



CHEMICAL AND PHYTOCHEMICAL ANALYSIS OF SOME ANTI DIABETIC PLANTS IN YEMEN

Al Jawfi Yaser^{1*}, Alsayadi Muneer^{2,3}, Benmansour Abdelhafid³, Chabane Sari Daoudi³, Lazoni Hammadi A³¹Department of Food safety, Station of Agriculture researches, Sana'a -Yemen²Department of Food science and Technology Ibb University, Ibb-Yemen³Laboratory of natural products- Department of biology Faculty of Sciences University Abou Bekr Belkaïd – Tlemcen, Algeria

*Corresponding Author Email: ymj2010@gmail.com

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ABSTRACT

There are many hypoglycemic plants known through the folklore in Yemen and some of Arabic Countries. The study was carried out to analyze the nutritional composition, mineral content and phytochemicals of the seeds of *Trigonella foenum* L, *Apium graveoleus* L and risen of *Commiphora myrrha* which are used traditionally in Yemen to treat diabetic patients. For different plant species the crude fat content ranged between (9.61 to 0.54 g/100 g) and crude fiber (14.77 to 1.51 g/100 g). The crude protein content was determined high in the seeds of *T. foenum* L (26.78 %), seeds *A. graveoleus* L (16.37 %) and risen *C. myrrha* (10.45 %) while the carbohydrate content was highest in the *C. myrrha* (71.21 %). The nutritive value ranged from 362.35 - 306.49 cal/100 g in the various plants. Calcium was present in the highest quantity (14000 ppm) in the *C. myrrha*. The content of total phenol varied from 1422.622 mgGAE/100 g to 505.286 mgGAE/100 g in the extracts.

Keywords: nutritional composition, diabetic, mineral content, total phenol

INTRODUCTION

The use of medicinal and aromatic plants species in Yemen goes back thousands of years, and form an important part of the culture, some of the plants traditionally still play important role in the health and body care system. Fenugreek commonly used in Yemen, it is an annual herb native to the Mediterranean region, North Africa, India, and Yemen, It is now widely cultivated in these areas. The material of commerce comes exclusively from cultivated plants mainly from Morocco, Turkey, India, and China. (Wichtl *et al*, 1994; Bruneton, 1995; Bhp, 1996; Leung *et al*, 1996, Al-Mamary *et al.*, 1997), Fenugreek is stated to possess mucilaginous demulcent, Laxative, nutritive, expectorant and orexigenic and vulnerary properties¹. In addition to its use in flavoring foods, the antifungal and antibacterial properties of fenugreek are now being applied to food preservation, some studies also show that serum cholesterol levels in diabetics and perhaps in others are reduced by fenugreek. There is some evidence that internal use of fenugreek seed can decrease some stone-forming substances in the kidney, particularly calcium oxalate. Fenugreek may encourage a flagging appetite. Cancer researchers are also studying fenugreek for its potential effectiveness as a cancer preventive. It is thought shows that fenugreek may help to prevent cancer by raising the levels of vitamin C, vitamin E, and other antioxidants in the bloodstream². Myrrh (*Commiphora myrrha*) is a close relative and member of the Burseraceae family, Myrrh is an Arabic word meaning bitter, the highly valued aromatic gum resin of myrrh has a bitter, pungent taste and a sweet, pleasing aroma. Myrrh grows to a height of about 9 ft (2.7 m). The light gray trunk is thick and the main branches are knotted with smaller branches protruding at a right angle and ending in sharp spines. The hairless, roughly toothed leaves are divided into one pair of small, oval leaflets with a larger, terminal leaflet. The yellow-red flowers grow on stalks in an elongated and branching cluster. The small brown fruit is oval; tapering to a point.³ It is native to the eastern Mediterranean, Ethiopia, the Arabian peninsula, in particularly Yemen and Somalia. This plant is used in folk

medicine as an expectorant, anti-inflammatory and antispasmodic for the treatment of oro-dental in infections, it is used also as amulet, analgesic, treat emotional and psychological.³ It is employed as a mouth wash and gargle as emmenagogue. In West Africa the gum of Myrrh resin is boiled for treatment of inflammation of the eyes by holding the face over the steaming pot; the myrrh resin has antimicrobial properties and acts to stimulate macrophage activity in the blood stream. The herb is being studied for its potential as an anticancer medication², Anesthetic, Antiemetic, Antioxidant, Fungicide⁴, antidiabetic.⁵ Celery is a biennial vegetable, although grown as an annual crop. it is a member of the Apiaceae family.⁶ Its seeds are used as an antispasmodic, also it is to treat asthma, bronchitis, disease of the liver, spleen, kidney failure, bladder and kidney calculi, edema, arthritis, dizziness, gout, weight loss, lowering blood pressure, relief of anxiety, insomnia, and reducing blood sugar. In the European tradition, the seeds have been used as carminative, stomachic, emmenagogue, diuretic, and laxative, also for glandular stimulation, rheumatic complaints, nervous unrest, loss of appetite and exhaustion.⁷⁻⁹

MATERIALS AND METHODS

Plant sources and preparation

Fenugreek seeds were collected in 2011 from region (Bit Al Ashawal –Ibb-Yemen), Myrrhresin were obtained in 2011 from (island Soqatra–Yemen) and celery seeds were collected also in 2011 from (Lahj–Yemen). Plant samples were collected and transferred in separated sterilized plastic sacks). Fresh plant material was allowed to air dry at ambient temperature (25°C) in the laboratory for approximately 15 days. The completely dried seeds and resin were crushed to powder by a hammer mill and stored at 4°C until analysis.

Chemical analysis

Determination of the lipid content

The lipid content was determined using petroleum ether in Soxhlet reflux extractor.¹⁰

Determination of crude fibre content

Defatted sample (1 g) was placed in a glass crucible and attached to the extraction unit (InKjel, D-40599, behr Labor-Technik GmbH, Dusseldorf, Germany). 150 ml boiling 1.25 % sulphuric acid solution was added. The sample was digested for 30 minutes and then the acid was drained out and the sample was washed with boiling distilled water. After this, 1.25 % sodium hydroxide solution (150 ml) was added. The sample was digested for 30 minutes, thereafter, the alkali was drained out and the sample was washed with boiling distilled water. Finally, the crucible was removed from the extraction unit and oven dried at 110°C overnight. The sample was allowed to cool in a desiccator and weighed (W1). The sample was then ashed at 550°C in a muffle furnace (MF-1-02, PCSIR Labs., Lahore, Pakistan) for 2 h, cooled in a desiccator and reweighed (W2). Extracted fibre was expressed as percentage of the original undefatted sample and calculated according to the formula¹¹

$$\text{Crude fibre (\%)} = \frac{\text{Digested sample (W1)} - \text{Ashed sample (W2)}}{\text{Weight of sample}} \times 100$$

Determination of crude protein

The crude protein was determined using micro Kjeldahl method.¹² Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H₂SO₄ was added followed by the addition of 10 g copper sulphate. The mixture was heated first gently and potassium sulphate and 1 g then strongly once the frothing had ceased. When the solution became colorless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and 100 ml of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N is receiving flask and distilled. When two-thirds of the liquid sulphuric acid was taken in; had been distilled, it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content.

Determination of moisture

Approximately 2 g of the material under test is accurately weighed (to 0.001 g) into a small dish. This is then placed in the oven for 1 hour, removed from the oven and put in the desiccator to cool. It is then weighed. The dish is replaced in the oven for 30 minutes and the process repeated to constant weight.¹³ The moisture content is found using the following formula

$$\% \text{ moisture} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Determination of total ash

For determination of ash content, 10 g of each sample was weighed in a silica crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3–5 h at 600°C. It was cooled in a desiccator and weighed to ensure completion of ash. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or greyish white). Weight of ash gave the ash content.¹²

Determination of minerals

Weigh 2.0000 g of the dried and milled (to 1 mm) sample into a silica crucible and place in a cold muffle furnace with the chimney vent open, and allow to heat up to 450°C. Close the vent and maintain at this temperature overnight. Remove from the furnace and allow to cool, then add 15 drops HCl from a polythene Pasteur pipette, being careful to moisten the entire sample. Using a fume cupboard, gently evaporate off all the HCl on a hotplate at moderate heat, then remove and cool. Dissolve the residue in 0.1 M HCl, and transfer quantitatively to a 10-ml volumetric flask. Make up trace element standards in 0.1 M HCl covering the expected ranges in the sample solutions and analyses by (spectrophotomètre d'absorption atomique. AI 1200. Aurora Canada) according to the instrument manufacturer's instructions Calculation. The sample solution is of 2 g in 10 ml, therefore the concentrations in µg ml⁻¹ of the trace element should be multiplied by 5 to give the concentration in µg g⁻¹ of the trace element in the dried sample.¹⁴

Determination of total carbohydrate

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein).¹⁵

Determination of alkaloids

alkaloids contents of the plants were determined using the method that described by Harborne (1998)¹⁶ by soxhlet, ten gramme of the powdered sample was extracted with 250 mL of ethanol period five hours, extracted of ethanol was evaporated to dryness with a rotary evaporator, under reduced pressure at 40°C. dry residue repeat by 150 mL of chloroform and acidify by HCl 5 % pH 3, it let pillow during 30 minutes in the room temperature, the phase acid aqueous were extracted by 150 ml of chloroform, basify by the NaHCO₃ 5 % pH 9 and lit it during 15 minutes in the room temperature. The chloroform phase was evaporated to dryness with a rotary evaporator under reduced pressure. The dry residue is the total alkaloids.

Determination of Tannin

Five grams of each part (seeds resin) was milled into powder. The powder was extracted with 100 ml acetone–water (70/30, V/V), and the mixture was stirred continuously for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40°C to remove acetone. The washed with 30 ml dichloromethane to remove lipid soluble remaining solution was substances. After that, the solution was further extracted with ethyl acetate at a ratio of 30/30 (V/V). The water layer was separated and extracted twice more similarly. Then the resulting water layer was evaporated to dryness, and the resulting substance was weighed.¹⁷

Determination of total phenols

The powdered plant material (2 g) was extracted with methanol, at room temperature overnight. The methanol extracts were combined and concentrated under reduced pressure on a rotary evaporator. Total phenolic content of each plants extract was determined with the Folin–Ciocalteu's reagent (FCR) according to the published method.¹⁸ Each sample (0.5 ml) was mixed with 2.5 ml FCR (diluted 1:10, v/v) followed by 2 ml of Na₂CO₃ (7.5 %, v/v) solution. The absorbance was then measured at 765 nm after incubation at 30°C for 90 minutes. Results were expressed as Gallic acid equivalent (mg Gallic acid /g dried extract).

Determination of flavonoid

The total flavonoid content of plants extracts was determined by a colorimetric method as described in the literature¹⁹. Each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO₂ solution (15 %). After 6 minutes, 0.15 ml of aluminum chloride (AlCl₃) solution (10 %) was added and allowed to stand for 6 minutes, then 2 ml of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was then determined at 510 nm versus prepared water blank.

Results were expressed as Catechin equivalent (mg Catechin/100 g dried extract).

RESULT

Results show that Fenugreek seeds contain a highest percentages of proteins and calories and lowest percentage of moisture and ash, while the highest content of carbohydrate and moisture appears in myrrh, and it contains the lowest percent of Protein, Crude fat and Crude Fiber. Whereas Celery was superior in its content of ash, fats and fibers, Table 1.

Table 1: Nutritive Values of Experiment's Plants Parts

Plant Sample	Moisture %	Total Ash %	Protein % N × 6.25	Lipid (%)	Crude Fiber %	Carbohydrate %	Nutritive value (Cal/100 g)
Celery	7.60	13.2	16.37	9.61	14.77	38.63	306.49
Myrrh	10.09	6.20	10.45	0.54	1.51	71.21	331.5
Fenugreek	6.57	4.03	26.78	6.35	6.75	49.52	362.35

The concentration of Ca, Na, Zn, Ag, Cu, Fe and Pb in Celery, Myrrh and Fenugreek samples is given in Table 2. The highest concentrations of Zn, Ag, Cu, Fe and Pb found in Celery, while that of Ca and Na were in Myrrh and Fenugreek respectively. And the lowest concentrations of Na,

Zn, Ag and Fe were in Myrrh, and they were of Ca, Ag, Cu, and Pb in Fenugreek. In each plant samples Ca present in great amount followed by Na, Fe, Zn, Cu, and Pb respectively. While Ag present in slightest amount in Celery and weren't found in both of Myrrh and Fenugreek.

Table 2: Mineral Composition of Plants Samples

Plant Sample	Ca (ppm)	Na (ppm)	Zn (ppm)	Ag (ppm)	Cu (ppm)	Fe (ppm)	Pb (ppm)
Celery	6425	1425	45.5	0.025	18.75	162.25	6.25
Myrrh	14000	900	6.5	0.00	16	432.5	5.25
Fenugreek	3225	1500	25.5	0.00	15	101.25	3.5

Table 3 Expose the photochemical structure of experiment's plant samples (Mg/ 100 g). Tannin was quite low in myrrh it was higher in celery seeds, while total flavonoids were higher in myrrh and celery. Alkaloids were least amount in all

plants. Fenugreek possesses the upper value of total phenols followed by Myrrh and Celery respectively.

Table 3: Photochemical Content of Experiment's Plants

Plant sample	Total phenol mg/100 g	Alkaloids g/100 g	Tannin g/100 g	Flavonoids g/100 g
Celery	720.879	0.1645	5.216	9.15
Fenugreek	505.286	0.7555	2.032	4.99
Myrrh	1422.622	0.1645	0.84	9.4

DISCUSSION

The aqueous extracts of seeds and leaves of fenugreek have been shown to possess hypoglycemic activity and are nontoxic.²⁰ Fenugreek seed contains 45-60 % carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30 % proteins high in lysine and tryptophan; 5-10 % fixed oils (lipids); pyridine-type alkaloids, mainly trigonelline (0.2-0.36 %), choline (0.5 %), gentianine and carpaine; the flavonoids apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; free amino acids, such as 4- hydroxyisoleucine (0.09 %); arginine, histidine and lysine; calcium and iron; saponins (0.6-1.7 %); glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin, tigogenin, neotigogenin); cholesterol and sitosterol; vitamins A, B1,C and nicotinic acid; coumarin compounds and 0.015 % volatile oils (nalkanes and sesquiterpenes).²¹ Our results showed that the concentration of proteins, lipids and carbohydrates of fenugreek were in the same ranges of that of the previous study. But protein contents in this study Higher than the results of Randhir.²² The lipid content of experimental plant samples was low relatively, especially in Myrrh which is in

the recommended value by the British Pharmacopoeia.²³ The presence of Ash in these plant in such quantities are satisfying, because of the high importance of mineral for health maintenance and development. Where the minerals are essential for human body, they are basic content of many of body tissues, such as calcium and phosphor for bone and iron for blood and muscles. They consider basic element of biomolecules (proteins, enzymes, phospholipids) in addition to their roles in connectivity process, and in all of biochemical reactions.²⁴ The content of fiber and ash in the plants of our experiment was adequate particularly in Celery. These dietary fiber in food have been shown to be useful in reducing blood glucose levels in diabetes, in reducing blood cholesterol levels, for treatment of cardiovascular disease and also in preventing bowel cancer.^{25,26} Some literatures suggested that abnormal zinc metabolism play a role in the pathogenesis diabetes and/or its complications.²⁷ The complexes of zinc and insulin in varying ratios are stored in pancreatic β-cells and released into the circulation via the portal vein.²⁸ Enzymes that do not contain a trace element as an integral part but are activated by metals such as Cu, Fe,

and Ni respond to *in vitro* addition of several transition elements with a dose-dependent activation.²⁹ Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting, and the regulation of cell permeability. It also plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system. Inorganic elements like Zn, Cr, V, Fe, Cu, and Ni play basic role in the improvement of impaired glucose tolerance and their indirect role in management of diabetes mellitus are being increasingly recognized.³⁰ The results of minerals in our study for fenugreek were approximately similar with Choudhury³¹ with exception of Ca and Fe where it was the double in our results for Ca and the infers for Fe. And Ca content in fenugreek in turkey was higher in comparing with the same plant in Yemen, while the other minerals contents were similar found lower concentration of Zn and Cu in Myrrh.^{32,33} That can be referred to the difference of soil and water structures, the climate and weather conditions. Phenolic in plants are usually found in conjugated forms through hydroxyl groups with sugar as glycosides.³⁴ The phenolic compounds may contribute directly to the antioxidant action³⁵. It has been suggested that phenolic are secondary metabolites, and in part, are produced as a result of the plant's interaction with the environment.³⁶ In addition, a wealth of other classes of compounds, such as polyphenols including Flavonols glycosides are also suggested to contribute to the health promoting properties of these species.³⁷ Studies show that fenugreek seeds have antioxidant Properties.³⁸ Some studies have suggested a possible protective effect of flavonoids against vascular diseases.³⁹ The presence of tannins may be responsible for ability to cure diseases such as diabetes.⁴⁰ The alkaloids may be responsible for the anticancer, anti diabetics, anti-aging and antiviral activities of this herbal plant.⁴¹ The tannins have been reported to inhibit digestive enzymes, affect the utilization of vitamins and minerals and are capable of binding and precipitating protein causing a reduction in nutritional value.⁴² They have therefore been regarded as antinutrients and considered nutritionally undesirable,⁴² however, these compounds are also believed to have some favorable effects on human health, such effects as the lowering of human low-density lipoprotein, reduction of heart diseases and cancer.⁴² Total phenolic was 171, 3 of the fenugreek extract, this result agreed with the results of previous study.^{36,43} While the result of Flavonoid content in fenugreek in this study is agreed with the earlier study by Gupta and⁴⁴ which had shown that the fenugreek seeds are rich in flavonoids (>100 mg/g). and it was higher comparing with that of Bukhari.³⁶ Celery leaves have total phenolic range between 5100-1637.1 mg/100g,⁴⁵ 233.1 in roots,⁴⁶ while total phenolic content was 2486 mg/100 g in seeds.⁴⁷ In this study total phenolic was 720.879 mg/100 g.

CONCLUSION

The plants of this experiment contain elevated concentrations of Fe, Cu and Zn, that essential element of importance in diabetes. The components of Celery, Myrrh and fenugreek, such as phenolic compounds, especially phenolic acid and flavonoid derivatives, carotenoids, tocopherol and vitamin C, minerals, fibers possess Antioxidant and Antidiabetic activities.

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