ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 6 No. 2 June 2020*

Assessment of Antioxidant Activity and Mineral Elements Composition of Fenugreek Seed Extract

Ibrahim A. M[1*], Anwar A.Y[1], Sani M.A[2],Ya'u S.A[3] Tasi'u A.M[1], Sani M.Y[3], Abdulmumin Y[1], Murtala M[1], Musa H[3]., Sadiya A.B[1], Abdullahi N[1]., Maimuna D.M[1], Salisu A.A[4], Tasi'u M[5].

[1]Department of Biochemistry,
Kano University of Science and Technology
Wudil, Kano Nigeria

[2]Department of Clinical Research,
School of Allied Health Sciences,
Sharda University,
Greater Noida India.

[3]Department of Biology,
Kano University of Science and Technology
Wudil.Kano Nigeria

[4]Department of Food Science and Technology,
Kano University of Science and Technology
Wudil, Kano Nigeria

[5]Department of Microbiology,
Kano University of Science and Technology
Wudil, Kano Nigeria

Email: ibrahiem4real@gmail.com

Abstract

The use of fenugreek as traditional medicine has been a practice with long history. It has been used for quite a number of indications such as diabetes, hypertension, anemia, labor induction and improvement of general body metabolism among others. However, the underlying scientific bases for its health benefits are limited and need further exploration. In this study, atomic absorption spectroscopy was used to analyze mineral elements content of fenugreek seed while2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine antioxidant activity of fenugreek seed extract. The amounts of mineral elements found are: copper (0.734±0.015 mg/100 ml), zinc (0.961±0.136 mg/100 ml), manganese (0.503±0.035 m g/100 ml) and calcium (0.403±0.056 mg/100 ml).Antioxidant activity expressed as percentage DPPH inhibition at different extract concentrations are: 31.33 % (20 mg/ml), 61.03 % (40 mg/ml), 62.13 (60 mg/ml), 39.51 % (80 mg/ml) and 60.00 % (100 mg/ml). Zinc appears to be relatively higher (0.961±0.136 mg/100 ml) out of

**Author for Correspondence*

the mineral element determined while the highest antioxidant activity of 62.13 % was achieved at extract concentration of 60 mg/ml. This study showed that fenugreek seed could be harness as an important source of dietary micronutrients and antioxidants with tremendous health promoting effect.

Keywords: Antioxidants, Fenugreek, free radicals, medicine, mineral elements

INTRODUCTION

Consumption of functional foods has been recently increasing due to consumers' enlightenment about the significant impact of diet on health and diseases (Pająk *et al.,* 2018). Seeds and sprouts of different plants are used as vital ingredients for functional foods owing to their numerous health benefits such as prevention of cardiovascular diseases, reduction of cholesterol and triglycerides level, bowel regulation, prevention of type 1 and type 2 diabetes and anti-obesity activity (Randhir *et al*., 2004; Kaviarasan *et al.,* 2007; Marineli *et al.,* 2014; Paiva *et al*. 2016). Several health benefits of plants seeds and sprouts is the result of their bioactive compounds, vitamins and mineral element contents(Zieliński *et al.,* 2005) Mineral elements are naturally occurring inorganic chemical substances distributed throughout human body and are critical for the sustenance of physicochemical processes needed for the perpetuation of life (Soetan *et al*., 2010; Al-fartusie and Mohssan 2017). They account for about 5-6 % of the total body weight (Rastmanesh, 2017). Albeit they do not produce energy, mineral elements are required by virtually all living organisms and serve various functions in human being.

Mineral elements such as calcium, sodium, phosphorous and chloride are required by the body in an amount greater than 100 mg/dl and are termed as "major or macro elements". While micro-elements are required in smaller amount; usually less than 100 mg/dl and includes copper, iron, iodine, zinc, manganese, fluoride, magnesium, chromium, selenium, cobalt and sulfur (Rastmanesh, 2017). Some elements are neither macro nor micro but play an important roles in animals, these include silicon, boron, nickel and arsenic while lithium, cadmium, lead, tin and vanadium have no clear role in living organisms (Soetan *et al*., 2010).
Functions of mineral elements in living organisms including human, animals and plants have been well recognized and documented (Underwood, 1971). To mention but a few, their functions include facilitation of metabolic reactions by serving as cofactors of some enzymes, stabilization of some vital biological molecules such as heme in human and chlorophyll in plants, mediation of biological redox reactions that generate and utilizes energy, binding, transportation and release of oxygen during respiration, maintenance of osmotic balance and free radical scavenging activity (Neilson, 1989; Rastmanesh, 2017).

Proper balance of mineral element is required for normal physiologic functions as low or high level of these elements can lead to several diseases including cancer (Al-fartusie and Mohssan 2017). The deficiency of these elements has been one of the major public health concern in the developing countries espcially among pregnant women and infants (Batra and Seth 2002). Several literatures have reported the relationship between plant's seeds intake and decreased risk of development of various ailments bedevling mankind owing to the various bioactive substances content including vitamins, minerals and antioxidants among others.

Antioxidants are substances that slow, hinder or eliminate oxidation (Salehi *et al*. 2018; Muhammad *et al*., 2019). Oxidation is a chemical reaction that generate highly reactive species called free radicals which could damage cells by a cascade of reactions that oxidizes target molecules mostly lipids, proteins or DNA(Salehi *et al*., 2018; Muhammad *et al*., 2019;). The balance of oxidative state in both plants and animals is maintained by complex system of antioxidants produced within the cells such as catalase, superoxide dismutase, peroxidase, glutathione peroxidase (enzymatic antioxidants) or antioxidants taken from the diet such as carotenes, lycopene, zeaxanthin, astaxanthin, lutein and vitamin C (non-enzymatic antioxidants)(Sajilata *et al*., 2008; Gong and Bassi 2016; Galarza *et al*., 2018; Salehi *et al*., 2018).Dietary antioxidants are obtained mostly from fruits and vegetables and their consumption is associated with maintaenance of balance between free radicals and antioxidant status; thus aiding in the decrease in oxidative stress and diminished risk of cancer, aging and cardiovascular diseases (Kumar *et al*.,2016).

Fenugreek (*Trigonellafoenum-graecum*) otherwise known as "Hulba" in Arabic or most commonly called "Methi" in traditional system of medicine is a medicinal plant and one of the popularspices in human food(Dixit *et al*., 2005; Khan *et al*., 2015; Abeysekera; 2018). Its seeds and green leaves are used as food as well as in medicinal application in the middle east and south-east Asia for over decades (Moradi *et al*., 2013; Khan *et al*., 2015). It provides natural food fiber and other nutrients required in human body (Thomas *et al*., 2011). Fenugreek has strong spicy and seasoning type sweet flavor (Blank, 1996). Aromatic and flavorful fenugreek is a popular spice and is widely used for well recognized culinary and medicinal properties (Sowmya and Rajyalakshmi 1999).

Although chemical and nutrients composition of many medicinal plant's seeds, leaves, stems roots and stem bark have been studied, literature on fenugreek seed composition particularly with respect to mineral elements and antioxidants are limited; thus we aimed at determination of mineral elements specifically copper, zinc, manganese and calcium as well as antioxidant activity of ethanol extract of fenugreek seeds.

MATERIALS AND METHODS

Collection and processing of sample

Seeds of fenugreek were purchased from Wudil market, Wudil local government area Kano state Nigeria. Fenugreek seeds were grounded into powdered form, mineral elements content of fenugreek seed powder namely; copper (Cu), zinc (Zn), manganese (Mn) and calcium (Ca) were determined according to the method of AOAC, 2000. The method is based on the fact that organic components of a biological material get incinerated completely when subjected to high temperatures (600°C for 5hours) leaving behind the inorganic component that could be used to identify and quantify mineral elements. About 2 g of the powdered sample was placed in a pre-weighted crucible. Along with the contents, the crucible was then placed in a muffle furnace and heated up to 600°C for 5 hours until a white ash was obtained and allowed to cool to room temperature using desiccator. Digestion of the ash was done by adding 5ml of 10% hydrochloric acid (HCL). The volume of the resulting mixture was made up to 50 ml with distilled water. The resulting sample was then subjected to atomic absorption spectroscopy using atomic absorption spectrophotometer (SchimadzuAAS6300-model) to determine actual concentration of the minerals. All reagents

used were of analytical grade and all the equipment (glassware) used were cleaned by soaking overnight in 10 % nitric acid and thereafter rinsed three times with deionized water.

Determination of Mineral Elements

Atomic Absorption Spectrophotometry (AAS)

This is an important technique used to measure the amount of chemical element in the sample by measuring the amount of light absorbed by a specific element.Atoms from the lower energy state could absorb energy in the form of ultraviolet or visible (radiation source) light and get excited to the higher energy level. The wavelength of transmitted light is measured by the detector which is then compared with the wavelength of light passing through the sample (Figure 1). The change in the wavelength absorbed is integrated by processor and displayed as peak of energy absorption at discrete wavelength(Farrukh, 2012)

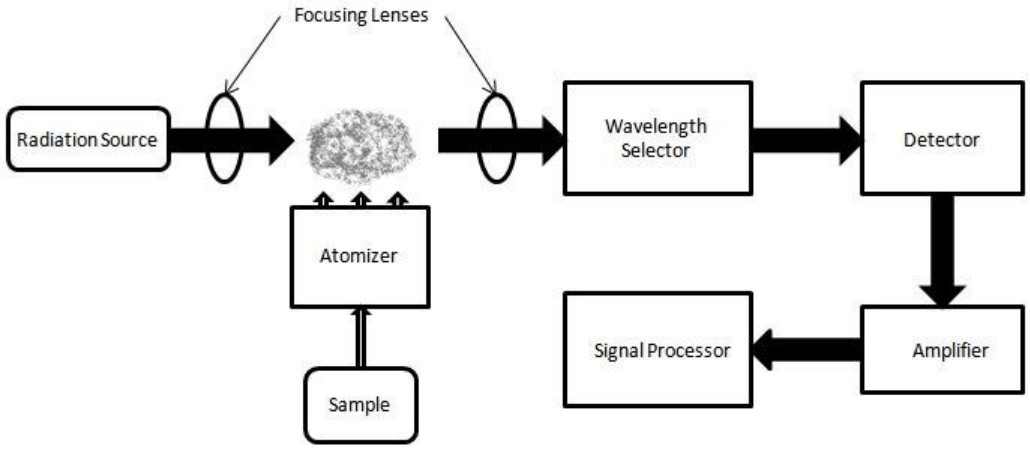

Figure 1: Atomic Absorption Spectrophotometer
(https://www.wikiwand.com/en/Atomic_absorption_spectroscopy)

Procedure for AAS

The standard solutions of Cu,Zn Mn and Ca were prepared according to standard protocol. The AAS machine (Schimazdu AA-6300 model) was set up in accordance with the manufacturer's instructions for each element to be analyzed. The standards, blank and samples were aspirated into the flame. TheElemental ions were then atomized and the atoms then absorbed radiation of a characteristic wavelength from a hollow-cathode(Ooi *et al.*, 2012). The absorbance measured is proportional to the amount of analyte in the sample solution.

Preparation of Ethanol Extract of Fenugreek Seed

For antioxidant activity determination, 20g of the sample was dissolved in 100 ml absolute methanol and soaked overnight. The resulting solution was filtered and transferred to clean vessel and subsequently evaporated to dryness in order to obtain the extract**.**

DPPH Radical Scavenging Activity:

Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was measured using the method of Blois (1958) with some modifications. The reaction mixture up to 3 ml containing 0.2 ml of DPPH and 2.8 ml of test solution at various concentrations i.e.(20, 40, 60, 80,100mg/ml) of the extract fractions was incubated at 37°C for 30minutes. The absorbance

of the resulting solution was measured at 517nm using spectrophotometer (Beckman model DU-40) The percentage inhibition of DPPH radical scavenging activity was calculated by comparing the results of the test with those of the control (not treated with extract) using the following equation:

DPPH scavenging activity = $(A_c - A_s)/ A_c \times 100$

Where A_c= Absorbance of control, A_s= Absorbance of sample (Molyneux 2004)

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA). The difference of mean value of the micronutrients (mineral elements) and percentage DPPH inhibition were determined at $P < 0.05$ level of significance.

RESULTS

Mineral elements

The result of four mineral elements in fenugreek seeds is presented in table 1 following ashing and atomic absorption spectroscopy.

Table1: Mineral elements found in fenugreek seed

Minerals	Concentration(mg/100ml)
1. Copper	0.734±0.015
2. Zinc	0.961±0.136
3. Manganese	0.503±0.035
4. Calcium	0.403±0.056

Results are expressed as mean ± SD of four mineral elements found in fenugreek seed.

Antioxidant activity assay

Diphenylpicrylhydrazyl radical (DPPH•) scavenging assay was carried out with different extracts concentration. In this method, a commercially available, stable free radical, DPPH•, soluble in methanol, was used. In its radical form, DPPH• has an absorption maximum at 515 nm, which disappears on reduction by an antioxidant compound(Dixit *et al.* 2005). The antioxidant activity of fenugreek seed methanol extract expressed as percentage DPPH inhibition is presented in table 2.

Table 2: Result for antioxidant activity of fenugreek seed at different concentrations.

Concentration (mg/ml)	Percentage inhibition (%)
20	31.33
40	61.03
60	62.13
80	39.51
100	60.00

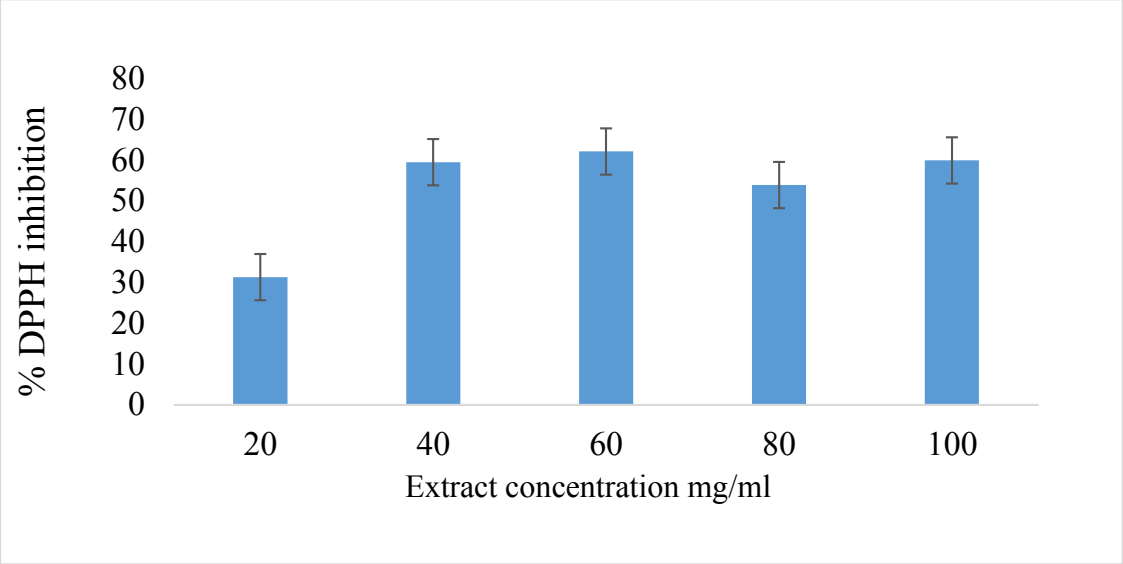

Figure 2: DPPH radical scavenging activity of methanol extract of fenugreek seeds

DISCUSSION

The result for mineral analysis indicates that fenugreek seeds contain different concentrations of copper, zinc manganese and calcium (Table 1). These elements are needed in trace amounts. Copper is a constituent of enzymes like cytochrome c oxidase, amine oxidase, catalase, peroxidase, ascorbic acid oxidase, plasma monoamine oxidase, ceruloplasmin, lactase, uricase, tyrosinase, cytosolic superoxide dismutase etc. and it plays a role in iron absorption (Chandra, 1990). Cu is an essential micro-nutrient necessary for the hematologic and neurologic systems (Tan *et al*., 2006), and it is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in haemoglobin, assists in the absorption of iron from the gastrointestinal tract (GIT) and in the transfer of iron from tissues to the plasma (Soetan*et al*., 2010). Deficiency of copper is associated with some health complications such as heart failure and abnormal reproductive system. Copper is also important in the regulation of cholesterol biosynthesis (Lei *et al*., 2017). In our study, the amount of copper observed was 0.734 mg/100ml (Table 1).Other studies have evaluated antioxidant activity and micronutrients composition of twenty five different fenugreek seed extract(Pant *et al*. 2018). The extract contain micronutrient such as iron (Fe), copper (Cu), zinc (Zn), calcium (Ca) and manganese at varying amount among different fenugreek genotypes (Pant *et al*. 2018). Among the different genotypes tested, RMt-143 displayed highest content of iron, calcium and manganese of 184.11±1.639, 781.20±4.790 and 25.65±0.695 µg/g dry weight respectively(Pant *et al*. 2018). Our study thus corroborate previous studies which further suggested that fenugreek seed could be used to provide micronutrients needed for human well-being.

Zinc is mostly found in plant and animal tissues and occurs in all living cells, it plays an important role in facilitation of many enzyme activities such as lactate dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, DNA and RNA polymerases. Zinc dependent enzymes are involved in macronutrient metabolism and cell replication (Al-fartusie, 2017; Hays and Swenson, 1985). However, the zinc content of many foods is low although its availability depends on some physiological and dietary factors (Pant et al. 2018).

In our study, zinc content of fenugreek seed extract was found to be 0.961 mg/100ml (Table 1). Other study reported zinc content of fenugreek seed to be within the range of25.4 ± .02 µg /gm to 68.05 ± .02 µg /gm (Pathak and Agrawal 2014)

Manganese (Mn); a micronutrient required for the metabolism of carbohydrate and lipids. It is also essential for growth, reproduction and development of skeletal system (Pant *et al.* 2018). It is a cofactor of enzyme of lipids, proteins and carbohydrate metabolism. Specifically pyruvate carboxylasesuperoxide dismutase required manganese for their activity (Pant *et al*. 2018). It is involved in glycoprotein and proteoglycan synthesis and it is a component of phosphohydrolases and phosphotransferases involved in the synthesis of proteoglycans in cartilage. Mn is also a part of enzymes involved in urea formation, pyruvate metabolism and the galactotransferase of connective tissue biosynthesis (Chandra, 1990). It serves to protect the cells against free radical (Sanatamaria, 2008). In our study we found manganese content of fenugreek seed to be 0.503 mg/100ml (Table 1) consistent with Shakuntala *et al*(2011) who also reported the presence of manganese in fenugreek (Shakuntala *et al*., 2011).

Calcium (Ca) is an important nutrient in human and serves as a vital component of bone. It also regulates cellular metabolism (Pant *et al.* 2018) and iron utilization (Fleck, 1976). The main dietary sources of calcium in human are diary product and vegetables (Chan *et al*., 1995).Bioavailability of calcium is determined by the presence or absence of negatively charged compounds (chelators) such as oxalate and phytate (Weaver and Heaney, 1991). The calcium content within fenugreek in our study was 0.403 mg /100 ml (Table 1). This implies that fenugreek seed could serves as important source of calcium and other mineral elements that are crucial to human health.

The free radical scavenging activity of fenugreek seeds as determined using DPPH assay showed that fenugreek seeds exhibit this function. Higher inhibition level is an indicator of a strong antioxidant activity which is measured based on the reduction of DPPH free radical by an antioxidant. The decrease in absorbance of DPPH radical is caused by the reaction between DPPH free radical and antioxidants resulting in the scavenging of the free radical by hydrogen donation.

From our result, it was seen that the seeds has its highest antioxidant activity at concentration of 60mg/ml with percentage inhibition of 62.13% (Figure 2). The higher inhibition of DPPH radical at this concentration could be due to the higher reduction state of the compounds expected to be antioxidants in the extract or in other word, the DPPH radical is highly reduced at this concentration. Antioxidants are believed to play a very important role in the body defence system against reactive oxygen species (ROS), which are the harmful by-products generated during normal cell aerobic respiration (Salah *et al*., 1995). A previous study by (Abeed Al Mashkor 2014) have shown that fenugreek seed acetone extract contain phenolic compounds which were established to possess antioxidant activity.

CONCLUSION

This study showed that fenugreek seeds are good source of antioxidant compounds possibly phenolic and related compounds. The seed extract could inhibit DPPH radical at all concentration used but the highest inhibition of 62.13 % was observed at extract concentration of 60 ml/100 ml. The seeds are also important sources of minerals such as copper, zinc, manganese and calcium. Based on the antioxidant activity and mineral content

of fenugreek seed extract, the plant's seeds could thus be used as an ingredient that could add value to the functional foods and by implication improves human health.

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