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SEM of *Theobroma cacao*

Proximate and Elemental Composition of Black Seed and Jujube as Formulation for the Treatment of Peptic Ulcer

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ABSTRACT

Black seed (*Nigella sativa*) is a widely used medicinal plant throughout the world. Seeds and oil have a long history of folklore usage in various systems of medicines and food. The fruits of jujube (*Ziziphus jujuba*) are being consumed all around the world because of their health benefits, as both food and herbal medicine. The aim of this research was to evaluate Proximate and elemental compositions of Black seed and Jujube as formulation for managing peptic ulcer. Proximate composition of jujube, black seed and formulation of black seed and jujube was carried out and the contents of moisture, carbohydrate, fats, fibers, proteins and ash were determined according to standard AOAC, 2010 method. Mineral elements (Zn, Fe, Cu, Cr, Mg, Ca, Na, K, As, Pb) were also determined by atomic absorption spectrophotometric method. The result revealed that jujube has higher carbohydrate, fiber and ash contents of 46.60%, 32.17% and 12.87% respectively, while black seed has higher fats and crude protein contents (35.9% and 9.47% respectively). Elemental composition revealed that black seed has higher zinc, iron, copper, and sodium contents of 1.91 mg/L, 6.95 mg/L, 2.42 mg/L, and 27.23 mg/L respectively. Black seed and jujube can be consumed alone or in combination as they are highly enriched with nutrients which are essential for physiological body functioning and can facilitate healing in ulcer treatment.

INTRODUCTION

Black seed (*Nigella sativa*) belongs to Ranunculaceae family, and possibly one of the most significant medicinal plants in history. *N. Sativa* is widely referred to as black seed. *N. sativa* is native to Southern Europe, North Africa and Southwest Asia and is grown in many countries around the world, including the Middle East Mediterranean region, Southern Europe, India, Pakistan, Syria, Turkey and Saudi Arabia [1]. The seeds are extensively sold in markets to be used as a condiment and native medicine. The seeds oil is also considered as one among newer sources of edible oils [2]. *Nigella sativa* is an annual flowering plant. It grows to a height of 20–30 cm (7.9–11.8 inches) with linear lanceolate leaves. The delicate flowers have 5 - 10 petals and usually yellow, white, pink, pale blue or pale violet colors. Scientific investigations have depicted its composition i.e. moisture, oil, proteins, ash and total carbohydrates contents in the range of 3.8–7.0%, 22.0–40.35%, 20.85–31.2%, 3.7–4.7% and 24.9–40.0%, respectively [3]. Fatty acid compositions of black cumin were linoleic acid (40.3-70.8%) followed by oleic (15.2-28.1%), palmitic (9.4713.34%) and stearic (2.6-3.1%) acids [4]. *Ziziphus mauritiana* (L.) wild (Rhamnaceae) locally known as Jujube English Name, Magarya in Hausa, is for tropical evergreen tree grown in the East and West Africa, Nigeria [5,6].

The leaves can either be deciduous or evergreen depending on species and are aromatic. The flowers are small inconspicuous yellow green. The fruit is an edible drupe, yellow-brown, red or black, often very sweet and sugary, reminiscent of a date in texture and flavor [7], delicious with a lot of health benefits. The fruit is an excellent source of proteins, carbohydrates, and micronutrients, such as vitamin C, zinc (Zn), iron (Fe), copper (Cu), phosphorus (P), sodium (Na), potassium (K), and calcium (Ca) [8]. The dry weight of 100 gram of edible portion contains crude fibre (4.9 g to 7.3 g), crude proteins (7.9 g to 8.7 g), fat contents (0.8 g to 1.5 g), and carbohydrate content (79.5 g to

83.2) [8–10]. The fruits of *Z. mauritiana* were found to be rich in vitamin C (15 mg to 43.8 mg per 100 g) and have energy value of 1516-1575 kJ per 100 g [11].

Despite numerous research on the compositional analysis of *Nigella sativa* and *Ziziphus jujuba*, however, information on the compositional analysis of the of their formulations is lacking. Perhaps, the recent claim that their formulation may be a good treatment for peptic ulcer, makes it necessary to investigate the compositions of this formulation. Thus, this research investigates the proximate and elemental compositions of black seed and jujube formulations.

MATERIALS AND METHODS

Sample collection and preparation

The seeds of *Nigella sativa* and *Ziziphus* jujuba were purchased from local market, Brigade, Gama of Nassarawa L.G.A. Kano. The black seeds and jujube were sorted, false and inert materials were removed. Black seed and jujube were washed with water, air dried and pulverized into fine powder. Combination of jujube and black seed were prepared in ratio of 3:1 respectively, i.e., 75 g of jujube was mixed with 25 g of black seed making 100 g.

Proximate composition

Moisture, ash, fat, protein, carbohydrate and crude fibre were estimated by the standard procedure of the AOAC [12] as described below in Agro processing and Natural Product Division.

Determination of moisture

Moisture was determined by oven drying method. About 3 g of powder seed sample was accurately weighed in a pre-weighed Petri dish and dried in a hot air oven at 105 ℃ for 12 – 24 h. The dish with the sample was cooled in desiccators and weighed. This exercise was repeated till the difference in weight between two successive weighing becomes constant. From the weight loss during drying, amount of moisture was calculated using the following formula and the moisture can be represented in percentage.

$$MOISTURE\ \% = \frac{W1\ - W2}{W} \times 100$$

W1 = Weight of sample with petri dish before drying
W2 = Weight of sample with petri dish after drying
W = Weight of sample

Determination of ash content

For the determination of ash, 5 g of powder seed sample accurately weighed into pre-weighed were oven dried at 105 ℃ for 24 hr. The dried samples were weighed and then transfer in clean crucibles. The crucibles were heated to the point of charring of the samples on hot plates. The crucibles with the carbon residue obtained as a result of ignition were placed in a muffle furnace at a temperature maintained at 650 °C until the carbon residue disappears (6 hrs.). The samples are allowed to cool and then weighed. From the difference in weight obtained the ash content was calculated using the formula:

$$Total\ ash\ content\ w\ (\%) = \frac{\text{Weight of crucible with ash (g)}}{\text{weight of crucible with sample (g)}} \times 100$$

Determination of crude fat

Crude fat was determined by petroleum ether extract method using Soxhlet apparatus. Approximately 3 g of dried powder sample was taken in a thimble and plug the top of the thimble with a wad of fat-free cotton. Drop the thimble into the fat extraction tube of a Soxhlet apparatus. Attach the bottom the extraction tube to a Soxhlet flask. Pour approximately 75 mL or more of hexane through the sample in the tube into the flask. Attach the top of fat extraction tube to the condenser. Extract the sample for 6 h or longer on a heating mantle at 40 °C. At the end of the extraction period, remove the thimble from the apparatus and concentrate the extract at rota evaporator at 40 °C. Dry at 100 ℃ for 1 h, cool and weigh. The difference in weights gives the ether-soluble material present in the sample.

$$Crude\ Fat\ (\%) = \frac{\text{weight of hexane soluble material}}{\text{weight of sample}} \times 100$$

Determination of total protein

Protein in the sample was determined by Kjeldahl method (Kjeldahl KDIGB 4M). The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of digestion mixture. The nitrogen content in the plants mainly appears as proteins and amino acids and thus the total amount of nitrogen indicates the amount of total proteins and amino acids. About 0.15 g each of sample and digestion mixture catalyst (copper sulphate + potassium sulphate) was weighed into a Kjeldahl flask and 2 mL of concentrated H_2S0_4 was added. The Kjeldahl flask was then heated on a mantle (in slanting position) until colour of solution changes to pale blue-green. This clear solution was made up to 25 mL under cold condition.

The Kjeldahl apparatus was set up for protein estimation. 10 mL of 2% boric acid and 6 drops of mixed indicator (bromocresol green) was taken in a conical flask and placed under the condenser. 5 mL of sample with 15 mL of 40% NaOH and 10 mL water were added to distillation tube through funnel. When water starts boiling inside the round bottom flask, steam produced then passes into distillation tube. NH_3 evolved in distillation tube is trapped in boric acid. Upon ammonia evolution, the colour of boric acid changes to blue. For maximum ammonia evolution, the process is continued for 20 mins. The solution was then titrated with standard HCl (0.01 N) till pink colour of the solution disappears.
Amount of nitrogen in the samples was calculated by the following equation.

$$\%\ of\ nitrogen = \frac{\text{Titre value (ML)} \times 0.00056Gn\ \times 6.25\ \times 100}{0.15\ \text{g}}$$

% Protein = % of Nitrogen × 6.24

Determination of carbohydrate

Carbohydrate is found by difference method and expressed as percentage of carbohydrate.

Carbohydrate (%) = 100 − [Moisture + Ash + Fat + Protein + crude fiber].

Determination of crude fiber

2.0 g of the sample, (W1) was weighed into the fiber flask, and 100 mL of 0.25 M of H_2SO_4 was added and the mixture was heated under reflux for an hour with the heating mantle. The hot mixture was filtered through a fiber sieve cloth. The filtrate was thrown off and the residue was returned to the fiber flask to which 100 mL of 0.31 M NaOH was added and heated under reflux for another 1 hour. The mixture was filtered using a fiber sieve cloth and 10 mL of acetone was added to dissolve any organic constituent. Residue was rinsed with about 50 mL of hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven dried at 105 ℃ overnight to drive off moisture. The oven dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W2. The crucible with weight W2

was transferred to the muffle furnace at 5500 ºC for 4 hours. Sample was cooled in desiccators and weighed after cooling to obtain W3. The difference W2 – W3 gives the weight of the fiber.

$$\%\ crude\ fiber = \frac{W2 - W3}{W1} \times 100$$

Mineral composition

About 10 g of powdered sample was oven dried at 105 ℃ for 24 hr, the dried sample weighed into crucibles and ash dried in a muffle furnace maintained at 650 ℃ for 6 hr. the ash obtained from the muffle furnace was dissolved in HNO_3 (10 mL) for the determination of mineral content present in the sample of black seed (*Nigella sativa*), jujube, and combination of black seed and jujube. The minerals present in the sample were determined by the help of atomic absorption spectrophotometry (AAS) as in the method given by AOAC, 1990 [13].

Statistical analysis

The results obtained were expressed as mean ± standard deviation (SD). Data were analyzed using One-way Analysis of Variance (ANOVA) using INSTAT3. Values were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of Jujube, Black seed and combination of Black seed and Jujube were presented in **Table 1**. The result revealed that jujube has higher ash, fiber and carbohydrate content of 12.87%, 32.17% and 46.60% respectively. Whereas, black seed has higher fats and protein contents of 35.9% and 9.47% respectively. Whereas, combination of jujube and black seed found to have higher moisture content (4.66%). Combination of black seed and jujube and black seed was found to have similar fats contents (35.77% and 35.9% respectively).

Table 1. Proximate analysis of Jujube, Black seed and combination of Black seed and Jujube.

Parameters	Jujube	Black seed	Formulation
Moisture %	$3.33 \pm 0.93^{a, b}$	0.7 ± 0.10^{c}	4.66 ± 0.16^{a}
Ash %	12.87 ± 0.45^{a}	8.55 ± 0.51^{b}	6.16 ± 0.26^{c}
Fat %	0.74 ± 0.08^{c}	35.9 ± 0.10^{a}	$35.77 \pm 0.41^{a, b}$
Fiber %	32.17 ± 1.13^{a}	21.37 ± 1.27^{b}	15.4 ± 0.65^{c}
Protein %	$4.28 \pm 0.68^{b, c}$	9.47 ± 0.29^{a}	4.4 ± 0.82^{c}
Carbohydrate %	46.60 ± 0.99^{a}	24.0 ± 1.41^{c}	33.49 ± 1.85^{b}

Values are expressed as mean ± standard of triplicate readings. Values within the same row having different superscripts are statistically ($P<0.05$) different.

The result of proximate analysis conducted revealed the presence of moisture, crude fat, ash, crude fibre, crude protein and carbohydrate. The determination of ash is considered essential for mineral element analysis, as mineral elements are responsible for preventing many diseases [14]. Ash content present helps determine the amount and type of minerals in the samples important because the amount of minerals can determine physiochemical properties of foods, as well as retard the growth of microorganism [15].

The higher the ash contents of a food, the higher its mineral contents. Total ash content of jujube (12.87%) was found to be higher than the result reported by [10] and [3]. The ash content of black seed (8.55%) in this study were found to be higher than the result reported by [2] and [3]. In this study combination of black seed and jujube were found to have lower ash content. Dietary fiber are healthy and essential for living body and have a role in the treatment of type 2 diabetes as well as in decreasing cholesterol level in the blood [12,16]. The fiber content of jujube fruit reported by [7] was very low compared to what was found in this study (6.00% and 32.17% respectively). The result was also consistent with that of [2] who found that black seed has a lower fiber content than in this study (6.39% and 21.37% respectively). The fiber content in combination was found to be lower than in jujube and black seed. Fiber besides its greater contribution to the health and wellbeing of humans, by preventing the gastrointestinal problems such as constipation and therefore it is regarded as a natural anticolon cancer. The result of the moisture content of jujube (3.33%) was found to be lower than 5.15% reported by [5].

Black seed showed low moisture content compared to [3] (0.7% vs. 7.12% respectively). In this study black seed was having lowest moisture content. The low moisture content of the sample is an indication of good storage quality with minimal fungal or microbial activity which does not permit the growth of moulds [11,17]. The fat content of the black seed in this research (35.9%) was in agreement with the content (32.74%) reported by [2]. Jujube (0.74%) have a higher content of fat than that of [10] (0.5%). In this study the fat content of black seed and combination were found to be very similar (35.9% and 35.77% respectively), jujube was found to have very low-fat content (0.74%). Fats play many important roles in human body ranging from providing energy to hormones production. As body fat, triglycerides play a role in energy storage [18]. They also provide a layer of insulation under the skin and protective cushioning around the organs.

The protein content in jujube found agrees with the result found by [10] in which the values were very similar (4.28% vs. 4.78 respectively). Both jujube and combination were found to have similar protein content (4.28% vs. 4.4% respectively), however, black seed was found to have higher protein content (9.4%). The protein content found in black seed (9.4%) was lower than in [2,3] (18.09% and 20.3% respectively). Proteins are basic nutritional compounds for the human body. They are considered the main units which make up the body tissues, but can also work as an energy source [19].

Carbohydrates in jujube fruits are responsible for the sweetness index [20]. The major function of carbohydrate in human body is to produce the body with energy and *Ziziphus mauritiana* fruit has enough energy available for the daily metabolic activity for the human body [11]. The carbohydrate content in jujube (46.60%) was found to be lower than the result reported by [10]. The black seed in this study was found to have lower carbohydrate content than in [2] with 29.18% carbohydrate content. In this study jujube was found to have highest carbohydrate content followed by formulation, whereas, black seed revealed the least carbohydrate content.

Table 2: Elemental composition of jujube, black seed and formulation of black seed and jujube.

Parameters	Jujube (mg/ L)	Black Seed (mg/L)	Formulation (mg/L)
Zn	1.13 ± 0.00^{b}	1.91 ± 0.00^{a}	1.15 ± 0.00^{c}
Pb	0.20 ± 0.02^{c}	0.96 ± 0.01^{a}	0.31 ± 0.03^{b}
Fe	2.83 ± 0.02^{c}	6.95 ± 0.02^{a}	3.74 ± 0.03^{b}
Cu	1.13 ± 0.00^{c}	2.42 ± 0.01^{a}	1.30 ± 0.01^{b}
Cr	$0.20 \pm 0.02^{b, c}$	0.32 ± 0.02^{a}	0.21 ± 0.04^{b}
Mg	24.32 ± 0.08^{a}	24.38 ± 0.10^{a}	24.29 ± 0.10^{a}
Ca	111.0 ± 13.82^{a}	$29.14 \pm 0.18c$	54.63 ± 0.33^{b}
Na	18.89 ± 0.19^{b}	27.23 ± 0.09^{a}	10.73 ± 0.27^{c}
K	561.1 ± 5.42^{a}	345.87 ± 1.31^{c}	503.17 ± 2.50^{b}
As	3.93 ± 0.17^{a}	$3.02 \pm 0.20^{b, c}$	2.68 ± 0.37^{c}

Values are expressed as mean ± standard of triplicate readings. Values within the same row having different superscripts are statistically ($p<0.05$) different.

The results of Mineral contents were given in table 2. Potassium, calcium, magnesium and sodium were the major mineral content. Meanwhile, arsenic, copper, iron, zinc, lead and chromium were found in minor amounts. The mineral content of black seed was observed to be higher in zinc (1.91 mg/L), iron (6.95 mg/L), copper (2.42 mg/L), and sodium (27.23 mg/L). Magnesium was observed to have no significant difference ($p<0.05$) in jujube, black seed and formulation of black seed and jujube (24.32 mg/L, 24.38 mg/L, 24.29 mg/L).

Black seed content in calcium (29.14 mg/L) and potassium (345.87 mg/L) was observed to have a lower content compared to jujube and formulation of black seed and jujube. In zinc (1.13 mg/L), iron (2.83 mg/L), and copper (1.13 mg/L) content, jujube was observed to have the lowest content value. While in formulation of black seed and jujube, sodium (10.73 mg/L) and arsenic (2.68 mg/L) were observed to have the lowest content than black seed (27.23 mg/L, 3.02 mg/ L), and jujube (18.89 mg/L, 3.93 mg/L).

The results of mineral element analysis showed that potassium is the most abundant element in jujube, black seed and formulation of black seed and jujube, followed by calcium, magnesium, sodium, iron, arsenic, copper, zinc, lead and chromium (**Table 2**). The results agree with the reports of [10] in which jujube was observed to have the most abundant element as potassium followed by calcium, sodium, iron, zinc, and copper respectively. Potassium is essential for controlling blood sugar level, so diabetic patients are advised to keep blood potassium level at natural ratios [11]. The content of calcium in jujube was larger in value than formulation of black seed and jujube, followed by black seed (111 mg/L, 54.63 mg/L, and 29.14 mg/L). Calcium is necessary for maintaining strong bones and blood pressure [11].

It was observed that sodium present in jujube is present in a lower level than black seed and formulation of black seed and jujube (18.89 mg/L, 27.23 mg/L, and 10.73 mg/L) respectively. Relatively, magnesium level in jujube, black seed, and formulation of black seed and jujube have similar value (24.32 mg/L, 24.38 mg/L, and 24.29 mg/L). Sodium is an important nutritional element that regulates fluid balance in the body [11]. It was observed that black seed has a higher content of iron followed by formulation of black seed and jujube, and then jujube alone. This can be attributed to black seed having higher mineral content. Black seed, jujube, and formulation of black seed and jujube have low contents of the elements, arsenic, chromium, copper, and lead from the study.

Zinc helps in wound healing, body growth, building proteins and manufacturing nucleic acids. Copper is good for regulating the heart rate and blood pressure. The results of mineral elements are in general consistent with those reported by [2], [3] and [21]. According to [22] black seed are nutritionally rich and they provide high amount of dietary minerals especially iron, they also contain good amount of minerals like copper, phosphorus, and zinc. The nutritional constituents found in black seed are necessary for the human body as they supply the body with the amount of nutrient such as proteins, fats and carbohydrates required. The presence of the nutraceutical compounds which actively contribute to the nutritive value of the seed makes them even more potential food source providing health benefits [13].

CONCLUSION

In conclusion, the finding of this study observed that nutritional contents are high and differ significantly in the black seed, jujube and formulation of black seed and jujube in most cases. Black seed, jujube and their formulation can be consumed as they are rich sources of nutrients which are essential for physiological body functioning.

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