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Short communication

Bioactive lipid constituents of fenugreek

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ABSTRACT

Neutral and polar lipids of fenugreek were investigated. Triacylglycerol and phosphatidylethanolamine were the major molecular species identified in the neutral and polar lipid fractions, respectively. The fatty acid profile was dominated by unsaturated acids, namely oleic, linoleic and linolenic acids accounting for 16.3%, 50% and 24.4%, respectively of the total fatty acids. Besides the major molecular species, N-Acyl phosphatidylethanolamines (NAPE) and fatty acid amides were isolated and identified for the first time in this spice. N-linoleylphosphatidylethanolamine was found to be the major NAPE while oleamide was demonstrated to be the major fatty acid amide in the lipid fraction. The possible role of oleamide, an endogenous sleep-inducing factor, as well as NAPE in contributing to the pharmacological properties of fenugreek is discussed.

1. Introduction

Fenugreek (*Trigonella foenum-graceum*) is a spice possessing amazing therapeutic and medicinal properties. The spice is used to relieve skin irritation, to reduce swelling and pain, as a laxative and in the treatment of indigestion and flatulence (Sauvare, Pett, Baissao, & Ribes, 2000). The seeds are used for the treatment of diabetics, anaemia and respiratory disorders (Kaviarasan, Naik, Gangabhagirathi, Anuradha, & Priyadarsini, 2007; Kaviarasan, Vijayalakshmi, & Anuradha, 2004). Fenugreek seed is an excellent source of fibre that is mainly comprised of galactomannans (Madar & Stark, 2002). It facilitates slow absorption of carbohydrates, thus resulting in feeling of fullness and aid in relieving stomach disorders. The unique amino acid, 4-hydroxyisoleucine stimulates the release of insulin thereby controlling blood sugar levels (Gupta, Gupta, & Lal, 2001). Fenugreek is rich in flavonoids such as apigenin, luteolin, orientin, quercetin, vitexin and isovitexin (Blumenthal, Goldberg, & Brinckmann, 2000; Sauvare et al., 2000; Shang et al., 1998). These natural antioxidants help to strengthen the immune system, improve cellular health and reduce signs of ageing (Kaviarasan et al., 2004). The spice seeds contain 0.1–0.9% diosgenin and are extracted on a commercial basis (Elujoba & Hardman, 1987; Sauvaire & Baccou, 1978). The seeds also contain the saponin fenugrin B (Gangrade, Mishra, & Kanshal, 1979). Several coumarin compounds have been identified in fenugreek seeds (www.drugs.com/npp/fenugreek.html) as well as a number of alkaloids (e.g., trigonelline, gentianine, carpaine). Fenugreek seed also contains 5.5–7.5% lipids constituting mainly of neutral lipids (85%) followed by phospholipids (10%) and glycolipids (5%). Unsaturated acids comprising mainly of linoleic (40%), linolenic (25%) and oleic (14%) acids dominate the fatty acid profile (Baccou, Sauvaire, Olle, & Petit, 1978; Sulieman, Ali, & Hemavathy, 2000).

In recent years, N-acylethanolamines (NAEs) and their precursors, N-acyl phosphatidylethanolamines (NAPEs) have been identified as phospholipid constituents in desiccated seeds of different plant species (Chapman, 2004). These minor membrane lipid components have been implicated in lipid signalling pathway that regulates an array of physiological processes in multicellular eukaryotes including plant defence response and seedling root development (Chapman, 2004). Long chain fatty acid amides are another class of signalling lipids with a physiological role in mammalian nervous system (Herbert et al., 2002). Oleamide an important member of this class is a sleep-inducing lipid with diverse action such as antinociceptive (pain reducing) properties and stimulates increased food uptake (Boger, Henriksen, & Cravatt, 1998; Martinez-Gonzalez et al., 2004). There is an increased interest in identifying natural sources of NAEs, NAPEs and fatty acid amides for therapeutic applications such as agents to modify feeding behaviour, as topical analgesics and as modulators of neuronal transmission. Fenugreek is traditionally assumed to increase appetite and to have pain-reducing properties (Kaviarasan et al., 2007; Puri, 1998). Since NAEs and oleamide are known for imparting such properties, it was of interest to ascertain the presence of these constituents in the lipid fraction. The present work therefore aims at isolating and identifying the major and some minor lipid constituents of fenugreek.

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2. Materials and methods

2.1. Materials

Three varieties of fenugreek, procured from the local market were divided into three 2 kg lots. Two hundred grams samples from each of the lots in triplicate were used for analysis. Solvents of analytical grade (Emerck, India Ltd., Mumbai) were distilled before use. Lipid standards were procured from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Methods

2.2.1. Extraction of Lipids

Fenugreek (200 g) was finely powdered to pass through a No. 40 sieve in an electrical grinder (Sumeet, Mumbai, India) at room temperature. The powder was soaked for 2 h in chloroform/methanol (2:1, v/v). The mixture was then stirred (3 × 2 min) in an omnimixer. The slurry was filtered under suction and the residue re-extracted (5 × 100 ml) with chloroform/methanol (2:1, v/v). The filtrate obtained in each case was combined and evaporated to dryness under vacuum and the residue made to 10% in chloroform/methanol. This extract was used for the analysis of both neutral and polar lipids.

2.2.2. Separation of lipid constituents

Analytical TLC of the lipid isolate obtained above was carried out on ammonium sulphate (3%) impregnated silicagel G plates (10 cm × 25 cm × 0.25 mm thickness, procured from Merck, Darmstadt, Germany) using various solvent systems. The solvent systems employed were (1) petroleum ether/diethyl ether/acetic acid (80:20:2, v/v/v) for neutral lipids, (2) methyl acetate/2-propanol/chloroform/methanol/0.25% aq. KCl (25:25:25:10:9, v/v/v/v) for polar lipids, (3) chloroform/methanol/25% ammonia (80:20:2, v/v/v) for NAPE, (4) toluene/ethyl formate/formic acid (5:4:1, v/v/v) for identification of oleamide and (5) chloroform/methanol/ammonia (85:15:1, v/v/v) for NAE. The separated spots were visualised by exposing the plates to iodine vapour and also by heating the plate at 180 °C for 10 min. The lipid classes were identified by comparing their R_f values with that of standards as well as from literature values. Preparative TLC was carried out on silicagel G plates (20 cm × 20 cm × 0.5 mm thickness, procured from Merck, Darmstadt, Germany) and the individual lipid constituents of interest were scrapped, eluted with chloroform. Solvent was removed under vacuum and the residue in each case was made to 1% in chloroform. The isolate thus obtained was further analysed for their chemical identity.

2.2.3. Estimation of lipid classes

Quantitative distribution of individual lipid classes on TLC plate was carried out using a dual wavelength flying spot scanning densitometer CS-9301PC (Shimadzu, Kyoto, Japan). The density of the spots was determined in the reflectance mode at a wavelength of 529 nm. Oleic acid and phosphatidylethanolamine were used as external standard for the estimation of neutral and polar lipid constituents, respectively. Aliquots of suitably diluted sample were spotted on the plate in increasing concentration ranging from 0.02 to 1.0 mg/mL. The plot of spot density versus concentration was drawn to obtain a standard curve. The plot was found to be linear in the range of 0.02–0.5 mg/mL. The concentration of individual components in the sample was obtained from the standard curve.

2.2.4. Separation of free fatty acids

The total lipid extract was saponified (2 M KOH, 80 °C, 1 h). The hydrolysate after removal of unsaponifiable matter was acidified (2 M HCl) and the free fatty acids liberated were extracted into diethyl ether. The ether layer was washed with distilled water until free of acid and then dried over anhydrous sodium sulphate. The free fatty acids obtained after removal of solvent was converted to their methylated derivative (diazomethane, room temperature) and then analysed by GC/MS.

2.2.5. GC–MS analysis

This was carried out on a Shimadzu GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph and provided with a DB-5 (J&W Scientific, 91 Blue Ravine road, Folsom, CA, USA) capillary column ((5%-phenyl)-methylpolysiloxane, length, 30 m; i.d., 0.25 mm and film thickness, 0.25 μm). The operating conditions were: column temperature programmed from 140 to 280 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min and further to 280 °C at the rate of 10 °C/min, held at final temperature for 10 min; Injector and interface temperatures, 210 and 280 °C, respectively; carrier gas helium (flow rate, 0.9 ml/min); ionisation voltage, 70 eV; electron multiplier voltage, 1 kV. Samples (0.1 μl) were injected in the splitless mode. Peaks were identified by comparing their mass fragmentation pattern with that of standard compounds as well as from the data available in the spectral library (Wiley/NIST Libraries, Shimadzu Corporation, Kyoto, Japan) of the instrument.

2.2.6. Estimation of free fatty acids

Aliquots of standard linoleic acid ranging in concentration from 1–10 μg/μl were injected into the GC and the plot of peak area versus concentration was then drawn. The plot was found to be linear in the range of 1–8 μg/μl. The concentration of individual components in the sample was obtained from the standard curve.

2.2.7. Isolation and characterisation of NAPE

The chloroform/methanol extract was subjected to prep TLC using chloroform/methanol/25% ammonia (80:20:2, v/v/v) as the solvent system. The band at R_f 0.66 was isolated. A part of this isolate was subjected to GC/MS analysis while the remaining was acid hydrolysed in order to identify the nature of the fatty acids linked to nitrogen. The hydrolyzate containing free fatty acid was extracted successively with diethyl ether. The extracts after removal of solvent was methylated and then subjected to GC/MS.

2.2.8. Isolation and characterisation of oleamide

The chloroform/methanol extract was subjected to prep TLC using toluene/ethyl formate/formic acid (5:4:1, v/v/v) as the solvent system. The band at R_f 0.51 was isolated (white powder) and subjected to spectral analysis (UV, IR, NMR and MS). A weak absorbance at 280 nm was noticed. The melting point was determined as 73–74 °C. The IR spectrum was scanned with a JASCO FTIR 4100 spectrophotometer (Jasco Corporation, Tokyo, Japan). ν_{max} (CCl_4): 3524 (asymmetric stretching of N–H), 3403 (symmetric stretching of N–H), 3007 (=C–H stretch), 2920, 2856, 1658 (amide C=O), 1628, 1462, 1405 cm^{-1}. The NMR spectra were recorded with a Bruker AC-200 MHz FT NMR spectrometer (Bruker, Fallanden, Switzerland). The usual abbreviations employed are: br = broad s = singlet, t = triplet, m = multiplet, J = coupling constant (in Hz), δ = chemical shift in ppm. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 5.86 (1H, br S, CONH*H*), 5.52 (1H, br S, CON*H*H), 5.33 (2H, m, C*H*=C*H*), 2.19 (2H, t, J = 7.4 Hz, CH_2CONH_2), 2.01 (4H, m, CH_2CH=CH CH_2), 1.59 (2H, m, CH_2CH_2–$CONH_2$), 1.33–1.14 (20H, m), 0.85 (3H, t, J = 6.6 Hz, CH_3). ^{13}C NMR ($CDCl_3$) d 176.3 (C=O), 129.8 (C=C), 129.6 (C=C), 36.3, 31.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 27.2, 27.1, 25.5, 22.7, 14.3. EIMS was recorded using DI probe facility in the GC/MS equipment described above. m/z (%, rel. int.) [M^+] 281(0.8), 41(40), 43(33), 44(15), 55(34), 57 (13), 59 (100), 60(10), 69 (14), 72 (52).

2.3. Data analysis

All data are an average of three independent analyses with each analysis carried out in triplicate. Thus a total of nine replications were performed. Statistical analysis was carried out using analysis of variance method (Origin 6.1 software) and means were expressed as significantly different or not at 5% level of confidence.

3. Results and discussion

The quantitative distribution of the various lipid constituents as estimated by TLC-densitometry is presented in Table 1. Triacylglycerols accounted for 85% of the neutral lipids. Other constituents at concentrations greater than 1% include free fatty acids (3.2%), diacylglycerols (5.5%) and monoacylglycerols (2.1%). The polar fraction was dominated by phosphotidylcholine (18.5%) and phosphotidylethanolamine (6%) of this lipid class followed by phosphotidylinositol (1.5%). The results obtained in this study are similar to that reported by Sulieman et al. (2000). Qualitative and quantitative estimation of the major free fatty acids identified by GC/MS is listed in Table 2. Linoleic acid was the major fatty acid identified accounting for 36% of the total fatty acids, followed by linolenic (18%), oleic (13%) and palmitic acid (9%). These results are in agreement with the reported literature values (Sulieman et al., 2000).

Besides the major lipid classes there has been an increased interest in recent years in the composition of minor lipid classes that are known to have important physiological function in plants/animals. Two such minor membrane lipid components implicated in lipid signalling pathway are the N-acylethanolamines that have been identified as phospholipid constituents in desiccated seeds as well as fatty acid amides with a physiological role in mammalian nervous system. Oleamide a member of the latter class has also recently been reported in some plants (Wu, Charles, & Huang, 2007). No report exists so far on the occurrence of these minor lipid classes in fenugreek.

TLC chromatogram of the total lipid extract in chloroform/methanol/ammonia (80:20:2, v/v/v) solvent system is shown in Fig. 1. The region corresponding to NAPE at R_f 0.66 could be clearly distinguished. GC/MS analysis of the isolated band resulted in a major peak at R_t 59.9. Acid hydrolysis of this band obtained from prep TLC, revealed the presence of linoleic acid as the major fatty acid moiety linked to the nitrogen atom. Total NAPE content was found to be 7.23 ± 1.5 μg/g of the spice. Fatty acids identified include palmitic acid, oleic acid and linolenic acid. To the best of our knowledge this is the first report on the occurrence of NAPEs in fenugreek seeds.

Recent studies have suggested NAPEs as precursors for the release of NAEs, biologically active signalling molecules. Long chain N-acylethanolamines are endogenous signalling components in plant system. Their formation from NAPEs was demonstrated to be a part of the signal transduction of pathogen elicitor perception, leading to plant defence response. Potential biological activities of some NAE types on seedling root cell development also suggest a physiological role of these metabolites in establishment of normal seedling growth and development. The total content of NAE in fenugreek seeds was found to be 728 ± 42.8 ng/g of fenugreek. The NAE band isolated from preparative TLC (R_f = 0.8) was subjected to acid hydrolysis and the liberated fatty acids were analysed by GC/MS. The major fatty acids identified were linoleic,

Table 1
Composition of neutral and polar lipid constituents in fenugreek.

Lipid species identified	Amount (g/100 g of fenugreek)
Triacylglycerols	4.330 ± 0.011
Diacylglycerols	0.280 ± 0.008
Monoacylglycerols	0.180 ± 0.005
Phosphotidylcholine	0.110 ± 0.002
Phosphotidylethanolamine	0.036 ± 0.003
Phosphotidylinositol	0.009 ± 0.001
Free fatty acids	0.160 ± 0.001

Data are the mean of nine replicates ± standard deviation.

Table 2
Fatty acid composition of total lipids of fenugreek seeds.

Fatty acid methyl ester	Amount (mg/g of fenugreek)
$C_{14:0}$	0.16 ± 0.03
$C_{16:0}$	8.95 ± 0.02
$C_{16:1}$	0.15 ± 0.01
$C_{18:0}$	3.67 ± 0.04
$C_{18:1}$	12.93 ± 0.05
$C_{18:2}$	35.81 ± 0.07
$C_{18:3}$	18.10 ± 0.02
$C_{20:0}$	1.17 ± 0.04
$C_{20:1}$	0.06 ± 0.03
$C_{22:0}$	0.36 ± 0.01
$C_{24:0}$	0.07 ± 0.01

Data are the mean of nine replicates ± standard deviation.

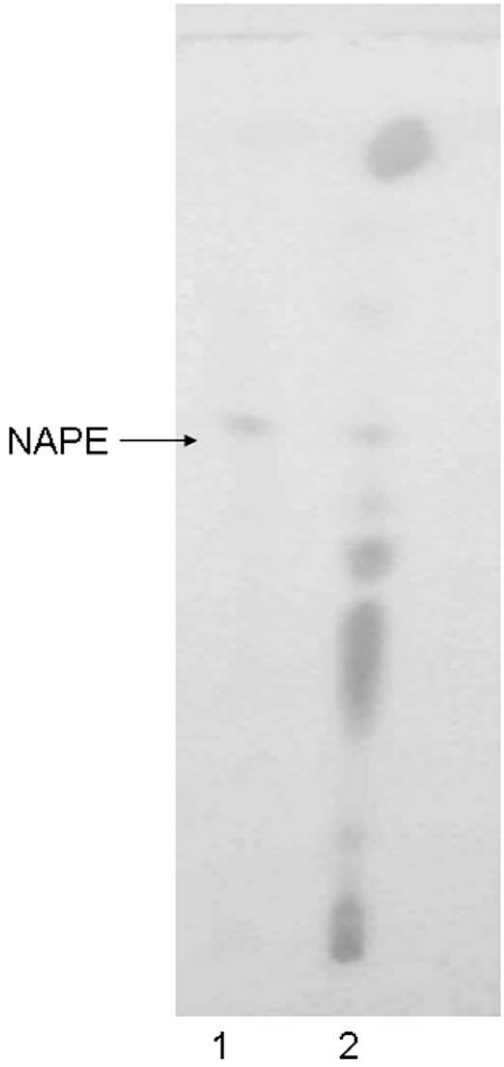

Fig. 1. TLC chromatogram of polar lipids of fenugreek in solvent system chloroform/methanol/ammonia (80:20:2, v/v/v), (1) isolated NAPE and (2) chloroform/methanol extract.

Table 3
Fatty acid composition generated from NAE.

Fatty acids	Amount (ng/g)
Palmitic acid	163.13 ± 2.15
Oleic acid	154.24 ± 1.79
Linoleic acid	221.16 ± 1.84

Data are the mean of nine replicates ± standard deviation.

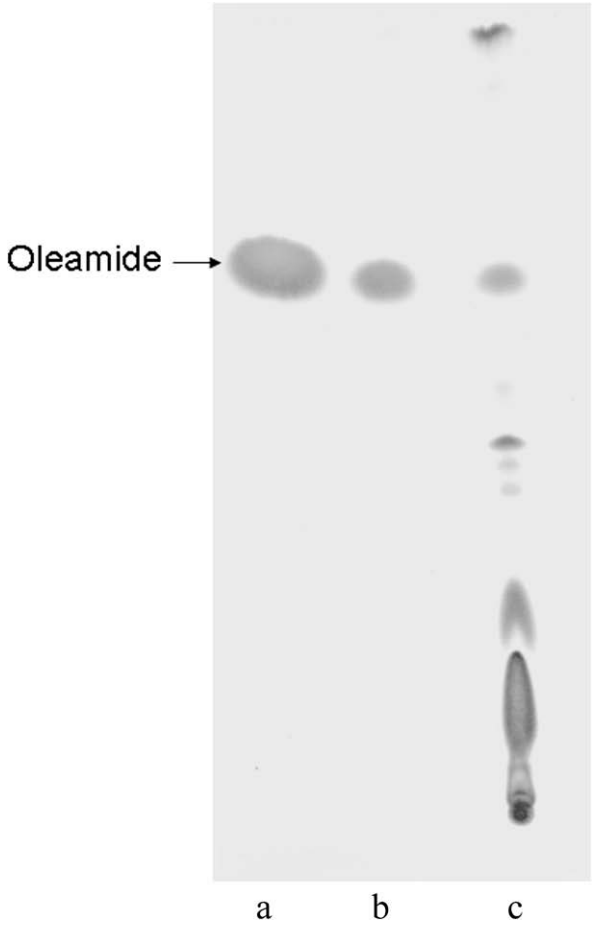

Fig. 2. TLC chromatogram of spice extract in toluene/ethyl formate/formic acid (5:4:1, v/v/v) solent system, (a) standard oleamide, (b) oleamide isolated from fenugreek and (c) chloroform/methanol extract.

oleic and palmitic acids. This suggests the presence of N-linoleoylethanolamine, palmitoylethanolamine and oleoylethanolamine (Table 3) as the NAEs in the spice. NAEs are fundamental regulatory molecules in mammalian physiology with several pharmacological properties. Many of these compounds termed as endocannabinoids due to their cannabinoid-like properties produce neurobehavioural effects and have important signalling roles in central nervous system (CNS) especially in the perception of pain and in control of appetite. Some of them have anti-inflammatory and anti-cancer properties and help regulate many physiological and pathological processes in the reproductive system. Oleoylethanolamine is an endogenous regulator of food intake and is suggested as a potential anti-obesity drug.

Fatty acid amides are another class of minor lipid constituents that are known to have important pharmacological properties. Oleamide (cis-9,10-Octadecenamide) is one of the most important primary fatty acid amides formed in mammalian CNS. This compound has been identified as the signalling molecule responsible for causing sleep in mammals. The molecule has also been shown to induce a decrease in core temperature, hypolocomotion, reduction in pain perception and increase in food uptake. Oleamide and related fatty acid amides belong to a group of compounds that have potential as clinically useful antidepressant drugs. The band at R_f 0.51 isolated from prep. TLC (Fig. 2) gave a single peak at R_t 51.8 min when subjected to GC/MS. This compound having a molecular weight of 281 as obtained from its mass spectral data was identified as oleamide by comparison of its GC retention time and NMR data with that of standard oleamide. This is the first report on the occurrence of oleamide in fenugreek. The content of this compound was 1.8 mg/100 g of fenugreek. Wu et al. (2007) in their study on the biochemical compounds of adlay (*Coxi lachrymal-jobi*) also reported oleamide content ranging from 18.9 to 45.8 mg/kg of the plant material. Our results are also comparable with this published data. In a study on the activation effects on choline acyltransferase and scopolamine-induced amnesia by Korean traditional natural plants, Heo et al. (2003) have demonstrated highest activity in methanolic extracts of *Zizyphus jujube*. Oleamide was shown to be the active compound responsible for these activities. Administration of the compound to mice significantly reversed the scopolamine-induced memory and/or cognitive impairment. It was also suggested that oleamide could be a useful chemopreventive agent against Alzheimer disease.

Thus the presence of NAPE/NAE as well as oleamide with wide ranging pharmacological action could have a significant impact on the medicinal properties of fenugreek. Since fenugreek is traditionally known for its anti-inflammatory and antinociceptive (pain reducing) properties and stimulates increased food uptake the possible role of the above minor lipid constituents in contributing to the therapeutic properties of fenugreek needs further investigation.

References

Baccou, J. C., Sauvaire, Y., Olle, M., & Petit, J. (1978). L'huile de Fenugreek: Composition, properties, possibilities d'utilisationdsans l'indust rie des peintures et vernis. *Revue Francaise des Corps Gras, 25*, 353–359.

Blumenthal, M., Goldberg, A., & Brinckmann, J. (2000). *Herbal medicine: Expanded commission E monographs* (pp. 130–133). Newton, MA: American Botanical Council. Integrative Medicine Communications.

Boger, D. L., Henriksen, S. J., & Cravatt, B. F. (1998). Oleamide: An endogenous sleep-inducing lipid and prototypical member of a new class of biological signaling molecules. *Current Pharmaceutical Design, 4*, 303–314.

Chapman, K. D. (2004). Occurrence, metabolism, and prospective functions of *N*-acylethanolamines in plants. *Progress in Lipid Research, 43*, 302–327.

Elujoba, A. A., & Hardman, R. (1987). Saponin-hydrolyzing enzymes from fenugreek seed. *Fitoterapi, 58*, 197–199.

Gangrade, H., Mishra, S. H., & Kanshal, R. (1979). Antimicrobial activity of the oil and unsaponifiable matter of red rosette. *Indian Drugs, 16*, 147–148.

Gupta, A., Gupta, R., & Lal, B. (2001). Effect of *Trigonella foenum graecum* (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: A double-blind placebo controlled study. *Journal of Association of Physicians of India, 49*, 1057–1061.

Heo, H., Park, Y., Suh, Y., Choi, S., Kim, M., Cho, H., et al. (2003). Effects of oleamide on choline acetyltransferase and cognitive activities. *Bioscience, Biotechnology, and Biochemistry, 67*, 1284–1291.

Herbert, S., Lani, B. J., Jack, L., Kent, C., Mary, M. C., Andrea, G., et al. (2002). Evidence that anandamide-signaling regulates human sperm functions required for fertilization. *Molecular Reproduction and Development, 63*(3), 376–387.

Kaviarasan, S., Naik, G. H., Gangabhagirathi, R., Anuradha, C. V., & Priyadarsini, K. I. (2007). In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. *Food Chemistry, 103*, 31–37.

Kaviarasan, S., Vijayalakshmi, K., & Anuradha, C. V. (2004). Polyphenol-rich extract of fenugreek seeds protect erythrocytes from oxidative damage. *Plant Foods for Human Nutrition, 59*, 143–147.

Madar, Z., & Stark, A. H. (2002). New legume sources as therapeutic agents. *British Journal of Nutrition, 88*(Suppl. 3), S287–S292.

Martinez-Gonzalez, D., Bonilla-Jaime, H., Morales-Otal, A., Henriksen, S. J., Velazquez-Moctezuma, J., & Prospero-Garcia, O. (2004). Oleamide and anandamide effects on food intake and sexual behavior of rats. *Neuroscience Letters, 364*(1), 1–6.

Puri, D. (1998). Therapeutic potential of fenugreek. *Indian Journal of Physiology and Pharmacology, 42*, 423–424.

Sauvaire, Y., & Baccou, J. C. (1978). Extraction of diosgenin, (25R)-spirost-5-ene-3beta-ol; problems of the hydrolysis of the saponins. *Lloydia, 41*, 247–256.

Sauvare, Y., Pett, P., Baissao, Y., & Ribes, G. (2000). Chemistry and pharmacology of fenugreek. In G. Mazza & B. D. Oomah (Eds.), *Herbs, botanicals and teas* (pp. 107–129). PA, USA: Technomic Publishing Company Inc..

Shang, M., Cai, S., Han, J., Li, J., Zhao, Y., Zheng, J., et al. (1998). Studies on flavonoids from fenugreek (*Trigonella foenum-graecum* L.). *Zhongguo Zhongyao Zazhi, 23*(61), 4–639.

Sulieman, A. M. E., Ali, A. O., & Hemavathy, J. (2000). Lipid content and fatty acid composition of fenugreek (*Trogonella foenum-graceum* L.) seeds grown in Sudan. *International Journal of Food Science and Technology, 43*, 380–382.

Wu, T., Charles, A. L., & Huang, T. (2007). Determination of the contents of the main biochemical compounds of adlay (*Coxi lachrymal-jobi*). *Food Chemistry, 104*(4), 1509–1515.