**Nutrient and Phytochemical of Fenugreek *(Trigonella Foenum graecum*) seeds**

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**Abstract:**

The aqueous extract and alcoholic extract were used for analyzing the main phytochemical , nutrient and active groups composition of fenugreek seeds powder (*Trigonella foenum graecum*).The preliminary tests of active groups in extracts were carried out. It appeared to contain (alkaloids , flavonoids , steroids , carbohydrates, trepenes , tannins , saponins , glycosides , free amino acid , crude protein and phenolic compounds ) . The extracts were different in their content of active groups quantitatively and qualitatively . The amount of moisture and crude fiber , total ash , total oil , concentration of crude protein , carbohydrate , nitrogen content and caloric value on a dry weight basis , were found to be (6.833±0.531) humidity ,solid material (93.166±0.531) , crude fiber (17.0±0.2) , the percentage of total ash were (3.566±0.478) , the percentage of total oil (7.15±0.25) ,concentration of crude protein (28.45±0.15) , while the concentration of carbohydrates (1340±0.029 mg/100g) , and the caloric value for fenugreek seeds powder is (5544.9) kcal/100g. The quantitative content of (alkaloids , flavonoids , steroids , tannins , free amino acid , saponins ) were estimated. The results indicated that the percentage alkaloids (1.8±0.1%) , percentage of flavonoids was (12.135±0.465%) , while the concentration of steroids (214±0.024 mg/100g ) , tannin concentration (63.69±1.67 mg/100g) , and amino acid concentration (70±0.064 mg/100g) , and percentage of saponine was (25.65±0.69%) . The present study showed that fenugreek seeds is a very rich energy and antioxidant .So that is very important to be entered the system of human nutrition , are economic nutritional source can be used as human food supplement, which contains important amounts of carbohydrates, protein, fat and amino acids.

**Key word: fenugreek seeds , Active compounds , Nutritive**.

**1-Introduction:**

 Fenugreek (*Trigonella foenum-gracum*) is an annual leguminous bean , and belongs to *Fabaceae* family, Its seeds and green leaves used as food posses medicinal applications, and is an old practice of human history [1,2,3] , it has been used for diverse medicinal benefits that include wound healing , aid in digestion , treatment of sinus and lung congestion, inflammation and infection , mitigation, hair treatment, breast enhancement and aphrodisiac effects [4]. In India, it is extensively used as Ayurvedic medicine and in China as traditional medicine [5]. Interestingly, in herbal medicine, it is used in the treatment of diabetes [6]. Fenugreek is consumed in various parts of the world in different forms (Figure1) and has been regarded as a treatment for many ailments known to man [7]. Recent advances in nutraceutical and phytochemical research stimulated a renewed interest in fenugreek to be used as a functional food . The research has led to identification of specific health benefits of this novel crop through extensive research and clinical trials [8]. Latest research reports indicate fenugreek to posse’s immunomodulatory, anti-carcinogenic, anthelmintic, anti-nociceptive, antioxidant, anti-microbial, anti-ulcer, and hepatoprotective, anti-obesity, anti-hyperglycemic, anti-diabetic and hypocholesterolemic effects [4]. It has been shown to normalizes the blood circulation , thereby making body active and energetic [9]. Medicinally ,the fenugreek seeds are the most important and useful part of fenugreek plant. These seeds are golden-yellow in colour , small in size , hard and have four-faced stone like structure. The biological and pharmacological actions of fenugreek seeds are mostly attributed to the variety of its bioactive chemical constituents that serve as raw materials for the manufacture of various hormonal and therapeutic drugs [10,11].



**Figure 1 various forms of fenugreek**

**2-Experimental**

**2-1-Materials and Methods:**

 The purest chemical materials are used to analyzing the main phytochemical , nutrient and estimate some of the active groups composition of fenugreek seeds study.

**2-2-Gathering the Plants:**

The seeds fenugreek plant are gathered from local marked in Samarra . The seeds fenugreek are ground by a special grinder and conserved in the sealed container at the room temperature before use.

**2-3-preliminary phytochemical screening :**

A chemical tests are carried out on the powdered of seeds fenugreek to their aqueous extract and alcoholic extract are subjected to the tests to identify their chemical constituents alkaloids , saponins [12,13] , flavonoids [14, 15] and, carbohydrates , glycosides [16] , steroids, terpenes [17] , free amino acid , crude protein, tannins and phenolic compounds [12] by using standard procedures topreliminary phytochemical screening , as preparation of extracts by weighting ten grams of fenugreek seeds powder are macerated in 300 cm3 of Boiled and cooled distilled water and ten grams from seed powder to plant are macerated in 100 cm3 of ethanol 97% at room temperature , then extracts are collected after 24 hr. [16] , Special statements were then made on prepared extracts.

**2-4- Proximate Nutritive Values of fenugreek seeds:**

The moisture content was determined by measuring the mass offenugreek seeds before and after the water is removed by heating in an oven, the total solids content is a measure of the amount of material remaining after all the water has been evaporated , crude fiber was estimated by acid-base digestion known as Coarse fiber , dry ash determination according to the standard procedure[18]. The crude fat content was extracted by soxhlet determined according to the procedure[19] , and total protein in the sample was determined by kjeldahl method used stem distillation , in which the ammonia released from digested protein was titrated against standard HCl[20], while Carbohydrates was determined by phenol sulphuric acid method , this forms a yellow brown coloured product with phenol and has absorption maximum at 488nm .The sulphuric acid causes all non reducing sugar to be converted to reducing sugar so that this method determines the total sugar present in foods. The method detects all classes of carbohydrates, including mono, di, oligo and polysaccharides. Although the method detects almost all carbohydrates, the absorptivity of the different carbohydrates varies [21]. This method is non stoichiometric and so it is necessary to prepare a calibration curve using a series of standards of known concentration of carbohydrate as shown in Fig.2.

The total amount of carbohydrate was determined according the following standard glucose calibration curve:

**Fig 2Standard Curve of Carbohydrates**

**2-5-Evaluation of Energy Value:**

 Energy value was finally determined by the following equation:

Energy value (Kcal/100g) = ( 4 X % Protein) + (9 X% Fat) + (4 X % Carbohydrate) [22).

**2-6- Quantitative Estimation of some Secondary Metabolites by Colorimetric Methods:**

 Estimation percentage of alkaloids was by used Harborne method [23] , estimation percentage of content flavonoids according to procedures [24], estimation percentageof saponine was by used procedures Obadoin and Ochuko [25] .While estimation of steroids in fenugreek seeds was carried out by using colorimetric method by Liberman–Burchard reagent. In this reaction the acetic anhydride in the Liberman –Burchard reagent is reacted with the steroids in the sample, which gives a green colour their absorbance were determined on spectrophotometer at 640 nm. [26] , The total content of steroids was calculated from standard graph of cholesterol as shown in Fig.3**.**

The total content of steroids was calculated from the following standard graph of cholesterol :

**Fig. 3 Standard Curve of cholesterol**

 Estimation of concentration tannin was by used Boham and Kocipai [27] preparation of extract , Five grams of powdered seeds fenugreek was weighed into 100ml conical flask , 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker , the solution was filtered.

**Table 1** **assay estimation of concentration tannin**

|  |  |  |  |
| --- | --- | --- | --- |
| Reagents | Sample | Stand. | Blank |
| 1-Filtrate | 5ml | - | - |
| 2-Standard | - | 5ml | - |
| 3-DW | - | - | 5ml |
| 4-Tannin reagent | 3ml | 3ml | 3ml |

 Mixed and let to stand for 10 minutes and the absorbance were read for sample and stand. against blank at 620 nm , and estimation of amino acid in fenugreek seeds was carried out by using colorimetric method by nenhydrin reagent is reacted with the amino acid in the sample, which gives a violet colour their absorbance were determined on spectrophotometer at 570 nm. [16]. The total content of amino acid was calculated from standard graph of argnin acid as shown in Fig.4.

The total content of amino acid was calculated from the following standard graph of Argnin acid:

**Fig. 4 Standard Curve of Argnin acid**

**3-Results and Discussion**

**3-1-Preliminary Phytochemical Screening**

 The concentration of phytochemical compounds in the tow solvent extracts the aqueous and alcoholic were significantly different from each other. The aqueous extract was found to contain a rich amounts of alkaloids , flavonoids , carbohydrates, phenolic compounds and tannins, terpenoids, saponins, amino acids, protein , with a less amount of glycosides , steroids , and absence of Oil , fats table2. This result aqueous extract to fenugreek seeds was in agreement except tannins compounds were absent from the findings from aqueous extract to fenugreek seeds of Kumari [28] , and this result identified were absent alkaloids , steroids , phenolic compounds and glycosides , While corresponding to the presence of flavonoids , tannins , saponins and Terpenoids with the findings from aqueous extract to fenugreek seeds of Chalghoume [29]. In addition to the presence of alkaloids , flavonoids , phenolic compound and tannins , carbohydrates , amino acids, protein , with absent of glycosides in the water extract to fenugreek seeds of Yadav and Chowdhury [30]. The ethanol extract of fenugreek seeds were also rich in flavonoids , carbohydrates , steroids , glycosides , amino acid , terpenoids and less amount of alkaloids , phenolic compound and tannins , protein and saponins. The results were consistent with the presence of these compounds in the seeds of fenugreek in the results of phytochemical analysis Kumari [28]. It was also not compatible with the absence of some compounds that are flavonoids , phenolic compound and tannins, terpenoids , glycosides with the results ethanol extract to fenugreek seeds of Nandagopal [31] , and this result identified were absent alkaloids , flavonoids , steroids , While corresponding to the presence of tannins , saponins and Terpenoids with the findings from ethanol extract to fenugreek seeds of Chalghoume [29 ]. The solvents relatively showed the same ability for extraction of phytochemical compounds but at different rates.

**Table 2 Phytochemical Screening for aqueous and alcoholic Extracts of fenugreek seeds**

|  |  |
| --- | --- |
| **Phytochemical tests** | **Extracts** |
|  | **Aqueous e** | **Ethanol** |
| **Alkaloids** | **Mayer** | +++ | + |
| **Dragendoff** | +++ | + |
| **Flavonoids** | **Lead acetate**  | \_ | \_ |
| **Ferric chloride** | +++ Deposit | +++ Deposit |
| **Carbohydrate** | **Molish** | +++ | +++ |
| **Bendict** | - | + |
| **Glycosides** | **Killer-Llni** | ++ | ++++ |
| **steroids** | **Salkowskis** | \_ | +++ |
| **Libermann-Burchard** | +++ | +++ |
| **Protein** **and Amino** **acids** | **Ninhydrin** | ++++ | +++ |
| **Biuret** | \_ | \_ |
| **phenolic compounds and tannins** | **Ferric chloride** | \_ | \_ |
| **Gelatin** | +++ | + |
| **Lead acetate** | ++++ Deposit | + |
| **Terpenoids** | **Trim-Hill** | \_ | \_ |
| **Libermann-Burchard** | +++ | +++ |
|  **Saponins** | **Foam test** | +++ |  \_ |
| **Mercuric chloride** | +++ | + |

 ++++ Very large quantity +++ large amount + small amount - absent

**3-2-Proximate Nutritive Values of Fenugreek seeds:**

 In this study,Fenugreek seeds were analyzed for their constituents of moisture, total solids, fiber, ash, crude fat, total protein and carbohydrates, as shown in table3.

**Table3 Proximate Nutritive Values of Fenugreek seeds**

|  |  |
| --- | --- |
| **Parameter** | **Mean ± S. D.****(% or g/100g dry weight)** |
| Moisture\*\* % | 6.833 ± 0.531 |
| Total solids\*\* % | 93.166 ± 0.531 |
| Crude fiber\* % | 17.0 ±0.2 |
| Ash\*\* % | 3.566 ± 0.478 |
| Crude fat\* % | 7.15 ±0.25 |
| Crude protein\*% | 28.45±0.15 |
| Crude carbohydrates\*\* | 1340±0.029 |
| Nutritive value (Kcal/100g) | 5544.9 |

 - Values are means of two or \*\*three replicates : \*duplicate

 Which indicates that moisture content of Fenugreek seeds was 6.833± 0.531% , which was less than those results obtained by Buba , Burham and Agrawal [32,33,34] , with to higher than Dilshad [35] through the proximate analyses results as obtained from Fenugreek seeds collected from four different origins or countries , while the total solids was 93.166 ± 0.531% and this value is higher than Dry matter 89.77 % by Burham. Values 17.0 ±0.2% and 3.566 ± 0.478% were estimated for crude fiber and ash contents of Fenugreek seeds respectively. These values are higher than the value of fiber 6.28% and higher than the value of ash 0.28% obtained by Burham , and values of fiber 7.06% obtained by Sharara [36].The ash content was also similar to results than the value of ash 2.99% , 3.0 % obtained from previous studies respectively Buba and Agrawal. Crude fat content of fenugreek seeds was 7.15 ±0.25, this value was compares favorably than values 7.13% of Burham, and the value 7.0% , 6.33% of Agrawal and Buba. Crude protein content value was 28.45±0.15%, which was comparable to those reported by Agrawal , who found that the crude protein value 23.30 %. However, the values of protein obtained from the present study were similar to those reported 29% by Burham , and higher than the value of protein 2.74% obtained by Buba. The value obtained for carbohydrate content was 1340±0.029 g/100g of dry weight. This value was 1ower than values obtained for fenugreek seeds Burham, Buba and Agrawal 46.38%, 77.04% and 55.49% respectively. The energy value was 5544.9 Kcal/100g. This value defined the fenugreek seeds as rich source of energy.

 **3-3-Quantitative Estimation of some Phytochemical Compounds in fenugreek seeds:**

 The quantitative contents of alkaloids , flavonoid , saponin , tannin , steroids and amino acid of fenugreek seeds , which indicated concentration of alkaloids in fenugreek seeds 1.8±0.1 % , flavonoid 12.135±0.465% and saponin 25.65±0.65% are shown in Fig.5 , which alkaloid lower than that recorded for Sharara [36] in raw fenugreek seeds 2.42% , and in support with the study conducted by Al-Maamari [37] , who reported the presence of flavonoid and saponin in the mean vlues in various accessions of Omami fenugreek seeds ranged for flavonoids 8.46 to 32.81 mg/100g , for saponins from 7.27 to 17.03 mg/100g.

**Fig.5: Quantitative Phytochemical Composition** **(alkaloids , flavonoids , saponin) of fenugreek seeds**.

While the tannin 63.69±1.67 mg/100g , steroids was 214±0.024 mg/100g and amino acid 70±0.064 mg/100g of fenugreek seeds , are shown in Fig. 6.

**Fig.6: Quantitative Phytochemical Composition** **(tannin, amino acid , steroids ) of fenugreek seeds**.

 which was comparable to those reported by Al-Maamari, who found that the tannins ranged from 30.21 to 74.54 mg/100g. That content amino acid 70±0.064 mg/100g of fenugreek seeds , which was less than those results obtained by Dilshad of Fenugreek herb in different environmental conditions .These variations in the phytochemical concentrations may be due to cultivars and the differences in soil composition and method of analysis.

**Conclusions**:

 From these results it may be concluded that fenugreek seeds are economic nutritional source can be used as human food supplement, which contains important amounts of carbohydrates , protein , fat , and amino acids. The phytochemical constituents of fenugreek seeds are: phytosterols , flavonoids , alkaloids , amino acids , protein, carbohydrates , glycosides , phenolic compounds , tannins , terpenoids , saponins , oil and fats.

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