Variability in the Content of 25 α - and 25 β -Steroidal Sapogenins among Thirty Morphologically Different Sudanese Seed Accessions of Fenugreek

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Abstract: Seeds of Fenugreek (*Trigonella foenum-graecum* L.), an international spice commodity, are much consumed in Sudan as a food, for flavoring and as a folk-remedy for several ailments, together with local beliefs in nutraceutical benefits. The authors have noted an immense variability in colour and other morphological characters of Fenugreek seeds offered in local Sudanese markets and wondered whether this variability is extended to their chemical composition. Steroidal sapogenins are important chemical constituents of Fenugreek seeds in view of their potential as precursors for the commercial synthesis of steroid drugs and their continually revealed beneficial biological activities. In this study, thirty Sudanese Fenugreek seed accessions collected from different geographical regions in Sudan were analyzed for their 25 α -, 25 β - and total sapogenin content using a simple and specific infra-red spectroscopic method. The seed accessions exhibited much morphological variability particularly in outer seed coat color and size. Preparative TLC followed by gravimetric analysis showed that steroidal sapogenins, mostly diosgenin and yamogenin, represented more than 70% of the steroids of Fenugreek seeds. Infra-red spectroscopic analysis showed that total 25 α -sapogenins (calculated as diosgenin) varied from 0.65% to 1.68%. Total 25 β -sapogenin (calculated as yamogenin) varied from 0.38% to 2.03%. The content of total sapogenins ($\alpha + \beta$ epimeric forms) varied between 1.24% and 3.0% of the oven-dry weight of seeds. β -sapogenins (mostly yamogenin) were dominant over α -sapogenins (diosgenin) in most Sudanese Fenugreek seed accessions.

Key words: Fenugreek seeds, *Trigonella foenum-graecum*, steroidal sapogenins, seed morphology, variability.

1. Introduction

Fenugreek (*Trigonella foenum-graecum*) is an annual crop belonging to the family *Fabaceae*. Seeds and leaves of the plant are characteristically aromatic, the seeds constituting as an item of the international spice trade. Fenugreek is now emerging as a multipurpose legume crop.

Numerous reports mention its uses in the traditional medicine of several nations of the world, such as for weight gain, treatment of arthritis, for fever lowering, for menstrual and labour pain, among others [1]. In Sudan, seeds, available in every home, are used as a

food in porridge or tea forms and according to local folk medicinal and nutraceutical beliefs the seeds are used to improve general well-being, to lighten skin colour, as a lactation stimulant, for stomach troubles, as antimalarial, for kidney stones, for giardiasis, as anti-acid (whole seeds swallowed) and to treat diabetes (seeds soaked in lime juice overnight, dried and powdered before use).

Fenugreek, as a legume crop, is gaining more importance as human needs for more proteins increase [2]. The plant was also reported to be suitable for cultivation as a forage crop [3].

Fenugreek seeds contain several important constituents including essential oils, steroids, proteins, mucilages and phenolic compounds. Fenugreek saponins,

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the aglycones of which are of steroidal nature (called sapogenins), are medicinally important (reviews on saponin classification [4, 5]). The extensive work of Hardman and his group at Bath University, UK [6], heralded the plant as a potential source for steroidal sapogenins, commercially needed precursors for the partial synthesis of steroid drugs, such as oral contraceptives. The plant, however, never reached commercial status in view of the lower sapogenin content compared with the existing commercial source, *Dioscorea* species. Nevertheless, interest in this potential aspect of the plant continues [7-10].

Fenugreek seed currently enjoys much research interest and studies have revealed that the seeds possess several health beneficial biological activities that can make the plant a potential source of drugs or at least a good nutraceutical. Reported biological activities include anti-cancer [11-16], anti-diabetic [17], anti-obesity [18], hypocholesterolaemic [19], antiplasmodial [20], antioxidant [21], anti-inflammatory, antipyretic and antinociceptive activities [22, 23] as well as inhibition of hyperpigmentation [14] and inhibition of the enzyme acetyl cholinesterase [24], a biological activity of potential significance in the treatment of memory disorders associated with Alzheimer's disease. Also of interest are biological activities bestowed by germination of the seeds, such as increased antioxidant potential [25]. Furthermore, even a plant-plant related allelopathic biological activity that of phytotoxicity to lettuce seed germination and growth was reported for Fenugreek seeds [26].

In most of the above studies, the active chemical ingredient responsible for the particular biological activity was not identified beyond doubt. However, some reports single out the steroidal sapogenins, which are major constituents of fenugreek seeds [6], as responsible for at least some of these biological activities. Some of these studies went even further to describe the mechanism of action. Isolated diosgenin, a major saponin aglycone present in Fenugreek seeds, was shown to inhibit melanogenesis (hyperpigmentation)

by acting through the phosphatidyl inositol kinase signalling pathway [14]. Diosgenin was also reported [12] to preferentially inhibit proliferation and induce apoptosis in HER 2-overexpressing cancer cells by suppressing fatty acid synthase expression through modulating identified specific signalling pathways. In yet another study [15], diosgenin treatment of human colon carcinoma cells resulted in a dose dependent decrease in the viability and growth of the cells with concomitant suppression of the expression of 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis. In view of the reported chemopreventive and chemotherapeutic effects of diosgenin against several cancers, it was anticipated [16] that diosgenin-rich foods would become useful nutraceuticals. Also, a purified fraction prepared from Fenugreek seeds which contained more than 90% steroid saponins enhanced food consumption and induced hypercholesterolaemia in experimental rats [19]. Recently [27], spirostanol saponin aglycones, obtained from Fenugreek seed by enzymatic hydrolysis, were shown to inhibit platelet aggregation in rats. The potential of plant (and animal) steroids as antiviral agents was reviewed elsewhere [28].

The authors had observed that Fenugreek seeds commercially offered in local markets in Sudan displayed marked differences in colour and size and wondered if these differences extend to chemical content, particularly the content of steroidal sapogenins. It is this chemical aspect we report here.

Infra-red spectroscopy was one of the earliest methods used to analyse sapogenins [29] by measuring the characteristic steroidal sapogenin infra-red absorption at 900, 915 and 980 cm^{-1} , mainly the latter frequency. The method was later modified [30] to allow for the mutual absorption of 25 α and 25 β epimeric sapogenins at 900 and 915 cm^{-1} , by introducing the "ratio method" (explained in Materials and Methods). More modern methods are currently used to assay sapogenins e.g., HPLC [10], capillary gas chromatography [31].

Thin-layer chromatographically separated diosgenin of extracts of *Tribulusterrestris* L. was quantified by densitometry at 430 nm, following spraying TLC plates with anisaldehyde/H₂SO₄ reagent [32].

The modified I.R. method [30] was used in this study because of its simplicity and sensitivity towards α and β epimeric sapogenins.

2. Materials and Methods

2.1 Seeds Source

Fenugreek seeds used in these studies were obtained

directly from different local markets in Sudan or from the gene bank of the Horticulture Section, Agricultural Research Corporation (ARC), Wad-Medani, Sudan. These gene bank accessions, 13 in number (Table 1, gene bank code number bracketed), represent seed accessions originally collected from different parts of Sudan and cultivated in the ARC Research Farm at Wad-Medani.

2.2 Solvents, Chemicals and Equipment

Analytical grade solvents and chemicals or equivalents

Table 1 Accession codes, seed collection areas and some seed characteristics of the thirty seed accessions of the study.

Accession Code	Seed collection area (in Sudan)		Seed moisture content (%)	100-seed-wt. (g)	Outer-seed coat color prevalence (%)***
	Locality name*	Region**			
A1	Wad-Medani (local market)	C	6.8	2.12	GB (71), B (28), Black (1)
A2	Abu-hashim (HSD.0637)	C	7.4	0.78	GB (100)
A3	El-alyab (HSD.0461)	N	7.4	1.38	GB (85), dB (15)
A4	Kadogli (HSD.0302)	SW	7.0	1.21	GB (89), dB (11)
A5	Niyala (HSD.0608)	W	6.2	1.30	C (91), B (6), G (3)
A6	El-Silaim (HSD.0509)	N	6.2	1.33	GB (79), B (21)
A7	Nyerteti (HSD.0604)	W	6.6	0.96	GB (100)
A8	Abu-hashim (HSD.0479)	N	6.8	1.03	YB (86), B (12), G (2)
A9	Ed-dammer (HSD.0421)	N	7.0	1.07	GB (94), B (6)
A10	Sinnar (HSD.0301)	C	6.6	1.05	GB (93), B (7)
A11	Karima (HSD.0478)	N	6.8	1.24	YG (97), B (3)
A12	Wad-medani (local market)	C	7.0	2.18	B (75), dB (10), GB (15)
A13	Sinnar (HSD.0300)	C	7.8	1.10	GB (97), B (3)
A14	El-goraiba (HSD 0487)	N	8.6	1.32	YG (97), B (3)
A15	El-goraiba (HSD 0724)	N	8.2	1.17	YG (94), B (6)
A16	El-hassahisa (local market)	C	6.4	1.15	YB (100)
A17	Wad-medani (local market)	C	4.4	1.41	GB (98), dB (2)
A18	Khartoum (local market)	C	6.4	1.47	GB (99), dB (1)
A19	Omdurman (local market)	C	6.6	1.07	GB (77), dB (10), G (13)
A20	Omdurman (local market)	C	7.0	1.23	YG (100)
A21	Soaba (local market)	C	7.0	2.00	dB (99), Reddish B (1)
A22	Wad-medani (local market)	C	7.2	1.82	GB (97), dB (1), GB with black tip (1)
A23	Wad-medani (local market)	C	7.2	2.06	GB (83), dB (10), Y (7)
A24	Wad-medani (local market)	C	7.4	2.04	YB (99), GB with black tip (1)
A25	Omdurman (local market)	C	6.8	0.86	GB (98), B (2)
A26	Omdurman (local market)	C	7.8	1.03	GB (96), YB (4)
A27	Omdurman (local market)	C	6.6	1.01	GB (100)
A28	Omdurman (local market)	C	4.6	1.96	GB (99), dB (1)
A29	Omdurman (local market)	C	4.6	0.98	GB (99), YG (1)
A30	Omdurman (local market)	C	4.6	1.88	YG (97), YB (2), dB (1)

HSD = Horticulture Sudan Genebank, Agricultural Research Corporation (ARC), Wad-Medani, Sudan.

** C = Central-, W = Western, N = Northern and SW = South Western-Sudan.

*** GB = Greenish-Brown, G = greenish, Y = yellowish, YB = Yellowish-Brown, YG = Yellowish-Green, B = Brown, C = Creamycolor, dB = dark brown

were used for analysis. Spectrosol grade solvents were used for spectroscopy. Silica gel G 60 was used as adsorbent for TLC. Precoated TLC plates were obtained from Merck Co. (Germany). When required, glass plates (20 $\times$ 20 cm) were coated manually with the above adsorbent using a TLC spreader (Camag, Switzerland).

A Shimadzu Infrared Spectrophotometer Model IR 435 was used for sapogenin analysis by measuring full IR spectra or, for routine analyses of crude Fenugreek extracts, the machine was set to measure IR absorptions in the region 1000-800 cm^{-1} .

2.3 Preparation of Fenugreek-seed Total Sapogenin Extract

After assessing optimum parameters such as volume and concentration of the acid used for hydrolysis, hydrolysis of powdered or whole seeds, etc., the following method was adopted. Whole Fenugreek seeds (typically 5 gm in weight for routine total sapogenin assays), were refluxed with 150 mL of 2N HCL in 500 mL round-bottom flask for 3 h to hydrolyze the steroidal saponins. After hydrolysis the mixture was cooled under tap water, filtered and the residue briefly washed with water before it was neutralized with NH_4OH (10%). The neutralized residue was washed again and dried over-night at 50 $^{\circ}\text{C}$. The steroidal sapogenins were then extracted with petroleum ether (b.r. 40 $^{\circ}\text{C}$ -60 $^{\circ}\text{C}$) in a Soxhlet extractor for 24 h. The extracts were concentrated in a rotatory evaporator. The light yellow semi-solid product obtained was dissolved in exactly 10 mL of spectrosol grade CHCl_3 and the sapogenins were quantified by infra red spectroscopy.

When required, sapogenin crystallization was carried out by adding methanol to petroleum ether crude extracts and the solution allowed to stand over-night in the cold (below 25 $^{\circ}\text{C}$).

2.4 TLC Separation and Identification of Fenugreek Seed Sapogenins

A definite weight (of a few mgs) of the light yellow

solid petroleum ether extract prepared above was dissolved in a small measured volume of CHCl_3 and used for TLC separations. Pre-coated plates of 0.2 mm layer were used. The plate was developed in a tank containing the solvent system: n-hexane/acetone (4:1). The separated compounds were detected using antimony trichloride spray reagent, when required. The R_f values were determined and compared with those published in the literature [33].

2.5 Separation and Recoveries of Diosgenin and Yamogenin

The two epimers diosgenin and yamogenin, which co-chromatographed in the above solvent system, were separated from each other according to the continuous-TLC development method described [34]. The crude total sapogenin extract, dissolved in CHCl_3 , was applied as bands onto 1.0 mm thick silica gel glass plates. The plates were developed in a tank containing a mixture of CH_2Cl_2 : $(\text{CH}_3\text{CH}_2)_2\text{O}$ (200:3) for 8 h. The tank was not completely covered; the lid was placed in such a way as to allow an open slit of about one mm wide to aid continuous solvent evaporation from the top edge of the plate as chromatographic development continued. After solvent drying at room temperature, the developed plate was covered with another clean glass plate exposing a silica gel zone of about 2 cm at one edge. The two plates were clamped together and provision was made to ensure that only this narrow zone has been reached by the detection reagent and the rest of the covered bands recovered uncontaminated (Fig. 1). The single band of diosgenin and yamogenin was eluted with CHCl_3 . Weight, m.p., i.r. spectroscopy and other physical parameters were determined for each epimer.

2.6 Infra Red Spectroscopic Assay of 25 α - and 25 β -Sapogenins

Quantification of diosgenin and yamogenin is based on the fact that 25 α -sapogenins and 25 β -sapogenins

Fig. 1 TLC separation of Fenugreek steroids (seed accession No. A1).

Solvent: n-hexane/acetone (4:1); adsorbent gel G; detection: antimony trichloride reagent.

absorb most intensely at wave numbers 900 cm^{-1} and 915 cm^{-1} respectively. The final chloroform solutions of crude sapogenins prepared were routinely assayed by scanning in the i.r. region 1000-800 cm^{-1} . Total 25 α -sapogenins and total 25 β -sapogenins present in Fenugreek extracts were calculated as diosgenin and yamogenin, respectively, from a calibration graph prepared using the pure compounds (Figs. 5, 6). The influence of the presence of one epimer on the absorption of the other epimer was routinely corrected for by using a graph prepared according to the "ratio method" [30] as shown in Fig. 7. Total sapogenin values were obtained by summing up values for total 25 α - and 25 β -sapogenins.

2.7 Calibration Graphs

2.7.1 Diosgenin and Yamogenin Calibration Graphs

Pure diosgenin (a 25 α -sapogenin) and yamogenin (a 25 β -sapogenin) were prepared from Fenugreek seeds as described above. Diosgenin (13 mg) was dissolved in CHCl_3 to yield the following concentrations; 0.07, 0.14, 0.29, 0.39 and 0.58%. Similarly concentrations

of 0.09, 0.18, 0.37, 0.49 and 0.73% were made using 10 mg of yamogenin.

The i.r. absorbances, measured at 900 cm^{-1} , 915 cm^{-1} and at 980 cm^{-1} for each sapogenin, were plotted against their concentrations (Figs. 5, 6).

2.7.2 The 25 α -, 25 β -ratio Graph

Chloroform solutions containing both purified diosgenin and yamogenin were prepared to give the following concentration ratios of diosgenin: yamogenin, 0.16:1, 0.27:1, 0.79:1 and 1.61:1. The ratios of the Absorbance of the mixed 25 α :- 25 β -sapogenins at 900 cm^{-1} : 915 cm^{-1} were plotted against their concentration ratios (Fig. 2). In routine work with Fenugreek seed extracts, the amount of total β -sapogenins was determined from the yamogenin calibration graph using the observed absorbance of the seed sample extract at 915 cm^{-1} . The observed Absorbance ratio for seed sample extract was used in the conjunction with the ratio graph to get the corrected concentration ratio of the two epimers. This value was used to calculate the amount of diosgenin from the value determined above for yamogenin. The method was described by Brain et al. [30].

3. Results and Discussion

3.1 Seed Morphological Variability among Sudanese Fenugreek Accessions

Table 1 includes data, namely seed collection area, seed weight, moisture content and color, of the 30 accessions of Fenugreek seeds subsequently analyzed for sapogenin content. The moisture content of air-dry seeds ranged from 4.4% to 8.6%. The outer-coat color of a single seed was uniform. However, outer-coat seed color within an accession was largely variable and included several shades mainly of green, brown and yellow. Five seed accessions (code numbers A2, A6, A16, A20 and A27) had uniform colors, the rest were of more or less mixed colors.

Variability in the appearance of Fenugreek seed was classically studied by Serpikhova (1934) [6] who classified seeds of *Trigonella foenumgraecum*

Fig. 2 Infrared spectra of individual steroids of Fenugreek seed accession No. A1 separated by TLC and designated as in Fig. 1 (I to VI). IR transmission (T%) was measured in the frequency range of 1000 to 800 cm^{-1} . The inset letters A, B and C denote absorbances at 900, 915 and 980 cm^{-1} , respectively.

according to their shape, size and color. Serpilkhova distinguished three groups: *Indicae* and *Anatolicae* with one variety each, in addition to the *Aethiopicae* group which contains 6 varieties. The varieties were assigned to specific geographical regions or countries. The group *Indicae* was reported to be confined to India and Ceylon, *Anatolicae* to Asia minor, the Mediterranean and central Europe, while the group *Aethiopicae* was claimed to be widely distributed in Africa, Asia and Europe.

Almost all the seed colors reported by Serpukhova for the different varieties of the world were encountered in the Sudanese fenugreek collections (as shown in Table 1, with the exception of a bluish-coloured variety reported by the authors to be endemic to Ethiopia and Eritrea.

Another study by the Hardman research group [6] described 52 Fenugreek seed samples collected from 18 countries, obtained directly from the country of origin, including two samples from Sudan. According to this report these two Sudanese samples were uniform in color (olive green) and shape (approaching

square). This is in contrast to our observations (Table 1) where all the colors reported by the above mentioned research group for Fenugreek seeds from 18 countries were displayed in our accessions. In addition we have encountered a rare seed color, a creamy color which was the major color for accession number A5.

The 100-seed-weight value for the accessions included in this study (Table 1) was quite variable and ranged from 0.78 gm to 2.18 gm. Moreover, the Sudanese Fenugreek seed accessions collected for our study (Table 1) showed a considerable variation in shape, both from collection to collection and within the same collection (accession), in contrast to previous reports [6]. Thus Sudanese Fenugreek seeds are remarkably variable in morphology, more so than previously reported, as far as color, shape and size are concerned. This raised the question whether or not this variability is extended to chemical constituents, the most important of which are the seed sapogenins. This aspect was the main concern of this report.

3.2 Steroidal Constituents of Fenugreek Seeds

3.2.1 Optimum Conditions for Acid Hydrolysis of Fenugreek Sapogenins

Infrared methods used for sapogenin assay required the prior removal of the sugar moiety of the saponin molecule by acid hydrolysis to give the free sapogenin [6, 29, 30]. In this study either powdered or whole seed material were tested in conjunction with different hydrolyzing reagent concentrations. The results (not given in detail) showed that optimum conditions for fenugreek seed saponin hydrolysis were achieved with 2N acid (HCl), at 30 mL acid/1 gm of whole seeds.

3.2.2 Thin-layer Chromatographic Separation, Partial Identification and Gravimetric Determination of Fenugreek Seed Steroids

Fenugreek seed extracts containing sapogenins and other steroids were prepared by *in situ* (as whole seeds) by acid hydrolysis of the seeds of Accession number A1, followed by neutralization and extraction with light petroleum ether (b.r. 40 °C-60 °C).

Fig. 1 Shows a typical TLC separation using the TLC solvent system: n-hexane: acetone, 4:1. Compared with other solvent systems tested (CHCl₃: acetone, 4:1 or CHCl₃: CCl₄: acetone, 2:2:1), this solvent gave the maximum number of separated spots (6 compounds). The steroidal nature of the separated compounds was indicated by comparing their R_f values with those published [33], color produced by detection reagents, U.V. absorption and infra-red spectra. Spots I, II, III and IV (Fig. 2) showed typical spirostan absorption in the region 800-1000 cm⁻¹. Spots I and III were 25 α -sapogenins (absorption at 900 > 915 cm⁻¹) and from their R_f data and literature reports [6, 30, 33], were identified as gitogenin and tigogenin, respectively. Spot IV comprised both 25 α - and 25 β -sapogenins according to their specific i.r. absorption and this taken with other data [6] were identified as a mixture of diosgenin and yamogenin. These two compounds could only be separated from each other by continuous chromatography. Previous work on the identity of Fenugreek seed sapogenin [6,

7, 30] is consistent with our findings, namely, the presence of diosgenin, yamogenin, tigogenin and gitogenin.

Spots V and VI, which did not show IR absorption at 900, 915 or 980 cm⁻¹ (Fig. 2) and were positive to the Liebermann-Burchard spray reagent, were tentatively identified as sterols.

Table 2 compares the relative abundance of sapogenins and sterols in fenugreek seed accession number A1. These steroids were separated by preparative TLC, using several TLC plates, the bands scraped, eluted and their weights appropriately determined gravimetrically. This gravimetric quantification indicated that the steroidal sapogenins constituted the dominant proportion of the steroid fraction recovered in the way described. Diosgenin and the co-chromatographing epimer, yamogenin, constituted more than a half of the total steroid compounds.

3.2.3 Preparative TLC Separation of Diosgenin and Yamogenin from Fenugreek Seed and Their Use as Standards

Diosgenin and its β -epimer yamogenin, which co-chromatographed in the TLC solvent system of Table 2, were separated from each other using continuous development TLC (see Materials and Methods). The extract was originally prepared from Fenugreek seed accession number A1. The two epimers were well separated from each other (mobility of yamogenin relative to diosgenin = 1.31), allowing easy recovery of each. Figs. 3, 4 show the full infra-red spectroscopic spectra of the two

Table 2 The relative levels of Fenugreek steroids (accession No. A1) separated by Preparative TLC and determined gravimetrically.

Spot/band No.	Sapogenin and sterol	W/W (%)
I	Gitogenin	8.6
II	25 β -sapogenin	19.4
III	Tigogenin	4.7
IV	Diosgenin + yamogenin	51.5
V	Sterol (1)	7.0
VI	Sterol (2)	9.6

Fig. 3 Full IR spectrum of diosgenin isolated and purified from Fenugreek seed accession No. A1.

Fig. 4 Full IR spectrum of yamogenin isolated and purified from Fenugreek seed accession No. A1.

compounds. In addition to the -OH absorption (around 3400 cm^{-1}), the two sapogenins showed typical spirostan absorption at 865, 900, 915 and 980 cm^{-1} . As expected yamogenin absorbed more at 915 than at 900 cm^{-1} while the 25 α -epimer, diosgenin, absorbed at 900 cm^{-1} , with only little absorbance at 915 cm^{-1} .

Purified diosgenin and yamogenin showed linear I.R. absorbance at wavenumbers 900, 915 and at 980 cm^{-1} (Figs. 5, 6). These two calibration graphs were

used to determine total 25 α - and 25 β -sapogenins. Since the two epimers interfere with each other's absorbance the actual concentration of each was determined from the ratio graph (Fig. 7) according to the method detailed by [30]. In the determination of sapogenins in Fenugreek seed samples, the concentration of total 25 β -sapogenin which absorb at 900 cm^{-1} and 915 cm^{-1} was determined directly from the absorbance at 915 cm^{-1} using the yamogenin

Fig. 5 Diosgenin calibration graph.

Purified diosgenin was prepared from Fenugreek seeds (accession No. A1). Infra-red absorptions (A) of different concentrations of the compound were measured at the wavenumbers indicated.

Fig. 6 Yamogenin calibration graph.

Purified yamogenin was prepared from Fenugreek seeds (accession No. A1). Infra-red absorptions (A) of different concentrations of the compound were measured at the wavenumbers indicated.

calibration graph (Fig. 6). The actual concentration ratio of 25 α : 25 β -sapogenins read from Fig. 7 using the observed absorbance ratio. The concentration ratio thus obtained was used to calculate the concentration of the total 25 α -sapogenins. Total sapogenin was obtained by summation of the values for 25 α -sapogenin (diosgenin) and 25 β -sapogenin (yamogenin).

3.2.4 Steroidal Sapogenins of Thirty Accessions of Sudanese Fenugreek Seeds

Fig. 8 (as well as Table 3) show values of total steroidal sapogenins ($\alpha + \beta$ epimers) of the thirty Fenugreek seed accessions as determined by IR

spectroscopy. The values varied considerably and ranged between 1.1 and 3.0% of seed dry matter (cf accessions A21 and A1). Seed accession A21 had been stored for 6 years before analysis, however, other accessions not subjected to storage (e.g., A9 and A16) also had low values. Nine accessions contained a total sapogenin of over 2.5% and 18 accessions had a value of 2.0% or more. The latter accessions were from the three Sudanese regions covered, viz. the northern, western and central regions. On the other hand, eight accessions had a total sapogenin value of 1.5% or less. Fazli and Hardman [6] analyzed 52 Fenugreek seed

Fig. 7 The ratio graph, solutions containing different weights (ratios) of diosgenin: yamogenin were prepared as explained in Materials and Methods. The ratios of IR absorbance at 900 cm^{-1} (characteristic of diosgenin) to that at 915 cm^{-1} (characteristic of yamogenin) were plotted against the concentration ratios prepared. The graph was used to evaluate the mutual IR absorption interference by the two epimers when assaying crude extracts of Fenugreek seed samples for sapogenins.

Fig. 8 Total sapogenin content of 30 seed accessions of Sudanese Fenugreek determined by IR spectroscopy (data of Table 3). Results were expressed as percentages of oven-dry Fenugreek seed powder.

samples obtained from 18 countries, including Sudan, and belonged to the African, European and Asian continents. They reported total sapogenin values that ranged between 0.77% and 2.18%. The two Sudanese samples included in this study contained 1.59% and 1.40% total sapogenin. These authors had also concluded that different seed batches obtained from

the same country had similar total sapogenin content but this value could differ from country to country. This is in odds with our findings.

The results (Table 1, 3) do not indicate any correlation between size, shape or color and total sapogenin among accessions. This is in agreement with the results of Fazil and Hardman [6]. For example the

Table 3 Sapogenin content of 30 accessions of Sudanese Fenugreek seeds determined by IR spectroscopy.

Accession Code Number	Total 25 α -sapogenin (percent of oven-dry seed powder, calculated as diosgenin)	Total 25 β -sapogenin (percent of oven-dry seed powder, calculated as yamogenin)	Total 25 α - plus 25 β -Sapogenin (percent of oven-dry seed powder; total 25 α + 25 β)	25 α -: 25 β -sapogenin concentration ratio
A1	0.97	2.03	3.00	0.48
A2	0.86	1.13	1.99	0.76
A3	1.10	1.56	2.66	0.71
A4	0.99	1.17	2.16	0.85
A5	0.62	1.11	1.73	0.56
A6	0.78	1.28	2.06	0.61
A7	0.92	1.32	2.24	0.70
A8	0.92	1.03	1.95	0.90
A9	0.71	0.53	1.24	1.34
A10	1.11	1.40	2.51	0.80
A11	1.02	0.58	1.60	1.76
A12	0.88	0.61	1.49	1.44
A13	0.68	0.76	1.44	0.89
A14	0.90	0.60	1.50	1.50
A15	1.00	0.70	1.70	1.43
A16	0.68	0.56	1.24	1.21
A17	0.88	0.81	1.69	1.09
A18	1.13	1.24	2.37	0.91
A19	1.10	1.45	2.55	0.76
A20	1.12	1.20	2.32	0.93
A21	0.37	0.73	1.10	0.51
A22	0.84	0.45	1.29	1.87
A23	0.65	0.78	1.43	0.83
A24	1.68	0.99	2.67	1.70
A25	1.09	1.37	2.46	0.80
A26	1.41	1.52	2.93	0.93
A27	1.05	1.33	2.38	0.79
A28	1.27	1.35	2.62	0.94
A29	0.91	1.12	2.03	0.81
A30	1.14	1.28	2.42	0.89

two accessions, number A1 and A23 had more or less similar “100-seed-weight” (~ 2.0 gm), however, their total sapogenin contents were 3.0% and 1.43%, respectively. The smallest seed (accession No. 2) contained 1.99% total sapogenin while the largest seed (accession No. 12) contained 1.49% total sapogenin. With the exception of greenish-brown color there was no correlation between seed colour and total sapogenin. Thirteen of the accessions analyzed had “greenish-brown” as the dominant seed color (Table 1). Twelve of these accessions had a fairly high total

sapogenin value (2% or over). However, one accession (A22) had a somewhat relatively low value of 1.29%.

It is possible that environmental conditions prevailing at the cultivation area played a role influencing the total sapogenin level observed with the seed accessions analyzed. However, the ARC Gene Bank samples analyzed, indicated in Table 1, which represent seed accessions that have been collected from different parts of the country and grown during the same season and in one area i.e., at the ARC

Research Farm in Wad-Meadni. Thus the environmental effect is greatly minimized. It is noteworthy that considerable differences were also observed in total sapogenin content within these ARC samples. Within these thirteen accessions total sapogenin varied from 1.24% to 2.66%.

The relative concentration of 25 α - to 25 β -sapogenins considerably varied among Sudanese Fenugreek accessions (Table 3). Only about 8 accessions contained more of the α sapogenin, diosgenin, than of the β epimer, yamogenin. Thus yamogenin is the dominant sapogenin in Sudanese Fenugreek seeds.

Fenugreek seeds were contemplated as alternative commercial sources of steroidal sapogenins for use by the pharmaceutical industry as precursors for the partial synthesis of steroid drugs. Low sapogenin content hindered this use. The results represent an encouragement towards this end which could be achieved by breeding and plant selection, given the immense variability in sapogenin content and the fact that some Sudanese seed accessions showed a total sapogenin content as high as 3%. This level (3%) is close to that required minimally for processing of *Dioscorea* tubers [35], the major commercial source of sapogenin precursors.

4. Conclusions

Fenugreek seed accessions collected from different geographical regions of Sudan displayed considerable morphological variability in outer seed-coat color, shape and size.

This study was mainly concerned with variations, among the seed accessions, in the content of steroidal sapogenins, chemicals contemplated as natural precursors for the commercial synthesis of steroid drugs, as well as gaining considerable research interest in their continually revealed useful biological activities.

A simple, specific, infra-red spectrophotometric method was used to assay total sapogenins as well as the proportions of 25 α - and 25 β -epimeric forms. Thus

the method has the advantage of distinguishing between diosgenin and yamogenin, epimers that differ only in the orientation of the methyl group attached to carbon-25 of the spiroketal side-chain, being below or above ring F.

Sapogenins constituted more than 70% of fenugreek seed steroids. Among the thirty accessions, large variations were observed in the seed contents of 25 α -, 25 β - and total sapogenins, suggesting the possibility of improving the seed content of these compounds by selection breeding.

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