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STUDIES ON *TERMINALIA BELLIRICA* SEED FOR PROXIMAL COMPOSITION, MINERAL ANALYSIS AND PHYTOCHEMICAL SCREENING

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ABSTRACT

Studies were carried out on the proximate analysis, phytochemical screening and mineral compositions of *Terminalia bellirica* seed (Bahera). The proximate analysis of the seeds revealed 8.65 % moisture, 91.35 % dry matter, 6.46 % ash, 93.54 % organic matter, 4.19 %crude protein, 32.02 % crude fiber, 40.12 % carbohydrate, and 0.67% nitrogen content respectively. From total ash, the water soluble part was 52.58 % and acid insoluble ash content was 3.47%. Food energy of the seed was found 187.35cal/gm. The presence of some phytochemicals like alkaloids, saponins and flavonoids explained the medicinal action of the plant encountered in its therapeutic uses. The seed samples contains reasonable amount of essential mineral contents needed for the body such as potassium, sodium, calcium, iron, magnesium, aluminum, zinc, copper, manganese, lead, nickel, chromium and cadmium content. The mineral elemental concentration carried out showed K having the highest concentration (190.84 mg/kg) while Cd and Cr concentration was the lowest (0.002 mg/kg).

KEYWORDS: *Terminalia bellirica*, Proximate analysis, Atomic Absorption Spectrophotometer, mineral compositions.

INTRODUCTION

In the recent years, there has been a gradual increase of interest in the use of medicinal plants in developing countries as herbal medicines are safe and without any adverse side effects compared to synthetic drugs. Thus a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lie in some chemical substance that produce a definite physiological action on the human body.[1-2] Plants are generally differentiated by human by their ability to manufacture food, the presence of cell walls, and their unlimited type of growth. The basic food of all organisms is produced by green plants. In the process of food manufacture, oxygen gas is liberated. This oxygen, which we obtained from air as we breathe in, is essential to life. The only source of food and oxygen is plant, no animal or man alone can supply these.

Terminalia bellirica, is known as "Bahera" or Beleric,[3] Sanskrit: Vibhitaka, Aksha is a large deciduous tree common on plains and lower hills in Southeast Asia, where it is also grown as an avenue tree. The leaves of the plant *Terminalia bellirica* (Gaertn.) Roxb are about 15 cm long and crowded toward the ends of the branches. It is considered a good fodder for cattle. *Terminalia bellirica* seeds are oil bearing seeds having an oil content of 40%. The seeds are called bedda nuts. In traditional Indian Ayurvedic medicine, Beleric is known as "Bibhitaki" (Marathi: "Behadaor Bhenda") (*Terminalia bellirica*). In its fruit form, it is used in the popular Indian herbal rasayana treatment triphala. The nuts of the tree are rounded but with five flatter sides. It seems to be the nuts that are used as dice in the epic poem Mahabharata. A handful of nuts would be cast on a gaming board and the players would have to call whether an odd or even number of nuts had been thrown.[4]

Terminalia Billerica (Bahera) is a very important herb. It is large tree with broadly epileptic leaves clustered at the ends of branches.[5] The leaves of the plant possess proteins which makes it promising angiogenic agent.[6] The fruits of this plant are spherical to ovoid. The dry fruit are widely used in aurvededa, Siddha and Chinese systems of medicine.[7] It has antidiabetic, anticancer and anti-microbial properties.[8] The fruit rind is an important

ingredient of triphala, an important Ayurvedic formulations.[9] The fruit extracts stimulates the secretion of insulin and enhance its action and inhibits starch digestion.[10] It possesses active compounds which can be used to develop antidiabetic drugs.[11] The fruit pulp possess active compounds phtosterols, triterpenoids, glycosides, tannins and phenolic compounds which accounts for its anti-inflammatory, analgesic, antibacterial, antioxidant and antitumor properties.[12]

Proximate analysis of food is the determination of the major components of food which include moisture, protein, solubility, ash. Proximate analysis is a system of analysis of nutrients also termed "conventional analysis" in which the gross components (protein, fat, carbohydrate, ash) of the food material rather than individual nutrients (amino acid, fatty acid, monosacharides) are determined.[13]

Phytochemical are chemical compounds derived from plants that are non-nutritive secondary metabolic compounds occurring in different parts of plants. They are important as protective and disease fighting compounds which help the body to prevent of fight against diseases and so are required by the human body to sustain life. Their therapeutic use in prevention or fighting a number of diseases is the basis of their extensive use in traditional medicine. Some of the phytochemicals are water soluble while others are not.[14]

The aim of current study was to determine proximate compositions, phytochemical screening and mineral concentration present in *Terminalia Billerica* seeds.

MATERIALS AND METHODS

Collection of sample

Fully matured fresh seeds of *Terminalia bellirica* were collected from local area of Rajshahi district, Bangladesh in the month of April 2017 and identified by the taxonomist of Bangladesh National Herbarium, Dhaka, where a voucher specimen (No.=4393) has been deposited.

Preparation of sample

The matured seeds were washed to remove dirt. Then it was oven-dried at reduced temperature less than 45^0C to make it suitable for grinding purpose. The screened (20 mesh) powder was then stored in air-tight container with marking for future experiment.

Proximate analysis of Terminalia bellirica seeds

Determination of moisture and dry matter content

5 grams of samples were weighed into pre-weighed Petri dish and dried to constant weight in an oven at 110°C for 6 hours. The oven dried Petri dish was then removed and placed in a desiccators to cool before weighing. After 30 min the Petri dish was removed from desiccators and weighed. This process of heating and cooling was continued until a constant weighed was obtained. From the final weight, the moisture content of samples was determined from the mean values of triplicate determinations.

$$\% \text{ moisture} = \frac{\text{initial weight (before drying) - final weight (after drying)}}{\text{Initial weight (before drying)}} \times 100$$

% dry matter = 100 -% moisture

Determination of ash and organic matter content

The crucible was first washed, dried in an oven at 110°C for 30 minutes cooled and then weighed (W_1). Three grams of sample was placed in the crucible and weighed, (W_2). Then the crucible was transferred into the muffle furnace, whose temperature was set at 650°C and allowed to stay for 3 hours, until the content became white after which the crucible was cool in a desiccators and weighed (W_3). The percentage ash content was then calculated using the relation below;

$$\% \text{ of ash} = \frac{weight\,of\,ash}{weight\,of\,sample} \times 100 = \frac{W_3 - W_1}{W_2 - W_1} \times 100.$$

%of organic matter = 100- ash%

Determination of acid soluble and insoluble ash

25ml of dilute hydrochloric acid was added to 0.5 gm of ash sample and boiled gently for 5 minutes. The insoluble matter was collected on an ash less filter paper and washed with distilled water and dried Then ignited for one hour, cooled in desiccator and weighed. Continuing ignition until a constant value is obtained.

$$\% \text{ of acid insoluble ash} = \frac{(\text{weight of ash taken} - \text{wight of acid insoluble ash})}{\text{weight of ash taken}} \times 100$$

% of acid soluble ash = 100 – acid insoluble ash%

Determination of water soluble and insoluble ash

1 gm of ash sample was mixed carefully with 25 ml of distilled water and boiled gently for 5 minutes. The insoluble matter was collected on ash less filter paper and washed with distilled hot water and dried Then ignited for 15 min at temperature not more than 450°C, cooled in desiccator and weighed. Continuing ignition until a constant value is obtained.

$$\% \text{ of water insoluble ash} = \frac{(\text{weight of ash taken} - \text{wight of water insoluble ash})}{\text{weight of ash taken}} \times 100$$

% of water soluble ash = 100– acid insoluble ash%

Determination of Nitrogen content

It was determined as total Kjeldahl nitrogen. Two grams of the sample were poured in a macro Kjeldahl flask and 20 ml of distilled water was added. The flask was swirled for a few minutes and allowed for 30 minutes to prevent foaming. 50 ml of concentrated H_2S0_4 was also added through an automatic pipette. The flask was heated cautiously at low temperature of 45°C on the digestion stand. When water has been removed and frothing has ceased, the temperature of the flask was increased until the digest was cleared. The digest was boiled for five hours. Heating was regulated during boiling so that

H_2SO_4 condenser is about half way up the neck of the flask. The flask was allowed to cool and 50 ml of distilled water was added to the flask. 10 ml of the aliquot was carefully transferred into a macro Kjeldahl flask. 20 ml H_3BO_3 indicator solution was added into 50 ml Erlemmeyer flask which was then placed under the condenser of the distillation apparatus. 20 ml of 40% NaOH was added to the macro Kjeldahl flask through a funnel on the stop cork and distillation was commenced. The condenser was kept cool at 300°C allowing sufficient cold water to flow through and heat was regulated to minimize frothing and prevent suck back. 40ml distillate was collected and distillation was stopped. NH_4-N in the distillate was determined by titrating with 0.1N standard H_2SO_4 using burette graduated at 0.1ml intervals. The color changed at the end point from green to pink. The percentage Nitrogen in the sample was calculated as follows:

$$\% \text{ of Nitrogen} = \frac{0.14 \times (V_1 - V_0) \times N}{p}$$

Here,
V_0 = Blank determination (volume of 0.1 N H_2SO_4in ml)
V_1 = Sample determination (volume of 0.1 N H_2SO_4in ml)
N= Strength of H_2SO_4.
P = Weight of sample in gm.

Determination of Protein content

The protein content was obtained by multiplying Total Kjeldahl Nitrogen (TKN) value by a conversion factor[15] of 6.25.

%Crude Protein= %N × 6.25

Determination of crude fiber

5 grams of sample was transferred into clean fitter crucibles. 200 ml of 0.255M H_2SO_4 previously pre-heated in the reagent system was added to prevent foaming. The contents of the beakers were boiled for 30minutes and filtered through a Buchner funnel with the aid of a suction pump. The residues were washed with hot deionized water until acid free. The residues left after acid digestion were carefully transferred into a 500ml beaker. 200ml of 0.313M NaOH solution was added to the sample. The contents of the beaker was filtered through a Buchner funnel and 15cm diameter whatman no. 4 filter paper on cooling. The residue was washed several times with hot water and once with methylated spirit until free of alkali. The residues was finally washed three times with acetone, carefully transferred into porcelain crucibles and dried at 110°C for 2 hours. They were allowed to cool in desiccators before weighing. (AOAC, 2000).

$$\% \text{ crude fiber} = \frac{\text{mass of dried fiber} \times 100}{\text{mass of sample used}}$$

Determination of carbohydrate contents

The content of the available carbohydrates is determined by subtracting from the sum of the values (per 100grams) for moisture, protein, fat content, ash, and crude fiber.

So, Carbohydrates content = 100 - (moisture of dry seeds % + ash% + protein % + crude fiber% + fat content%).

Estimation of Food energy

The energy value (kcal) of the samples was estimated by multiplying percentage crude protein, fat and carbohydrate by the recommended factor (4, 9 and 4 respectively) used in vegetable and seed analysis.[14]

Phytochemical content of *Terminalia bellirica* seeds

Phytochemical screening is a process of tracing plant materials. It confirms the presence of various phytochemicals which can be seen as a potential source of useful drugs. Standard phytochemical methods Sofowora.[16-17] were applied to detect the presence of different classes of constituents like alkaloids, flavonoids and saponins in seeds of *Terminalia bellirica*.

Elemental analysis by AAS technique

Among all elements only Sodium (Na) and Potassium (K) were estimated by using flame photometer (Model AnA-135, OSK, Japan). Most of the elements like Calcium (Ca),Magnesium (Mg), Chromium (Cr), Iron(Fe), Zinc (Zn), Aluminum (Al),Copper (Cu), Nickel (Ni), Lead (Pb), Cadmium (Cd) and Manganese (Mn) in seeds of our plant samples were analyzed by using Atomic Absorption Spectrophotometer (Varian, AA240FS, Australia) which was equipped with flame and graphite furnace. For our experiment, we choose air acetylene flame mode. The condition for fixed acetylene was 1.8 l/min and air 15 l/min along with argon gas flow for inert atmosphere. The instrumental default temperature parameters were automatically fixed for each element analysis.

RESULTS AND DISCUSSION

Proximate analysis and calorific value

The proximate analysis revealed the percentages of various nutrients in the seeds of *Terminalia bellirica*. The results for proximate composition and calorific values of *Terminalia bellirica* seeds are shown in Table 1.

Table 1: Proximate composition (%) and energy value (cal/g) of *Terminalia bellirica* seed.

Parameters	Concentration (%)
Moisture	8.65±0.02
Dry matter	91.35±0.02
Total ash	6.46±0.10
Organic matter	93.54±0.10
Acid soluble ash	96.53±0.02
Acid insoluble ash	3.47±0.10
Water soluble ash	52.58±0.10
Water insoluble ash	47.42±0.10
Nitrogen content	0.67±0.10
Protein content	4.19±0.10
Crude fibre	32.02±0.50

Carbohydrate contents	40.12±0.30
Food energy	187.35 cal/gm

The percentages of moisture and dry matter was found 8.65% and 91.35 %respectively. Moisture content is among the most vital factors considered in food processing, preservation and storage.[18] The low percentage of moisture obtained indicates that *Terminalia bellirica* seeds have low shelf-life, implying that its long storage could lead to spoilage due to susceptibility to microbial attack.[19] Ash content is useful in assessing the quality grading of seeds and also gives an idea of the amount of mineral element present in the seed.[20] The ash and organic matter content was found 6.46% and 93.54%, respectively. The total ash is particularly important in the evaluation of purity of drug *i.e*. the presence or absence of foreign organic matter such as metallic salts or silica.[21] From the present investigation it was found that acid insoluble ash is 3.47% as well as water insoluble ash is 47.42%. Crude fibre recorded in the present study (32.02%) indicates the level of non-digestible carbohydrate and lignin in corresponding seeds.[22-23] Crude fiber refers to the indigestible carbohydrate component that is present in plants. Fiber is characterized by low or no nutritional value but because of its effect on the digestive system, it is thought to help with such problems in diabetes and high levels of blood cholesterol.[24-25]

Proteins are major source of energy. It contains essential amino acids responsible for growth and repair of worn-out tissues in humans.[26-27] It was observed that the seed sample contain 4.19% protein and 0.67% nitrogen. Seed proteins should possess the requisite functionality for their successful utilization in various food products. These functional properties are intrinsic physio-chemical characteristics that affect the behavior of properties in food systems during processing, manufacturing, storage and preparation.[28-30]

Carbohydrate gives ready source of energy to the body. Carbohydrate content of the seed yielded (40.12%) showed that they may be a very good source of energy (Carbohydrate) for both man and animals if detoxified.[31,32]

Food energy of the seed is 371.61cal/gm. The high calorific values obtained indicate that this seed could constitute a major source of energy for many of the world's poor and least privileged people.[33-34]

Phytochemical content

The present investigation (Fig-1) showed that of seeds of *Terminalia bellirica* contain phytochemical such as flavanoids, alkaloids and saponins in appreciable quantities. The present investigation showed that seeds of this medicinal plant showed higher saponin content (28.89%), flavanoid content is (10.0%) and alkaloid content is (9.25%).

Saponins are group of chemicals with detergent like properties that plants produce to help resist microbial pathogens.[34] Alkaloids and saponins prevent excessive intestinal absorption of cholesterol and reduce the risk of cardiovascular diseases such as hypertension.[35] In medicine, saponins also used in antioxidant, anti-cancer, anti-inflammatory and weight loss etc.[36]

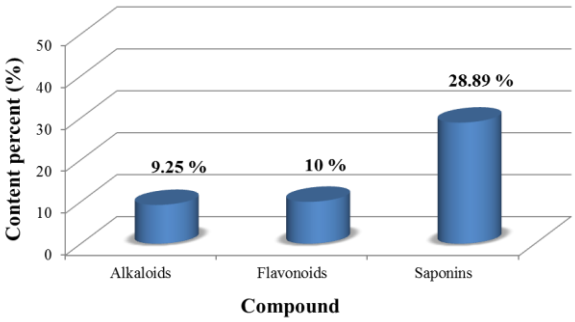

Figure 1: Phytochemical contents of *Terminalia bellirica* seeds.

Flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. The quantity of flavonoid obtained in this study were 10.00%.Flavonoids are a group of polyphenolic compounds with known properties including free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action.[37] Some flavonoids have also been reported to behave like the some coumarins in the inhibition of giant cell formation in HIV-infected cell cultures.[38] The presence of these secondary metabolites suggests that the plant might be of industrial and medicinal importance.

Elemental analysis

The result for the mineral composition for *Terminalia bellirica* seeds is shown in Table 2. The most abundant mineral found in the sample is potassium with the concentration 190.84mg/kg. High concentration of potassium in the body was reported to increase iron utilization[39-40] and beneficial to people taking diuretics to control hypertension and suffer from excessive excretion of potassium through the body fluid.[41]

Calcium is the next most abundant elements in the present investigations. Calcium, a constituent of bones and helps the body to contract correctly, blood to clot and the nerves to convey messages. When the calcium supply to the body becomes insufficient, the body on its own extract needed calcium from the bones. If the body continues to tear down more calcium than it replaces over a period of years the bones will become weak and break easily. Calcium is essential for disease prevention and control and may therefore contributes to the medicinal influences of the plant.[42]

Table 2: Mineral compositions of *Terminalia bellirica* seeds.

No.	Element name	Content (mg/kg)
1.	K	190.84±1.02
2.	Ca	81.42±0.88
3.	Na	30.42±0.53
4.	Mg	19.14±0.26
5.	Al	12.29±0.004
6.	Fe	2.71±0.53
7.	Zn	0.51±0.002
8.	Cu	0.19±<0.001
9.	Mn	0.13±0.009
10.	Pb	0.02±0.53<0.001
11.	Ni	0.01±0.001
12.	Cr	0.0020±0.001
13.	Cd	0.0008±<0.001

Measured values are mean ± Standard Deviation (SD) of three replicate analyses.

Sodium and magnesium are the next abundant mineral elements found in the sample of *Terminalia bellirica* seeds with the values of 81.42mg/kg and 30.42mg/kg, respectively. Sodium regulates fluid balance in the body and helps in the proper functioning of muscles and nerves.[43] According to,[44] the daily value for sodium is 2400mg for adults and children aged 4 and older. However, there is a need to judiciously consider this sample, especially in sodium and potassium restricted diets. This is important since high dietary sodium is implicated in cardiovascular and renal disorders.[45] Similarly, people who suffer from, or are prone to hypertension are discouraged from high dietary sodium.

Magnesium is beneficial to blood pressure and helps to prevent sudden heart attack, cardiac arrest and stroke. Magnesium deficiency results in uncontrolled twisting of muscles leading to convulsion, which may eventually lead to death[46] and it is common in people with chronic alcoholism.[47]

Aluminum is beneficial for the recovery of Alzheimer's diseases. Aluminum may share absorptive pathways with calcium.[48] In the present findings a good quantity of aluminum present which was 12.29 mg/kg. Iron and zinc are the next abundant mineral elements found in the seeds with the values of 0.987 mg/kg and 0.333 mg/kg, respectively. Iron helps in the formation of blood and in the transfer of oxygen and carbon dioxide from one tissue to another.[49] Iron deficiency results in impaired learning ability and behavioral problems in children, and also anemia.[50] Zinc boosts the health of our hairs, plays a role in the proper functioning of some sense organs such as ability to taste and smell, helps in carbohydrate and protein metabolism and also assists in metabolism of vitamin A from its storage site in the livers and facilitates the synthesis of DNA and RNA necessary for cell production.[46]

The values of manganese, and copper in the sample are 0.13 and 0.19mg/kg, respectively. The concentration of lead and nickel also estimated as 0.02 mg/kg and 0.01 mg/kg, respectively. Chromium and cadmium were found to be 0.002 mg/kg, the least of all mineral elements present in the sample.

CONCLUSION

Plants have contributed immensely to the medical field. It has been the source of most drugs used for combating infections. The proximate and phytochemical compositions of *Terminalia bellirica* seeds suggest that the seeds contain the important constituents needed to combat various kinds of infections in human beings. The high level of minerals elements in seeds make it useful as human diets or livestock feed and also be as raw materials in pharmaceuticals formulation.

Further investigations on the chemical compositions and possible isolation of the active ingredients for specific functions in order to standardize the formulation for efficient medical use would be carried out.

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