Evaluation of Proximate, Phytochemical and Heavy Metal in Black Cumin and Fenugreek Cultivated In Gamo Zone, Ethiopia

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Abstract

*Spices have been recognized to have medicinal properties. Among several spices and medicinal plants black cumin and fenugreek are very common. Geographical, climatic and cultivating soil type can change the chemical composition of these spices. The objective of this work is to determine proximate, phytochemical and heavy metals content in black cumin and fenugreek cultivated in Gamo zone, Ethiopia. The proximate composition (moisture, ash, fiber, fat, protein carbohydrate and gross energy) were determined; their contents (%) in black cumin 6.98 ± 0.12, 5.02±2.9, 6.90±0.21, 32.1±0.68, 20.9±0.09, 34.1±0.73and 498±4.4 respectively; and 6.30±0.35, 4.27± 0.17, 9.36±0.25, 12.8±0.41, 30.8±0.09, 46.1±0.52 and 422±1.6 respectively in fenugreek. The total mean of phenolics flavonoids and alkaloids in black cumin 193 ±5.3 mgGAe/100g, 87.6±4.3 mgQE/100g, 69.4±4.7 mgAE/100*g *respectively and similarly in fenugreek 382±11mgGAe/100g, 123±3.6 mgQE/100g and 37.6±2.2 mgAE/100g. In black cumin and fenugreek collected from Kamba, Daramalo and Dita Woreda nine heavy metals were determined by using FAAS. The total mean concentrations in mg/kg for detected heavy metals (Fe, Zn, Cu, Mn, Co,) in black cumin 78.5±5.9, 27.3±1.5, 3.06±0.26, 8.47±0.22, 10.1±0.37 respectively and Cr, Ni, Pb and Cd were not detected in black cumin; similarly the concentration of Fe, Zn, Cu, Mn, Co and Ni in fenugreek 168 ±5.2, 14.8±0.20, 4.76±0.1, 13.7±0.40, 3.66±0.21 and 2.12±0.03 respectively and Cr, Pb and Cd were not detected in fenugreek. All the results of determined parameters were compared to previous studies and the values were in agreement with slight variations. Therefore, black cumin and fenugreek cultivated in Gamo zone were free from heavy metal toxicity.*

Key Words: black cumin, fenugreek, proximate composition, phytochemicals, heavy metals

1. Introduction

The World Health Organization (WHO) recently estimated that 80% of the world's population relies in some way on medicinal plants for their basic healthcare [1]. Medicinal plants naturally create and accumulate secondary metabolites like phenols, alkaloids, steroids, terpenoids, flavonoids, saponins, glycosides, and tannins that fight disease [2]. Spices have been shown to have therapeutic effects because of their anti-oxidant, anti-microbial, anti-diabetic, anti-inflammatory, and chemoprotective properties [3]. Due to their accessibility, effectiveness, and cost, spices are frequently utilized as medicines in Ethiopia [4]. Two spices and healing plants, black cumin and fenugreek, have a long history of usage in traditional medicines all around the world [5]. Both culinary and therapeutic uses have been made of the seed of black cumin, *Nigella sativa*, a perennial flowering plant in the Ranunculaceae family. Numerous other names, such as black cumin (English), black-caraway (U.S.A.), habba-tusawda (Arabic), tikur azimud (Amharic), and karetta shuquwa (Wolaita & Gamo), are also widely used to refer to black cumin [6]. Because of their dark hue and bitter flavor, black cumin seeds are used as a flavoring in curries, sweets, cheeses, and soups. It can also be added to bread, tea, coffee, tinned products, and coffee. Back cumin has a long history of use as a medicinal herb in a number of traditional herbal systems to treat a wide range of ailments, including diabetes, paralysis, airway disorders, inflammation, infections, hypertension, and problems relating to the digestive tract, as well as pain conditions like chronic headaches and back pain [7].

The annual flowering plant known as fenugreek, or *Trigonellafoenum-graecum*, belongs to the Fabaceae family. Fenugreek is a native of the Eastern Mediterranean region and is widely cultivated in Central Asia, Pakistan, India, and China. Fenugreek is known by many different names in different parts of Ethiopia, including Sunqoo (Afan Oromo), Abake (Tigrigna), Abeshe (Guraginga), Abish (Amharic and Kore), and Shuquwa (Wolaita & Gamo). Around the world, fenugreek leaves and seeds are used for a variety of things, including food preparation (like making cheese soup and bread), dyeing, controlling insects in grain storage facilities, preventing blood poisoning from wounds, and medicinal purposes (like being anti-diabetic, lowering blood sugar and cholesterol levels, anti-cancer, anti-microbial, and preventing blood poisoning from wounds) [8].

Spices are functional foods, which are foods that, in addition to providing basic nutritional needs, have been found to positively affect a number of important body systems. Black cumin and fenugreek are used by more than 85% of Ethiopians to season food, improve the flavor of beverages like coffee and tea, and treat all of their medical conditions [9]. Spices may operate as a channel for the transfer of toxins and heavy metals from the environment to individuals through the food chain because they are derived from plants [10, 11]. Consuming medicinal herbs or spices that have accumulated high trace and hazardous metal pollution over an extended period of time may cause these compounds to bioaccumulate in the body's organs, which can result in a number of health problems [12]. Understanding the amounts of phytochemicals and heavy metals in these spices is essential given the widespread intake of spices. The results of this type of research may therefore serve as a guide or informational source for those who eat these spices, providing them with vital information regarding the safety of these substances in terms of their effects on nutrition and health [13, 14].

Black cumin and fenugreek grown in the Gamo zone must therefore be assessed for the level of proximate values, phytochemicals, and necessary and hazardous heavy metals in order to estimate (limit) the daily intake. There have been numerous studies on the topic of proximate, phytochemical, and metal determination in black cumin and fenugreek grown in various countries around the world, but fewer studies have been conducted in Ethiopia. However, there have been no studies on black cumin and fenugreek grown in the Gamo zone for their proximate, phytochemical, and level of heavy (essential and toxic) metals. Therefore the present study is intended to fulfill this research gaps. The aim of the present study was evaluation of proximate, phytochemical and heavy metal in black cumin and fenugreek Cultivated In Gamo Zone, Ethiopia

2. Materials and Methods

2.1. Sample Collection and Preparation

Three separate districts in the Gamo zone were used to gather the samples (i.e Kamba, Daramalo and Dita). Farmers in each chosen districts were asked to donate 500 grams each of black cumin and fenugreek, which were then taken to the chemistry lab at Arbaminch University. The samples were cleaned with tap water, dried in the sun for two days, and then ground into a fine powder using a mortar and pestle. They were then placed in clean polyethylene bags and kept in a clean, dry, and cool location until their next usage.

2.2. Proximate Composition determination

The proximate compositional analysis of black cumin and fenugreek seeds, including percentages of fat, fiber, protein, carbohydrate, moisture, ash, and energy, was determined by Using AOAC [17 - 20] guidelines.

2.3. Determination of Phytochemicals in Black cumin and Fenugreek

2.3.1. Sample Preparation for Phytochemical Determination

Because polar phytochemicals like phenols extract within polar solvents and non-polar phytochemicals like alkaloids extract more readily, methanol (a polar solvent) and petroleum ether (a non-polar solvent) were utilized separately for extraction purposes. 25 grams of each sample were put into a 400 mL extraction flask, which was then filled with 200 mL of methanol and petroleum ether. The mixtures were then agitated at room temperature for three days before being filtered through Whatman paper N°1 and evaporated using a rotary evaporator. For additional analyses, dried extract was stored in a refrigerator at 4°C. Finally, distilled water containing 1 mg/mL (w/v) of each extract was added for the phytochemical test. The crude extract of black cumin and fenugreek samples were tested for the presence of selected major bioactive compounds like phenolics compounds, alkaloids, flavonoids, saponins, tannins, and terpenoids by using following standard methods [21 -24].

2.4. Quantitative determination of some phytochemical

2.4.1. Determination of total phenolics contents (TPC)

The Folin Ciocalteu method, as described in [18], was used to quantify the total phenol contents of the methanol extract of the samples of cumin and fenugreek. Gallic acid was used as the reference phenolic component while drawing the standard calibration curve. The Folin Ciocalteu reagent was newly made and used at a ratio of 1:10 with distilled [25].

Preparation of standard solution: 10mg of standard Gallic acid was accurately weighed and dissolved in 100mL distilled water in a volumetric flask to obtain 100ppm and then pipette out (1.5, 3.75, 6.25, 8.75 and 11.25) mL of aliquots into 25mL volumetric flasks to prepare 5ppm, 15ppm, 25ppm, 35ppm and 45 ppm of atropine solution. Then 10 mL of distilled water and 1.5ml of Folin Ciocalteu reagent were added to each of the above volumetric flasks. After 5 min, 4mL of 0.7M sodium carbonate was added, and then distilled water was added to make the volume up to 25 mL. A dilute methanol extract (0.5 ml of 1:10 mg ml) was mixed with Folin Ciocalteu reagent (5mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 mL, 1M). The resultant mixes were allowed to stand for 15 min before the colorimetric technique at 765 nm was used to determine the total phenol content. The total phenol values are reported in terms of Gallic acid equivalent (mg GAE/100g of dry mass), and the total phenol levels of the methanol extract were determined using a standard calibration curve. The total phenol content of each sample was determined in triplicate and quantified using the Gallic acid standard curve. The results were represented as milligrams of Gallic acid equivalents (GAE) per 100 grams of extracts [25].

$$\text{Total phenol Content } \left(\frac{\text{mg}}{100\text{g}}\text{GAE}\right) = \frac{\text{Concentration}\left(\frac{\text{mg}}{\text{L}}\right)\times\text{Volume of flsk (L)}\times\text{Df}}{\text{Sample Mass (g)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(1)}$$

Where Df = Dilution factor

2.4.2. Determination of total flavonoid contents (TFC)

Preparation of standard solution: To create an intermediate standard solution containing 100 ppm of quercetin, 10 milligrams of the antioxidant was precisely measured and diluted in 100 mL of distilled water. Then, five series of quercetin working standards were created using the dilution equation ($C_1V_1=C_2V_2$); 2.5, 5, 7.5, 10 and 12.5 mL were pipetted into a 25 mL volumetric flask to provide standard solutions of 10, 20, 30, 40, and 50 ppm of quercetin. Plotting an absorbance versus concentration calibration curve was done. Using quercetin as a reference flavonoid component, the total flavonoids in petroleum ether extracts of black cumin and fenugreek samples were calculated [26]. Sample extracts 0.5mL (1mg/ml) were separately mixed with 0.15ml of 10% aluminum chloride, 0.5mL of 1M sodium acetate and the final volume of mixture was made 2.5mL with distilled water mixed thoroughly and incubated for 30 min. at room temperature. The absorbance of the mixture was measured at 415nm against blank solution with a UV/Visible spectrophotometer. The results were expressed as milligram of quercetin equivalent (QE) per 100 g of dry matter.

$$\text{Total flavonoid content (mgQE/100g)} = \frac{\text{Concentration}\left(\frac{\text{mg}}{\text{L}}\right)\times\text{Volume of flask (L)}\times\text{Df}}{\text{Sample Mass (g)}} \quad \ldots\ldots \text{(2)}$$

2.4.3. Determination of total Alkaloids contents (TAC)

Preparation of standard solution: Atropine 10 mg was measured and diluted in 100 mL of distilled water to make intermediate standards (100 ppm). Next, atropine intermediate standard solutions of 1.25 mL, 2.5 mL, 3.25 mL, 5 mL, and 6.25 mL were pipetted into 25 mL volumetric flasks to make working standard solutions of 5, 10, 15, 20, and 25 ppm. Finally, 5 mL of phosphate buffer (pH 4.7) Then a calibration graph was created after measuring the absorbance at 470 nm of wavelength. The total alkaloids in methanol extracts of black cumin and fenugreek were calculated using the atropine standard calibration curve. 25 mL of volumetric flask were filled with samples, 1 ml (1 mg/ml), and 2 M HCl, which was then dissolved and filtered to determine the total alkaloid content. By adding 0.1N NaOH, the PH of the extract was brought to neutral. 5 ml of bromocresol green and 5 ml of phosphate buffer were added to 1 ml of this solution, and the mixture was thoroughly mixed. The mixture was subjected to further extraction using 5 ml of chloroform, transferred to a 25 ml volumetric flask, and brought to the desired level with the same solvent. At 470 nm, the complex's absorbance was measured. Milligrams of atropine equivalent (AE) per 100 g of dry matter were used to express the results [27].

$$\text{Total alkaloid content (mgAE/100g)} = \frac{\text{Concentration}\left(\frac{\text{mg}}{\text{L}}\right)\times\text{Volume of flsk (L)}\times\text{Df}}{\text{Sample Mass (g)}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(3)}$$

2.5. Determination of Selected Heavy Metals

Sample Digestion

For determination of heavy metals in black cumin and fenugreek, samples collected from three different Woredas of Gamo zone (Kamba, Daramalo and Dita) were washed with tape water, dried in sun for two days, grinded with mortar and pestle (homogenized) to fine powder, sieved in 0.5 mm mesh, digested in acids and analysed individually without mixing. The sample digestion procedure was used using [28]; 0.5g of dry material prepared in a beaker was introduced to 6ml of concentrated nitric acid (69% HNO_3) and 4ml of concentrated perchloric acid (70% $HClO_4$). After 30 minutes of open acid digestion, the mixture was heated for 40 minutes on a hot plate by gradually raising the temperature from 100 to 1500°C, then cooled down. Next, 2 ml of $HClO_4$ and 1 ml of hydrogen peroxide (30% H_2O_2) were added, and the mixture was reheated to facilitate complete oxidation of organic substances until reddish-brown nitrous oxide fume disappeared, leaving colorless digestion solution. Finally, the mixture was cooled. After acid digestion colorless solution was obtained and filtered in to 50 ml volumetric flask by using filter paper and then diluted to the mark of flask with distilled water. Blank solution was prepared in the same way as sample preparation except sample addition.

Preparation of standard solutions of heavy metals

The intermediate standard solution of each metal was prepared from stock standard solution (1000 mg/L) by taking 10 mL in 100 mL volumetric flask. Five series of working standard solutions of each heavy metals were prepared from the intermediated standard solutions (100 mg/L) by using dilution equation ($C_1V_1=C_2V_2$) as indicated on table 1.

Table 1**:** series of working standard solutions of Metals

S.No	Metals	Series (in μL)
1	Fe	50, 150, 250, 350 and 450
2	Zn	50, 150 L, 250, 350 and 450
3	Cr	50, 150 , 250 , 350 and 450
4	Cu	125, 250, 375, 500 and 625
5	Ni	125, 250, 375, 500 and 625
6	Mn	50, 100, 150, 200 and 250
7	Co	25, 125, 250, 375 and 500
8	Cd	25, 125, 250, 375 L and 500
9	Pb	5, 10, 15, 20 and 25

2.6. Statistical Analysis

The variance analysis, a highly effective statistical method, was used to assess the differences between the means of three sets of measurements (ANOVA). Using software from a statistical package for the social sciences, the mean concentration of experimental data from the analysis of black cumin and fenugreek was compared using One-Way ANOVA at 95% confidence level (SPSS).

3. Results and Discussion

3.1 Proximate Composition of Black Cumin and Fenugreek

The proximate Composition of black cumin (*Nigella sativa*) and fenugreek (*Trigonella foenum-graceam*) seed collected from three different woredas of Gamo zone were determined and the results were reported as %mean ± %standard deviation as shown in table 2 below.

Table 1: The proximate composition of three cultivars of Bc and Fg cultivated in Gamo zone.

Parameters	Bc K	Bc Dr	Bc Dt	Fg K	Fg Dr	Fg Dt
Moisture (%)	6.48±0.16	6.69±0.06	6.92±0.04	6.08±0.05	5.90 ±0.07	5.99±0.15
Ash (%)	5.23±0.04	5.05±0.02	4.79±0.13	4.27±0.04	4.44±0.06	4.10±0.14
Fiber (%)	6.84±0.28	6.80±0.29	7.07±0.08	9.48±0.30	9.54±0.34	9.16±0.36
Fat (%)	32.1±0.53	33.5±0.24	30.7±0.18	13.3±0.91	11.5±0.22	13.5±0.27
Protein (%)	19.8±0.13	21.8±0.23	21.29±0.26	30.6±0.11	29.7±0.25	32.0±0.37
Carbohydrate (%)	34.4±0.93	33.6±0.95	34.4±0.34	46.9±0.75	46.1±0.50	45.1±0.56

Energy (Kcal/100g)	498±4.4	521±6.5	507±4.3	430±5.8	406±1.4	430±1.6

3.1.1 Moisture Content

The average moisture contents in percent of black cumin collected from Kamba, Daramalo and Dita were 6.40 ±0.10%, 6.69±0.0643% and 6.92±0.04% respectively. Black cumin of Dita has the highest and of the Dita has the lowest moisture content and the average of the three cultivars was 6.69±0.22%.The mean moisture content of three cultivar black cumin were not significantly different at the 0.05 level, (p = 0.53058 which is p≥0.05). The average moisture content in back cumin of this study (6.69±0.22) was lower than the literature mean value (7.12) studied in Bangladesh [30] and higher than (4.2± 0.3) reported by [31]. The moisture of fenugreek sample collected from Kamba, Daramalo and Dita were 6.08±0.05%, 5.90 ±0.07 and 5.99±0.14±respectively Fenugreek of Kamba highest and fenugreek of Dita has the lowest moisture content with average moisture content of of three cultivars was 6.29 ±0.34%. At the 0.05 level, the means are not significantly different (p = 0.6699 that means p > 0.05). When the moisture content value of black cumin and fenugreek were compared, at the 0.05 level, the means were significantly different (p = 0.01239 implies p<0.05). The moisture content of fenugreek (6.299 ±0.349) in this study was smaller than literature mean value (7.54) reported by [32] studied in Ethiopia and lower than literature mean value (8.1±0.3) reported by [33] studied in Ethiopia. This may be due to differences of concentration of minerals in cultivation area soil and climatic condition.

3.1.2 Ash Content

The ash content is a measure/reflection of the mineral contents present in the food material. The ash content of black cumin collected from Kamba, Daramalo, and Dita woreda were 5.23±0.04, 5.05±0.02, 4.78±0.13 respectively. At the 0.05 level, the means were not significantly different (p = 0.92009 which is p >0.05). The average ash content in black cumin (5.017%) of this study was smaller than the literature mean result (7.39) stated by [30]. Higher than literature mean value of ash content (4 ± 0.3) reported by [31] study in Ethiopia. The ash content of the three cultivars of black cumin was consistent with those reported for black cumin in Pakistan [34]. The ash content of the fenugreek samples collected from Kamba, Daramalo and Dita Woreda were 4.27±0.04, 4.44±0.06 and 4.104±0.14 respectively. At 0.05 level, there was no significant difference between mean ash content of three cultivars of fenugreek (p = 0.20483 which was p>0.05).The ash content of fenugreek was significantly smaller than that of black cumin. The mean value ash content in fenugreek of this study (4.27±0.17) greater than the literature mean value of ash content (3.00) reported by [32] studied in Ethiopia and also greater than literature mean value (2.6±0.2) reported by [33]. Thus there was a significant difference between literature mean value and this study ash content. This may be due to climatic, geographical and minerals content differences of cultivation area.

3.1.3 The Crude Fiber Content

Dietary fiber promotes the growth and protects the beneficial intestinal flora. The fiber content of black cumin collected from three Woreda of Gamo zone (Kamba, Daramalo and Dita) were 6.84±0.28%, 6.80±0.29%, and 7.068±0.083% respectively with average mean fiber content of three cultivars 6.90±0.21%. The highest fiber content (7.07±0.08) was investigated in black cumin of Dita and the lowest fiber content (6.80±0.29) was observed in the black cumin of Daramalo. At the 0.05 level, the means were not significantly different (p = 0.9832, which is p > 0.05).The average crude fiber contents of black cumin (6.902±0.209%) in this research was greater than the literature values (5.1± 0.3) reported by [31] and similar to literature values of [33] studied in Pakistan. The crude fiber of fenugreek samples collected from Kamba Woreda, Daramalo Woreda and Dita Woreda were 9.48±0.302%, 9.445±0.34%, and 9.16±0.36 % respectively with total mean fiber content in three cultivars of fenugreek 3.09±0.01%. Highest fiber content (9.48±0.30) % was determined in Fenugreek of Kamba and of Dita has lowest fiber content (9.16±0.36) %. At 0.05 level, the means were not significantly different (p = 0.65697 which was p>0.05). The total mean fiber content in fenugreek of this study (9.36±0.25%) was significantly greater than the fiber content of black cumin. The total mean fiber content in fenugreek of this study was greater than literature mean value of fiber content (7.00 %) reported by [34] in Ethiopia and very smaller than literature value (17.0 %) reported by [35]. Thus this significant difference between fiber content in literature mean value and this study mean value was due to the variation in mineral content of cultivation area. In general, the fiber content in black cumin and fenugreek cultivated in Gamo zone were similar to study reported by [36].

3.1.4 The Crude Fat Content

As shown from table 2 shown, the mean percentage of crude fat in black cumin of Kamba, Daramalo and Dita were 32.1±0.53%, 33.5±0.25% and 30.8±0.19% respectively with average mean of three cultivars 32.1 ± 0.68%. The, black cumin of Daramalo had the highest fat content and of Dita had the lowest mean fat as compared from their experimental results, and at the 0.05 level the means were not significantly different (p = 0.88556 which is p>0.05). When the crude fat content of this study was compared with different literature values, the average mean values of the three cultivars of black cumin (32.128%) was smaller than the mean fat content of black cumin (45.4%) reported by [29] studied in Bangladesh and crude fat content of this research was also in agreement with study result 32.74%) report in Pakistan [33]. The mean percentage of crude fat for fenugreek samples collected from Kamba, Daramalo and Dita were 13.3±0.92) %, 11.5±0.22%, and 13.5±0.27% respectively. The highest mean value fat content (13.5±0.27) % was observed in fenugreek of Dita and lowest mean fat content. 11.5±0.22% was determined in fenugreek of Daramalo. At the 0.05 level, the means are not significantly different, (p = 0.6991which was p > 0.05). The mean value fat content of three cultivars of fenugreek in this study (12.8±0.41%) was greater than literature mean value (7.92) reported by [32] studied in Ethiopia and also similar to another literature mean value (12±0.3) reported by [33] in Ethiopia. The total mean fat content of back cumin (32.13 %) was greater than fat contents of fenugreek (12.8%) cultivated in Gamo zone; at 0.05 level the means were significantly different.

3.1.5 The Crude Protein Content

Protein is essential for normal growth, body development and general repair of body tissues, enzymes, and hormones as well as other substances required for healthy functioning. As shown from the above table 2, the average crude protein content of the three cultivars of black cumin (Kamba, Daramalo and Dita) were (19.8±0.14%), (21.8±0.23%) and (21.2±0.26%) respectively. These showed that, black cumin of Darmalo has the highest (21.774%) and black cumin of Kamba has the lowest (19.8±0.14%) crude protein content. At 0.05 level the means are not significantly different (p = 0.972 which is p>0.05). The total average protein content of black cumin (20.9±0.09%) was smaller than average protein content of fenugreek (30.7±0.09%); and at 0.05 level the means are significantly different. The average crude protein content of three cultivars of black cumin (20.9±0.09) higher than the literature value (18.9 ±0.82 [33] and smaller than literature mean value (28.0±0.36) reported by [31] and in agreement with value (20.3%) studied by [30].The average crude protein content of fenugreek samples collected from Kamba, Daramalo and Dita Woreda were 30.6±0.11%, 29.7±0.25%. and 32.0±0.38% respectively. The result of study showed that fenugreek of Dita has highest crude protein content (32.0%) and fenugreek of Darmalo has lowest crude protein content. At 0.05 levels, the means are not significantly different. The total average crude protein content in fenugreek (30.8±0.05%) of this study was greater than literature mean values 29.31% and 19.8±0.3) [32, 33] respectively. This was because of the differences in climate, geographical and mineral conditions of cultivation area.

3.1.6 Total Carbohydrate Content

Carbohydrate is the main source of energy in human body. The average carbohydrate content of Black cumin samples collected from Kamba, Daramalo and Dita Woreda were 34.4±0.93%, 33.6±0.95 % and 34.3±0.35 % respectively. The highest carbohydrate content was observed in black cumin of Kamba and black cumin of Daramalo has lowest carbohydrate content. At the 95 confidence level, the means were not significantly different (p = 0.22036, which is p>0.05).The overall carbohydrate mean value 34.1±0.73% in black cumin for this study was greater than literature mean value (19.7% and 30) [30, 31]. The carbohydrate content of black cumin was significantly smaller than that of fenugreek. The average carbohydrate content in fenugreek samples collected from Kamba, Daramalo and Dita Woreda were 46.9±0.75%, 46.1±0.54% and 45.1±0.56% respectively. Fenugreek of Kamba has highest carbohydrate content (46.9±0.75) and of Dita has lowest carbohydrate content (45.1±0.56%). At the 95 confidence level the means were not significantly different (p = 0.91805 which p>0.05).The overall mean carbohydrate content in three cultivars of fenugreek (46.0±0.51%) was greater than literature mean value (45.21% and 45) studied in Ethiopia [32, 33] and significantly smaller than literature value [36].

3.1.7 Gross Energy Value

As shown in table 2, the gross energy content of black cumin collected from Kamba, Daramalo and Dita were 498±4.4 Kcal/100g), 521±6.4 Kcal/100g), and 507±4.3 Kcal/100g) respectively; and their average energy was 509±3.3 Kcal/100g. Black cumin of Daramalo has highest energy and of Kamba has lowest energy value. At the 0.05 level, the means are not significantly different. The gross energy value of fenugreek samples from Kamba, Daramalo and Dita were 430±5.8 Kcal/100g, 4062±1.4 Kcal/100g and 430±1.5 Kcal/100g respectively; with total average energy content of three cultivars 422±1.6 Kcal/100g. At the 0.05 level, the two means are not significantly different (p = 0.96548 which was p>0.05).The gross energy content of black cumin was significantly greater than gross energy content in fenugreek.

3.2 Determination of Phytochemical

3.2.1. Quantitative determination of Phytochemicals

The data are provided in table 3 as mean SD. The quantitative measurement of a few chosen phytochemicals (total phenolics, total flavonoid, and total alkaloid content) in black cumin and fenugreek was made by UV-Vis Spectrometer using different standard solution calibration curve. Since percentage relative standard deviation (% RSD) values for all quantification data were in the range of 4.506 to 9.081, which is less than 10, this shows good precision of measurement data.

Table 3: Content of selected phytochemicals (mean±SD).

Phytochemicals	Bc-K	Bc-Dr	Bc-Dt	Fg_K	Fg-Dr	Fg-Dt
TPC (mgGAE/100g)	179 ±11	182±12	218 ±10	326±29	358±18	461± 29
TFC (mgQE/100g)	83.2±7.0	88.7±8.2	90.9±4.6	108±8.9	120 ±2.8	120±6.5
TAC (mgAE/100)	62.2±3.4	73.7±5.2	72.5±6.9	37.9±9.1	30.0±2.8	44.9±3.5

Where TPC= total phenolics content, TFC= total flavonoid content, TAC= total alkaloid content

Total phenolics Contents in Black Cumin and Fenugreek

The results indicated that, black cumin of Dita has highest and that of Kamba has lowest phenolics content with total average phenolics content 193±5.3. At 0.05 levels, there was no significant difference between the means of total phenolics content in three cultivars of black cumin. The TPC in black cumin of this study was greater than literature mean value (160± 11mgDAE/100g) [37] and less than literature mean value (480 mg GAE/100 g, and 589±0.02 mg GAE/100 g) [381]. The total phenolics content in fenugreek of Daramalo was highest and of Kamba was lowest; but at 0.05 levels, there was no significant difference between the means of three cultivars of fenugreek. The total average phenolics content of this study was higher than literature mean value ((139.2 mg GAE/100g) [80] and significantly lower than literature mean values (589±0.02 mg GAE/100 g, reported in [39]. This was because of climatic, geographical and soil type differences. The phenolics content in fenugreek was higher than that of in black cumin. This was because of variation in plant variety.

Total Flavonoid Contents in Black Cumin and Fenugreek

Flavonoids have been reported to exert wide range of biological activities such as anti-inflammatory, anti-bacterial, anti-allergic, cytotoxic anti-tumor, treatment of neurodegenerative diseases, vasodilator activities and inhibition of lipid peroxidation [40].The total flavonoid content in black cumin was lower than that of in fenugreek. The total flavonoid content of black cumin cultivated in Dita was highest and that of Kamba was lowest At 0.05 level, the means were not significantly different ($p = 0.98765$ which was $p > 0.05$).Total flavonoid content in black cumin of this study was higher than literature mean values (14.0 ± 0.8) [81] and smaller than literature mean value (3.78% mg.) reported by [40]. The flavonoid content in fenugreek of Daramalo was highest and of Kamba was lowest; but at 0.05 level, the means were not significantly different ($p = 0.18629$ which was $p > 0.05$). The total average flavonoid content in fenugreek of this study was lower than literature mean value (145 mgQE/100g, 274 mgQE/100g) reported by [41]. In general, the result this study showed that black cumin and fenugreek cultivated in Gamo zone were good source of flavonoid compounds.

Total Alkaloid Content of Black cumin and Fenugreek

Alkaloids protect against chronic diseases (reducing headaches associated with hypertension) Alkaloids diverse group of secondary metabolites found to have anti-microbial activity by inhibiting DNA topoisomerase [25]. The total alkaloids content in black cumin of Daramalo was highest and that of Kamba was lowest. At the 0.05 level, the means are not significantly different ($p = 0.99156$ which was $p>0.05$). The total alkaloid content in black cumin was higher than alkaloid content in fenugreek and at 95% confidence level; the two means were significantly different. This was because of sample variate difference. The Alkaloid content in fenugreek of Dita was highest and that of Daramalo was lowest, but at 0.05 there was no significant difference between means.

3.3. Determination of Heavy Metals Using AAS

3.3.1 Calibration of Instrument

The intermediate standard solution (100 mg/L) was diluted with distilled water to create five calibration standard solutions for each heavy metal. These solutions were then tested using the same process as samples, and their absorbance readouts were recorded from FAAS. The linear regression equations (calibration curves) were derived from standard solution concentration and its absorbance readout. It was determined that the linearity of the established calibration curves is good, and the results are correct, because the correlation coefficients (R^2) of all the calibration curves were > 0.995, which demonstrated that there was good correlation (connection) between concentration versus absorbance.

3.3.2. Method Validation

Validation is one of the most important measures that analytical chemists use to predict whether a method meets the needs of intended purpose or not. Accuracy (recovery test), precision and method detection limit were used to check the validity of the instrument, sample preparation and measurement methods used through this research.

3.3.2.1. Precision

The precision is the measure of closeness of the results obtained from triplicate analysis of the same sample under the same condition. It is usually expressed as variance, standard deviation, or percent relative standard deviation of a set of measurements. For this research, the precision of the results were evaluated by the standard deviation and percent relative standard deviation of the results of triplicate analysis of the study samples. As indicated in table 5, the standard deviations (SD) of the determined heavy metals in study samples were ranges between 0.0682 and 5.7231 and percentage relative standard deviations (% RSD) were ranges from 0.1858 to 9.88 % which mean less than 10 % for all the analyzed elements. This indicates that there are good precision (agreement) between the measurements.

3.3.2.2 Recovery Test

The reliability and efficiency of the procedure was checked by spiking known concentration of heavy metals to the sample. As indicated in table 4, the percent recovery obtained lies in the range from 90.75 to 102.56 which was within acceptable ranges (100±15). These values indicate that the analytical procedure used in this research was appropriate and valid for the analysis of selected metals in study samples.

Table 4: Recovery test result of the heavy metals determination

Elements		Fg K	Fg Dr	Fg Dt	Bc K	Bc Dr	Bc Dt
Fe	%Recovery	98.6±5.5	97.1±3.2	97.6±2.7	105±0.86	101±5.6	100 ±6.5
Zn	%Recovery	100±2.8	95.0±0.69	104±3.9	102±1.5	99.3±5.6	106±2.9
Cu	%Recovery	95±1.2	102 ±0.82	96.5±0.81	93.4±2.4	94.2±1.5	92.2±1.1
Mn	%Recovery	99.6±2.2	95.7±1.8	93.8±1.9	100±1.3	95.8±1.9	99.7±2.5
Co	%Recovery	99.5±2 .2	95.7±1.8	93.8±1 .9	100± 1.2	95.79± 1.9	99.7±2 .5
Cr	%Recovery	---	---	---	---	---	---
Ni	%Recovery	101±2.5	98.7±0.71	93.5±1.7	---		---
Pb	%Recovery	---	---	---	---	---	---
Cd	%Recovery	---	---	---	---	---	---

3.3.3 The Level of Heavy Metals in Black Cumin and Fenugreek

The concentration of trace heavy metals (Fe, Zn, Cu, Mn, Co, Cr Ni) and toxic heavy metals (Pb and Cd) in black cumin and fenugreek collected from three different Woreda of Gamo zone were determined by using FAAS. The mean concentrations of these heavy metals in the study samples were determined from their triplicate analysis. The mean concentrations of metals in mg/kg by dry weight basis with their respective SD are reported in table 5. Thus significance difference of concentration of each metal among the study samples was checked by comparing the p values at 95% confidence level. If $p \geq 0.05$ at 95% confidence level, the means are not significantly different and $p<0.05$ the means are significantly different.

Table 5: Mean concentrations (mg/Kg) of metals in black cumin and fenugreek.

Samples	Fe	Zn	Cu	Mn	Co	Cr	Ni	Pb	Cd
Fg K	170±3.3	13.2±0.24	5.76±0.24	14.1±0.21	3.74±0.22	ND	1.74±0.10	ND	ND
Fg Dr	162±1.68	15.9±0.22	3.62±0.24	13.3±0.25	3.83±0.10	ND	2.40±0.07	ND	ND
Fg Dt	172±0.32	15.1±0.27	4.83±0.20	13.7±0.23	3.36±0.24	ND	2.22±0.17	ND	ND
Bc K	85.3±1.6	25.8±0.13	2.76±0.16	7.93±0.28	9.44±0.16	ND	ND	ND	ND
Bc Dr	75.2±5.72	27.3±1.0	3.14±0.20	8.55±0.18	10.3±0.18	ND	ND	ND	ND
Bc Dt	74.9±2.42	28.6±0.18	3.26±0.28	8.94±0.17	10.4±1.03	ND	ND	ND	ND

Key: ND = Not detected

As indicated in table 5, the concentrations of selected heavy metals were successfully determined in study samples except Cd and Pb which were not detected. The total average mean concentration of determination heavy metals in three cultivars of study samples represents the Gamo zone study area result.

Iron: iron has several key functions in the human body as it is a constituent of certain biological molecules like the hemoglobin and involved in various physiological activities including oxygen supply, energy production, and immunity [15]. However, Iron over dose is associated with adverse effect on various metabolic functions and cardio vascular system [16]. Iron concentrations in black cumin and fenugreek samples collected from three in Gamo zone were varied between 74.934±2.425-172.632±0.321 mg/kg these values were relatively higher than concentration of other heavy metals. Iron concentration in black cumin of Kamba, Daramalo and Dita were 85.3±1.6, 75.2±5.7 and 74.98±2.4 respectively with total average iron concentration 78.5±5.9. At 0.05 level, the means were not significantly different (p = 0.904 which was p>0.05). The iron concentration in black cumin (78.4612±5.8819 mg/kg) of this study higher than literature mean value (59.3 mg/kg) reported by [43] and significantly lower than literature mean value (150±1.0 mg/kg) reported by [85] studied in Bangladesh. Iron concentration in fenugreek of Kamba, Daramalo and Dita were 170±3.3 mg/kg, 162±1.7 mg/kg and 172±0.32 mg/kg respectively with total mean of three cultivars 168±5.2 mg/kg. At 0.05 level, the means were not significantly different (p = 0.8489 that was p>0.05). Iron content in black cumin was significantly smaller than that of in fenugreek; at the 0.05 level, the means are significantly different (p = 1.54103E^{-4}; p<0.05). The mean value iron concentration in three cultivar fenugreek of this study (168±5.2 mg/kg) was higher than literature mean value (72.7 mg/kg) studied in Ethiopia [45] and significantly smaller than literature mean value (540±2.6 mg/kg) [44]. The result for iron concentration in both black cumin and fenugreek were bellow tolerable daily intake (TDI) value (0.8 mg/kg bw per day) regulated by EFSA, FAO/WHO, 2010.

Zinc (Zn): It is known that zinc is an essential trace element not only for humans, but for all organisms, it is a component of over 300 enzymes and even greater number of other proteins, which emphasizes its indispensable role for human health [15]. Zinc concentration in black cumin and fenugreek found to be in range between 13.2±0.24mg/kg-28.7±0.18 mg/kg. Zinc concentration in black cumin collected from Kamba, Daramalo and Dita were 25.8±0.13mg/kg, 27.3±1.07 mg/kg and 28.7±0.18 mg/kg with total value 27.3±0.34 mg/kg. Zinc concentration in black cumin from Dita was highest and that of Kamba was lowest. At 0.05 level, the means were not significantly different (p = 0.77714 which was p>0.05). The total mean of zinc concentration in black cumin of this study was smaller than literature mean value (47.0 ±2.1 mg/kg) [42] and smaller than literature mean value reported by [46]. The result for zinc concentration in black cumin of current work was similar to much previous work. Zinc concentration in fenugreek samples collected from Kamba, Daramalo and Dita were 13.2268±0.2389 mg/kg, 15.9±0.22 mg/kg and 15.1±0.28 mg/kg respectively; the total mean concentration of three cultivars was 14.8±0.20 mg/kg. At the 0.05 level, the two means are not significantly different (p = 0.44621 which was p>0.05). Zink concentration in fenugreek of this study was higher than literature mean value (6.1±0.1mg/kg) reported by [44] and it was significantly smaller than literature value (45 mg/kg) result reported by [45]. Concentration of zinc in fenugreek was slightly greater than in black cumin; at the 0.05 level, the means are significantly different (p = 4.26778E-4; p<0.05, in both samples concentration was bellow tolerable daily intake (TDIV) values which is (0.43 mg/kg bw per day)

Copper (Cu): Level of copper in black cumin and fenugreek samples range in between 2.77±0.16 mg/kg-5.76±0.24 mg/kg. Copper concentration in black cumin collected from Kamba, Daramalo and Dita were 2.76±0.16 mg/kg, 3.13±0.20 mg/kg and 3.27±0.28 mg/kg respectively; with total mean concentration 3.06±0.26 mg/kg. Black cumin of Dita has highest copper content and that Dita has lowest copper content. At the 0.05 level, the means are not significantly different (p = 0.74467 which was p>0.05). Total mean concentration of copper in black cumin (3.06±0.26) of current work was compared to different literature values and it was smaller than literature mean value (13.7±0.24 mg/kg) [42] and it also lower than values (13.80 mg/kg) reported by [43] and slightly higher than literature mean value (2.34) [46].Copper concentration (mg/Kg) in fenugreek collected from Kamba, Daramalo and Dita were 5.76±0.24, 3.69±0.24 and 4.83±0.20 respectively with total average 4.76±0.07. At the 0.05 level, there was no significant difference between means (p = 0.70393 which was p>0.05). Copper content in black cumin was smaller than in fenugreek; at 0.05 level the two mean were not significantly different (p = 0.05059; p>0.05). The total mean concentration (4.76±0.07 of current finding was smaller than literature mean values (11.6±1.5) [44] and (8.25) [45] it was higher than other study values reported by [47].

Manganese (Mn): Concentration of manganese in black cumin collected from Kamba, Daramalo and Dita were7.93±0.27 mg/kg, 8.54±0.18 mg/kg, 8.94±0.17 mg/Kg respectively; and their total mean concentration was 8.47±0.21. At the 0.05 level, the two means are not significantly different (p = 0.25273 which was p>0.05). Total mean concentration (mg/Kg) of manganese in black cumin of current result (8.46±0.21) was smaller than literature mean value (45.0±1.2 mg/kg) studied by [42] and higher than other result reported by [43]. The results of this study finding similar with much previous studies [46] slight variations. Concentration (mg/kg) of manganese in fenugreek samples of Kamba, Daramalo and Dita were 14.1±0.21 mg/kg, 13.3234±0.2467 mg/kg, 13.7±0.24 mg/kg respectively; and total mean value was 13.72±0.40 mg/kg. t the 0.05 level, the two means are not significantly different (p = 0.08995; p>0.05). Concentration of manganese in fenugreek was significantly greater than that of in black cumin; at 0.05 level, the tow means were significantly different (p = $1.48947E^{-4}$ which was p<0.05). Manganese concentration in fenugreek of this study was smaller than previous studies result (73.0±1.34) reported by [44] and higher than other literature value reported by [47].

Cobalt (Co); Cobalt concentration ranges from 3.36±0.24 to 10.4±1.03 mg/kg. Cobalt content in black cumin of Kamba, Daramalo and Dita were 9.43±0.16 mg/kg, 10.3±0.18 mg/kg, 10.4±1.0 mg/kg respectively; with total mean value 10.0±0.37 mg/kg. At the 0.05 level, the two means are not significantly different (p = 0.92586 which was p>0.05). Cobalt concentration in fenugreek of Kamba, Daramalo and Dita were 3.74±0.22 mg/Kg, 3.83±0.10mg/kg, and 3.36±0.24 mg/kg respectively; their total mean concentration was 3.656±0.25 mg/kg. The means were not significantly different at 0.05 level, (p = 0.78222; p>0.05). Cobalt in fenugreek sample were significantly greater than that of in black cumin; at the 0.05 level, the means are significantly different (p = $4.67376E^{-5}$ which was p<0.05). Cobalt content obtained in black cumin was slightly greater than literature mean value (8.5±0.1) reported by [46] and it was smaller than other study result (15.3±1.2) [42]. Cobalt content in both black cumin and fenugreek samples were below the standards of tolerable daily intake value (0.023) regulated by FSA, 2003.

Nickel (Ni); Nickel only detected in fenugreek samples, but it was not detected in black cumin. Nickel Content in fenugreek samples of Kamba, Daramalo and Dita were 1.745±0.10 mg/kg, 2.40±0.07 mg/kg and 2.22±0.17 mg/kg, respectively; with total average 2.12±0.03 mg/kg. At the 0.05 level, the two means are not significantly different (p = 0.59255; p >0.05). The average nickel concentration in fenugreek was higher than literature mean value (0.340 mg/kg) [45].Nickel content was bellow tolerable daily intake value (0.0028 mg/kg bw per day).

Chromium (Cr), Lead (Pb) and Cadmium (Cd): Chromium (Cr), Lead (Pb) and Cadmium (Cd): were not detected in both black cumin and fenugreek samples. Thus black cumin and fenugreek cultivated in Gamo zone were free from the above metal toxicity.

4. Conclusion

This research looked at the phytochemicals, heavy metals, and proximate composition of fenugreek and black cumin grown in the Gamo region. The investigation's findings showed that fenugreek samples had higher fiber, protein, and carbohydrate contents than black cumin samples did in terms of moisture, ash, fat, and energy values.. We measured the amounts of various significant phytochemicals (phenolics, flavonoids, and alkaloids), and the results showed that fenugreek had higher concentrations of phenolics and flavonoids, and black cumin had higher concentrations of alkaloids than fenugreek. The quantitative findings of the identified phytochemicals agreed with earlier research. Fe, Zn, Cu, Mn, Co, Cr, Ni, Pb, and Cd were found in both black cumin and fenugreek, but Ni was only found in fenugreek and Cr, Pb, and Cd were not found in either sample, according to the analysis of heavy metals (Fe, Zn, Cu, Mn, Co, Cr, Ni, Pb, and Cd). The results were consistent with other research, and the concentrations of all determined heavy metals were below the limits of tolerable daily intake values. The concentrations of all detected heavy metals were in the range of nutritionally adequate levels. The nutritional composition of black cumin and fenugreek seed was found to be in close conformance with values already described in the literature with a few minor deviations, according to the study's findings. This little variation might be the result of several elements that vary depending on the type of seed purchased from the location. The temperature, geography, soil, and other variables may be to blame for these changes.

Acknowledgements

The authors acknowledge the laboratory facilities and financial support provided by Arba Minch University, College of Natural Science's, Department of Chemistry.

Conflict of Interest

The authors claim that there were no conflicts of interest in publishing this paper.

Ethical Approval

In this study, no human or animal testing is required. This research does not involve any human subjects. This study does not include any patients

Abbreviations

Bc= Black Cumin Fg = Fenugreek K= Kamba Dr = Daramalo Dt = Dita

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