolysis, at the beginning is separated only a part of the glucide and remains a simpler heteroside, called prosapogenine or sapogenine I. Boiling it with a concentrated acid, the rest of the glucides are separating, the aglicon or sapogenine remaining in free status. In the hydrolysis process the hexozes are separating at the beginning, afterwards the peptoses and at the end the uronic acids.

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By their chemical structure, the saponines are divided into two groups: steroidic saponines and tryterpenic saponines.

The spirostanic saponines are steroidic derivates with 27 carbon atoms in their molecule, which have linked on the 17^{th} carbon atom a spirocetalic rest. In plants, they can be found in glicosilat form and they are part of the steroidic saponine group.

The spirostanic saponines can be found only in the vegetal phylum. In Dicotyledonous they are less frequently spread, being found only in few genera, as Digitalis and Trigonella. They are more frequent in Monocotyledonous, Liliaceae, Dioscoreaceae and Amarylidaceae families.

The sterol and steroidic saponines accumulation was investigated in the fenugreek seed and fruit pulp, in the pod development and maturation period. The flowers and young pods contain high levels of these substances, which decreased in the seeds development and maturation period. The steroidic saponines (especially diosgenine, typogenine and their C-25 epimers) were accumulated massively in the seeds, time in which the growth of the sterol quantity was limited. The cholesterol level presented significant changes, less dramatic. Different other sterols, including polynastanol (14-α-metil-9-β, 19-ciclo-5-α-colestan-3-β-ol), appeared in the seeds development and maturation period.

In experiments, (2 – 14C) acetat titrations were performed on extirpated pods, in development status. The radioactivity on four saponine formes (mono- and dihidroxilate) was emphasized. The titrated diosgenine was isolated and purified till the specific, constant radioactivity. These results indicate the fact that the extirpated pods, in development status, may biosynthesize sterols and steroidic saponines (Brenac C, Sauvaire Y, 1996).

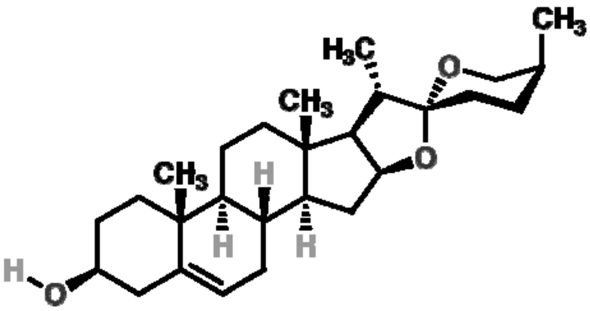

Fig. 3 Chemical structure of diosgenin created using Cambridgesoft ChemDraw Trial

The sterol and steroidic saponine content was studied on 7 species of the Trigonella genus. The fenugreek *(T. foenum graecum)*, considered as a typical representative of the genus, was used as reference species. It's sterolic composition is characterised by the almost complete lack of stigmasterol and by the presence of the Δ-sterols and the polinastanol, a less ordinary sterol. The steroidic saponines composition (11 different compounds, which present 1.5% dry mass) was also taken into consideration. The analyses results for these substances and their specificity for the studied forms are to be discussed. First, the obtained results support the idea of an unchanged allocation at *T. corniculata* (L.), *T. caerulea* (L.) Ser. And *T. melilotus caeruleus* (L.). the *T. monospeliaca* L. species transfer to the Medigago genus is also confirmed, these species presenting a different sterol composition in respect with the one of the Trigonella genus, but similar M. sativa L. species. However, the result don`t support completely the inclusion of the T. calliceras Fisch. taxon in the Medigago Bieb. genus, nor the *T. cretica* (L.) Boiss. species redistribution in the *Melilotus cretica* (L.) species. But, the composition of this species is similar of the other Trigonella species, from the point of view of the studied components.

As conclusion, the usage of the polynastanol and of the steroidic saponines was proposed as chemotaxonomic elements for the investigation of the *Trigonella, Medigago* and *Melilotus* genera separation (Brenac P, Sauvaire Y, 1996).

At the Montpellier II University in France, the effects of tride-morphine and pheno-propio-morphine on the fenugreek sterol composition was studied. It was observed that the tride-morphine addition in the plant development period or the pheno-propio-morphine addition in the same quantities and the same culture media had as result an important accumulation of 9-β, 19-ciclopropil sterol (about 50% of the total sterols), in the same time with a decrease of the Δ-5-sterol content (40-50% from the total sterol quantity, in comparison with more than 90% of the tested cultures). However, the 9-β, 19-ciclopropil sterols accumulated were different in function of the studied biologic material (radicular tissue or cells). The biggest quantity of ciclopropil sterols was obtained by treating the fenugreek roots with tride-morphine, polynastanol and 4-α, 14-α-dimetil-9-β, 19-ciclo-5-α-colestan-3-β-ol, mean while the cell suspensions treated with phenol-propio-morphine and 24-metilen-polynastanol the biggest sterol quantity was obtained. The results indicate the fact that these components inhibit the ciclo-eucalenol-obtusi-foliol-isomerases (COI). More, both in the roots and the cell suspensions these inhibitors have a lower accumulation of Δ-8-sterols, fact which shows that the isomerase was influenced too. Finally, it was observed that the phenol-propio-morphine has induced an accumulation of Δ-8, 14-sterols in the cell suspensions, demonstrating that the Δ-14-reductase may be inhibited too by this fungicide (Cerdon C and colab., 1996).

At Montpellier II University were also studied the effects of the tetcyclacis on the development and the sterol and steroids content of the fenugreek. The tetcyclacis is a sunstance with retarding effect on the plant development, which, in 10 mg doses induces a significant inhibition of the twigs development (60%) in comparison with the roots development (30%) and in the presence of a witness. This important retardation became reversible by treating the plants with a supplementary quantity of giberelin (200 mg). the total sterol composition of the witness lot and of the treated plantlets was analysed and quantified. Mostly in the roots, the plantlets treatment with tetcyclacis has as result the sterol profile modification, leading to an accumulation of 14-α-metil-sterols, possibly as consequence of inhibiting the 14-α-dimetilase,

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dependent of the P-450 cytocrom. In addition, tetcyclacis caused a major decrease of the cholesterol content in roots. However, it was proven that tetcyclacis is ineffective for S-adenosil-L-metionin inhibition from the fenugreek, fact which means that the cholesterol accumulation is not the result of enzymatic inhibition. More, it was demonstrated that the accumulation was simultaneous with a significant decrease of the saponine content present in the treated roots. The last result is to be discussed, if we get in consideration the idea that the cholesterol metabolism in saponine would take place (Cerdon C, 1996).

Another chemical compound of the fenugreek is the diosgenin, a spirostanic saponine from which steroids, cortico-steroids, estrogens, contraceptives and spirono-lactone may derive.

Nevertheless, some components may substitute to some of these steroids. Like this, alternative sources for steroids – cortico-steroids are stigmasterol and hecogenine, for contraceptives and estrogens, cholesterol, and for spirono-lactones, sitosterol. The natural source of these steroids is represented by the plant tubercule of the *Dioscorea genus* (about 1% diosgenin in the *D. deltoidea callus*).

Generally, more intense studies were performed with cell suspensions and less with callus cultures. Like this, cell suspensions were realised from other genera, among which was *Trigonella* also, obtaining 2.5% diosgenin.

Regarding the main steroids biosynthesis place, on the basis of biochemical and electron microscopy studies, is presumed that the synthesis is produced in the endo-plasmatic reticule. In the whole plant, it was shown that the highest biosynthetic activity takes place in the aerial caulinar system and is qualitatively different of that in the tubercules or rhizomes. In the case of steroids synthesis, the ways are less known, and their enzymology is at an incipient phase. The researches were performed using marked isotopes of some precursors, as the: acetate, cholesterol, sitosterol, squalen and cycloartenol. Some of the further precursors, as the mevalonic acid (AMV), geranolpirofosfate and farnesil-pirofosfate are common with the mono-terpenoids, sescvi-terpenoids and carotenoids biosynthesis ways. It looks that the squalen and the cyclo-artenol interfere in the steroids biosynthesis.

Indian researchers have analyzed the diosgenine synthesis in Trigonella corniculata artificial mutants. The diosgenine concentration was evaluated in the normal plants and in the mutant plants seeds, obtained by treatments with alchilant agents and exposure to γ radiations. It was registered a significant increase of the diosgenine level, mean while, at the mutant Virina xanthescens it was observed a significant decrease. At the dwarf mutants, excessively ramified, uni-foliated, with many nods or with less nods, it was observed an unsignificant variation of the diosgenine quantity. It was concluded that there is a possibility to increase the diosgenine quantities by inducing mutation (Mahna SK and colab., 1994).

It was also observed the effects of fertilisers and of leaf sprinkling solutions on the plant production and the diosgenine content of the fenugreek. At the Durgapura Experimental Centre it was performed a field experiment with the NLM variety (Prabha). The Trigonella seeds were harvested from plants treated with 48 phosphorus combinations (30, 60, 90 kg/ha), nitrogen (0, 20, 40 kg/ha) and leaf sprinkling solutions (water, NAA, phosphoric acid and sulphuric acid). Each treatment was repeated three times. The seeds were analysed from the productivity and diosgenine content point of view, the results being statistic analysed. The data showed that the productivity increased by treatments with increased nitrogen, phosphorus and leaf sprinkling solutions concentrations, in comparison with the cultures treated only with water. The diosgenine content increased proportionally with the increase of nitrogen and phosphorus doses. In the case of the leaf sprinkling solutions the biggest quantity was obtained at the plants treated with NAA, and the smallest quantity at the plants treated with sulphuric acid. The mix treatment, with phosphorus, nitrogen and leaf sprinkling solutions, as the one with nitrogen and solutions, have given the best results (Mehra P, Kamal R, 1995).

The Egyptian researchers from The Cairo University have experimented the interactive effects of the excess water treatment and giberelic acid over the nitrogen content at Trigonella foenum-graecum. The influence of the iso-osmotic solutions from sodium chlorine and polyetilen glycol, with osmotic potentials comprised between 0 and 8 bars, was tested regarding the 12 days fenugreek plant nitrogen content. These stress levels were applied from the beginning of germination (within the I treatment) and in the transplantation moment (II treatment). Also, the GA-3 effect was tested, as pre-watering media for the nitrogen content at plants with osmotic stress. The nitrogen content had increase in the whole plant system, as the external pressure increased, the increase being more obvious in the treatment with polyetilen-glycol than in the treatment with sodium chlorine. This accumulation was connected with the total nitrogen quantity increase, especially due to an important increase of free amino-acids (proline).

GA-3 has induced a high total nitrogen content, as the osmotic potential was reduced to 8 bars, especially in supra-solicitation with polyetilen-glycol conditions, due to an important and sudden increase of the soluble nitrogen and especially proline. The water supra-solicitation induced a marked decrease of protein and nucleic acids (DNA and RNA) content. This decrease was more pronounced in the case of II treatment, accompanied by a small accumulation of soluble nitrogen, especially due to the decrease of amino-acids and proline quantity, both to the water pre-moistened plants and to the plants pre-watered with GA-3, in comparison with treatment I (Hamed AA et al, 1994).

The same method was tested in India upon the fenugreek plants mineral composition. At inferior osmotic potential the studied plantlets for both treatments presented a high level of sodium and chlorine, but low levels of potassium, calcium, magnesium and phosphorus. The same chemical

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composition was affected at the plants treated with polyetilen-glycol, where the sodium, magnesium and chlorine levels were reduced and equilibrated by an increase of the potassium, calcium and total phosphorus levels, in comparison with the levels obtained in increased salinity conditions. The same ionic modifications were emphasized at the plants pre-moistened with GA-3, when the external osmotic potential decreased and by sodium chlorine and polyetilen-glycol treatments. However, the GA-3 induces an increase of the sodium, potassium, calcium, chlorine ions levels and of the insoluble phosphorus fractions, inducing in the same time a decrease of the magnesium ion content, both at the treated plants and at the witness. In the conditions of the II treatment, it was observed the accumulation of the sodium and clorine ions, and also soluble phosphorus, in addition to a growth of the potassium, calcium, magnesium and insoluble phosphorus ion levels in the supra-solicited plants, no matter if they were moistened with water or GA-3 (Al Wakeel SAM and colab., 1994).

Among the fenugreek chemical constitutes a series of oligo-elements were identified, which have a very important functional importance. The presence of some of them is mandatory for the good functioning of the biological processes, reason why they were called essential (iron, zinc, selenium, manganese, copper, sodium, molibden, cobalt, chrome and fluor), and other can be found in the organism by it's simple contamination (aluminium, bismuth, led etc).

CONCLUSIONS

The phyto-chemical and pharmaco-dynamic research allowed the discovery of an increased number of active principles isolated from plants and led to the understanding of their therapeutic usage. Along chemo-therapy, physio-therapy or alimentation hygiene, the phyto-therapy constitutes an alternative of high perspective for the modern therapeutics.

Presently, in Romania there are used over 200 medicinal plants. The use in phyto-therapy of the Trigonella foenum-graecum species consists in using it as integral seeds or humid macerates.

The use of this species in physio-therapy is based on its favourable effects on sacharate diabetes, exophtalmic goitre, digestive disorders, neural disorders, sub-nutrition and children anorexia; the pharmaceutic product has also an aphrodisiac action.

Trigonella foenum-graecum is a Fabaceae family species, originating in the Mediterranean region, Ukraine, India and China where is cultivated on large areas. In Romania, the species is found only in cultures, its seeds being used in phyto-therapy with the name of *Trigonallae* semen.

In the chemical composition of the fenugreek seeds the following compounds were studied: fat oil, lecytine, mucillages, steroidic saponines, diosgenine, proteins, free amino-acids and oligo-elements.

The fat oil was identified by extraction from vegetal powder, after removing the seeds peel, in the Soxhlet device, with petrol ether. The oil content was determined by gravimetry and reported to the no peel integral seed powder, obtaining valued of 7.75%, respectively 7.88%. Lecytine was identified from the purified vegetal extract, by thin layer chromatography. The mucillages of the seed tegument were determined by gravimetry, after their precipitation with 1% acetic acid acidulated alcohol, establishing a content of 8.10%.

Following the performed clinical studies, a world increase of the type 2 diabetes incidence may be observed, resulting a high increase of the obesity occurrence (Yale JF, 2000). In addition, the research discovers the importance of the "pre-diabetes" status or of the metabolic syndrome, when the insulin resistance leads to the increase of glucose metabolism destruction (Yale JF, 2000). The patience with metabolic syndrome or diabetes have an increase cardio-vascular morbidity or death risk (Ford ES and all, 2002). Like this, it is desired to use dietetic supplements which can modulate the glucose homeostasy and can improve the potential lipidic parameters. It is recommended especially to prevent the diabetes at patients with metabolic syndrome. These patients already manifest abnormalities of the glucose metabolism and may beneficiate of a low risk intervention, not expensive, based on alimentation for normalizing the metabolic status.

Trigonella is a dietetic supplement promising in the mentioned fields. The currently generated data are poor but they would lead to developing well arranged clinical trials, adequately conducted, randomised, evaluating the *Trigonella* extract effect regarding the insulin resistance, the insulin secretion and the cholesterol metabolism.

Some studies suggest the fact that the doctors do not present to their patients the options of the complementary and alternative therapy, and also the fact that the patients are reserved to discuss these treatment with the doctors. Other studies indicate the use of the complementary and alternative therapies among the patients which suffered a hearth surgical intervention. There are no comparative studies for the patients with hyper-cholesterolemie, despite the existence of a large category of medicinal products with lipid decreasing properties.

As conclusion, even if at least 11 vegetal products were identified, with hypo-cholesterol potential, the proofs remain limited. Along the cholesterol reduction property, some of these plants may have positive aspects for cardio-vascular disorders, by increasing the HDL level and inhibiting the lipid oxidation. The adverse effects of these cures are almost insignificant. Nevertheless, new researches are necessary to establish the therapeutic value of these plants in the hyper-cholesterolemy treatment.

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