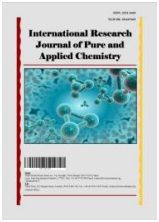

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Proximate Analysis and Evolution of Energy Value from Leaves and Stem of Sword Fern: *Nephrolepis exaltata*

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Authors' contributions

This work was carried out in collaboration among all authors. Author DKS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RSD and KRS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of present study was to prognosticate the amount of each nutrient in the Arial parts of sword fern "*Nephrolepis exaltata*". The study was conducted at Sarva Vidhyalaya Campus, Kadi, Gujarat (India) during the period of December-2019 to March-2020. The prognostication of nutritive component including ash%, moisture%, Crude Protein%, Crude Fat%, Crude fibers% and Carbohydrate was taken from AOAC and Standard Lowry method for estimation of protein. The proximate analysis indicates the monumental presence of crude protein and carbohydrates, moderate presence of crude lipid and fibers. The ash content was 8.65% and 12.42% in leaves and stem respectively, which manifests the existence of inorganic minerals. The Crude protein was recorded as 21.25% and 30.0% in leaves and stems respectively, whereas the crude lipid was 8.41% and 1.47% respectively. The leaves and stems were found to be a good source of energy value with 359.766 Kcal/100 gm and 313.266 Kcal/100 gm respectively.

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Keywords: Nephrolepis exaltata; proximate analysis; Lowry method; energy value; AOAC (Association of Official Analytical Chemists); pteridophyte; vascular plants.

1. INTRODUCTION

In 1860, Henneberg and Stohmann developed a method for quantitative analysis for discriminate the different macronutrients in feed, was explored as proximate analysis. Proximate analysis is not wholly nutrient analysis, rather it is a partitioning of both nutrients and non-nutrients into categories based on common chemical properties. In Proximate Analysis, based on the chemical properties, the compounds in a feed are partitioned into six different categories. They include: moisture, ash, crude protein, crude lipid, crude fibre, nitrogen-free extracts (digestible carbohydrates) [1].

To acquire the approximate amounts of substances within a material the Proximate analysis can be the best scientific inquiry. This method is traditionally utilized in contrasting scientific fields to study diverse materials such as animal feed, coal, and bio-fuels etc. The process is quite intricate involving extraction of one material through miscellaneous solvents or extraction of different materials through single solvent. The hazard potential of chemicals can be determined by proximate analysis. This information can be used to create quality controls for various materials which ensure that they are healthy enough to be consumed by humans or animals [2].

Proximate analysis of plant is the best way to ensure the composition of essential biomolecules such as protein, fats, and carbohydrates etc. In human food products, nutrient levels such as proteins, fats and carbohydrates are often determined [3].

Desert area is the most engrossing part of the mother earth and the point of origin for many plants' species, from ferns to modern dicots. Pteridophytes including ferns and fern-allies are non-flowering, vascular and spore bearing plants. They are ostentatious plants of the earth's vegetation and from evolutionary perspective they are important as they show the evolution of vascular system and exposure of seed habit in the plants. They are abundant in moist tropical and temperate forests and grow in different intermediate regions from sea level to the highest mountains [4]. Ferns are vascular plants that do not produce seeds. *Nephrolepis exaltata*, an epiphytic and terrestrial fern was taken for current study. There are around thirty species in the genus *Nephrolepis* [5]. *Nephrolepis exaltata* is a customarily found pteridophyte and vocalized as "Boston fern" or "sword fern" which is classified in the family *Nephrolepidaceae* [6].

Nephrolepis is easily elucidated and morphologically quite sui generis plant; the stem is a minuscule erect stock with closely tasseled fronds. It has a willowy lateral branch bearing numerous roots and occasional buds which develops into new plants. The genus *Nephrolepis* belongs to Family Nephrolepidaceae, which includes 35 Genera and 303 accepted taxa overall with 2 Subspecies. The order of plant is 'Polypodiales' [7]. The plants of this order are also known for their resistivity in desert area.

Different studies have established the presence of antioxidant activity and also anti-inflammatory property in many species of *Nephrolepis* [8]. Some studies on mice shows that the extract preparation of *Nephrolepis biserrata* given at 100 mg/kg within an hour interval can reduce the level of inflammation. The leaves of *Nephrolepis biserrata* are used for treatment of blisters, boils, abscesses, sores, abdominal pains, wounds and cuts [9].

The result of the proximate composition for some plants showed that the seed oil possessed good physicochemical properties which are the main component of edible oil. As such, this type of plant parts could be utilized successfully as a source of edible oil for human consumption and for industrial applications [10].

Despite the progress made in research of *Nephrolepis exaltata* during the past decades, some phytochemical constituents of the plants are yet to be discovered. As such, the plant yet retains further potential to provide the master stroke for resolving some of the globe's major health problems. However, this study seeks to examine the potential of *Nephrolepis exaltata* for nutritional use. Although some studies revealed that the aqueous extract of fern *Nephrolepis exaltata* may be cardiotoxic to some Arthropods like cockroaches in a dose-dependent manner. Some of unidentified terpene found in aqueous extract of *Nephrolepis exaltata* could play a part for the cardiotoxic effect of in cockroaches [11]. Some studies revealed facts about the allergic effects of Boston fern *Nephrolepis exaltata*,

which is commonly vocalized as 'Bostoniensis' [12]. Proximate analysis is most dependable strategy to find nutritional composition of numerous plants and their parts. Some proximate analysis methods have been used to interpret the nutrition relationship between monocots Maize and sorghum [13].

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The fresh plants were collected from a local surrounding in Kadi (23.2973°N, 72.3302°E) during the month of December 2019. Identification and authentication of the plants was done by Taxonomist and Associate Professor, Dr. K.J Bhatt at the Department of Biology, Pramukh Swami Science and H.D Patel Arts College, Kadi.

2.2 Processing of Plant Materials

Nephrolepis exaltata plant leaves and stem were air dried at 28°C to 40°C for 30 days. Thereafter, samples were milled into fine powder using electric blender. This was done in order to increase the surface area of sample for improved solvent penetration of cells and enhanced extraction of secondary metabolites.

Although fresh and dried samples can be applied in plant extraction studies, dried samples are mostly preferred when considering the time needed for experimental design. Vongsak compared the extraction of fresh and dried plant parts and concluded that fresh samples are fragile, with tendency to deteriorate faster than dried samples, hence extraction is faster in dried samples [14].

2.3 Proximate Analysis

The AOAC methods were used to determine proximate analysis of all plant samples. Powdered plant material was used for analysis of % Ash, % moisture, Crude protein, Crude lipid, Carbohydrates and elemental composition of the selected plant species.

The Proximate analysis was carried out by following scheme-

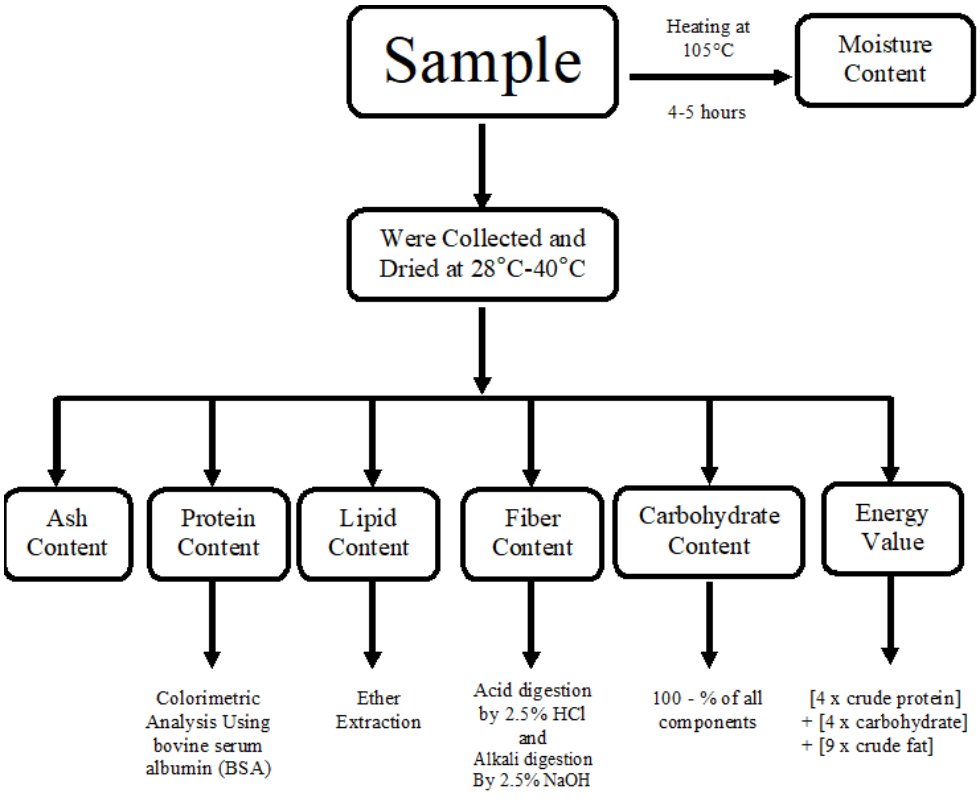

Fig. 1. Scheme for proximate analysis of *Nephrolepis exaltata*

2.3.1 Determination of % ash

Ash was determined according to AOAC (2000) method. At 660°C a clean crucible was heated in a muffle furnace for an hour, then in a desiccator it was cooled and weighed as (W_1). For the determination of ash content, 10 g dry sample was taken in the crucible. The sample was scorched over the burner with the help a blowpipe. Thereafter, the crucible containing sample was heated at 550°C for 6-8 hours in a muffle furnace. When6 the embers left in crucible after the complete ignition the furnace was turnoff. After cooling the crucible at room temperature, it was weighed as (W_2). Percent ash was calculated as follow.

$$\%\text{Ash} = \frac{Wt.of\ Ash\ (W_2 - W_1)}{wt.of\ sample} \times 100$$

2.3.2 Determination of the moisture

Standard AOAC method was followed to deduce the Moisture contents. A dry empty pre-weight (W_1) clean petri-dish (with lid) was filled with 1 gram of sample. Samples were oven-dried at 105°C for 4-5 hours until constant weight was obtained. After which Sample was removed and placed in desiccator for 30 minutes in order to cool it. After cooling the dish, the final weight (W_2) was measured was weighted. Percent was calculated as follows:

$$\%\text{Moisture} = \frac{Wt.of\ residue\ (W_2 - W_1)}{wt.of\ sample} \times 100$$

2.3.3 Determination of proteins

The content of proteins can be ascertained using different methods. From a comparative analysis to determine total protein using the Kjeldahl method and several spectrophotometric methods in a number of different samples, it was concluded that the Lowry method showed the lowest variance using BSA (Bovine Serum Albumin) as standard protein for precise absorption [15].

Due to simplicity and availability of reagents The Lowry method has been widely used for protein determination. However, besides aromatic amino acids, a wide range of other compounds react with the Folin–Ciocalteu reagent [16]. The modified Lowry protein measurement was conducted according to the method described by Hartree in 1972 [17].

2.3.3.1 Proteins extraction

For the estimation of protein in different plant parts, samples were homogenized separately. About 10% trichloroacetic acid (TCA) was applied to the plants since TCA has the property to dissolve macromolecules such as proteins, DNA, and RNA so it is widely used in biochemistry for the precipitation. After preparation of homogenized solution, centrifugation followed at 5000 rpm for 10 minutes. The Supernatant liquid portion was discarded and pellets containing the protein were recovered. Pellets were again suspended in 5 ml of 10% cold TCA and recentrifuged for 10 minutes. Supernatant was again discarded and the precipitate was dissolved in 10 ml of 0.1 N NaOH. The solution was used for protein estimation.

2.3.3.2 Quantitative estimation of proteins

Using the protocol of Lowry *et al.*, 1951, total protein content was estimated in about 1 ml extract.

1. For preparation of a stock solution of bovine serum albumin (1 mg/ml), 1 N NaOH was used.
2. The five-working solutions of different concentrations (2, 4, 6, 8 and 10 ml) from the working standard solution were taken in series of test tubes.
3. In another set of test tubes 0.1 ml and 0.2 ml of the sample extracts were taken and the volume was made up to 1 ml in all the test tubes using distilled water.
4. A mixture solution (A) was prepared by mixing 50 ml of 2% Na_2CO_3 in 0.1 N NaOH and 1 ml of 0.5% $CuSO_4$. $5H_2O$ in 1% sodium potassium Tartrate.
5. The 5 ml prepared mixture (A) was added to each test sample at room temperature.
6. The prepared test samples were left undisturbed for a period of 10 min.
7. Subsequently, A mixture of 0.5 ml of distilled water mixed with 0.5 ml FC reagent B was added to each mixture tube at room temperature (25°C) for 30 minutes until the blue color developed.
8. The Equip-Tronics colorimeter (µP based Inbuilt Filter-Model No-WQ-652) was adjusted at wavelength of 720 nm and set at 100% transmittance using blank before taking the readings of the standard and the test samples respectively.
9. Five replicates were examined in each case and their mean values were recorded.
10. A regression curve was worked out of various concentrations of the standard

solutions against their respective absorbance in accordance to the Beer's law.

2.3.4 Determination of crude lipid (fat content)

There are a variety of test methods for fats, but generally the method can be broken down into "crude" or total fats. "Crude" methods involve dissolving the material in a solvent such as ether or hexane followed by solvent evaporation. The material that remains is called "crude fat". In this case, ether was used as solvent to dissolve the lipid content.

1. A thimble with dry sample was prepared and its weight recorded as W_1.
2. The extraction was done using Soxhlet extractor with diethyl ether as solvent.
3. The thimble was placed into a thimble holder and the holder was clipped.
4. Then after a sufficient amount (one glass reclaiming tube full - approximately 40 ml) of diethyl ether was poured into RBF (Round bottom flask) of sohxlet apparatus, so the plant material in the thimble could be extracted.
5. The RBF was coupled to the extractor with the ring clamp tightly adjusted.
6. The temperature was set to 40^{o}C in heating element.
7. The heater switch, the main power switch, and the condenser water were turned on.
8. As the ether solvent boiled, glassware was inspected for leaks so as to ensure that there were no volumetric losses.
9. Extraction was carried out using standard methods for 16 hours at Low setting (condensation rate of 2 to 3 drops per sec).
10. After extraction, the temperature of heating element was lowered down by shutting down the power and water supply and allowed the ether to drain out of the thimbles (about 30 min).
11. Remove the thimble from the holder, and rinse the holder with small portions of diethyl ether from the wash bottle.
12. The extract was transferred into a pre-weighed beaker (W_2) for further evaporation at room temperature.
13. Room temperature drying was employed in order to avoid possible explosion from oven-induced drying of the ether solvent.
14. The weight of beaker and residue (W_3) was recorded on completion of the drying process.

    Note: Excessive drying may oxidize the fat and give high results.
15. The remaining residue in thimble was used for fiber analysis.

$$\%\text{Crude Lipid} = \frac{Wt.of\ extract\ (W_3 - W_2)}{wt.of\ sample\ (W_1)} \times 100$$

2.3.5 Determination of crude fibers

Crude fiber was determined by acid and alkali digestion method using fiber tec apparatus by following AOAC (2000) [18].

1. The weighed (W1) thimble residue samples were first digested with acid and then with alkali.
2. The sample was transferred into a clean glass beaker a clear beaker and 100 ml of 2.5% HCl was added to it.
3. The mixture was boiled with stirring for about half an hour. It was then drained into the beaker.
4. The fiber residue was again digested in 2.5% NaOH, similar to the acid digestion process.
5. The residue collected was transferred to a pre-weighted (W_2) dried crucible to remove the moisture.
6. The crucible was then kept in furnace for red dull heat till the formation of white and grey ash. The crucible was cooled in desiccators and weighed again. (W_3)
7. The loss in weight of the dry residue upon ignition was taken as the amount of crude fiber. Percent crude fiber was calculated as follows-

$$\%\text{Crude Fiber} = \frac{Wt.of\ dry\ residue\ (W_3 - W_2)}{wt.of\ sample\ (W_1)} \times F \times 100$$

Where,

F = Value of crude fat, W_1 = Weight of Sample, W_2 = Weight of dry crucible, W_3 = Weight of crucible after heating

2.3.6 Determination of carbohydrate contents

Carbohydrates were determined by subtracting the weights of protein fats, crude fibers, ash, and moisture contents from 100.

$$\text{TCH (\%)} = 100 - \%(\text{CP} + \text{A} + \text{CF} + \text{M})$$

2.3.7 Determination of energy value

Carbohydrates give average gross energy values of 4.2 kcal or 17.6 kJ per gram, fat gives 9.4 kcal, or 39.4 kJ per gram and protein gives 5.65 kcal or 23.7 kJ per gram. So, the energy values were

estimated by calculation method using the formula [19],

Energy value (g/100g) = [5.65 x crude protein] + [4.2 x carbohydrate] + [9.4 x crude fat]

3. RESULTS AND DISCUSSION

Data in Table 1 represents the "Proximate Analysis and Energy Value" of *Nephrolepis exaltata*. The dry sample of leaves was light green in color with specific agreeable odor whereas the dry sample of stem was subtle colored. The percentage yield of Ash content was found to be higher in stem, which indicates the presence of high mineral content. The moisture content in leaves and stem was found to be very high, ranging between 21.78% in leaves and 24.83% in stem. Apart from moisture the major chemical constituent found was carbohydrate, recording a maximum content of maximum content 38.32% in leaves and 30.94% in Stem. Crude protein content was estimated as 21.25% in leaves and 30.00% in stem, while crude fat (Lipid) content observed was 8.41% in leaves and 1.47% in stem. The crude fiber content with maximum concentration 1.59% was determined in leaves while minimum amount 0.34% was found in stem.

Table 1. Proximate analysis and energy value of *Nephrolepis exaltata* plant leaves and stem (% of dry samples)

Composition	*Nephrolepis exaltata* (Leaves)	*Nephrolepis exaltata* (Stem)
Ash %	8.65%	12.42%
Moisture %	21.78%	24.83%
Crude Protein %	21.25%	30.00%
Crude Lipid %	8.41%	1.47%
Crude Fiber %	1.59%	0.34%
Carbohydrate %	38.32%	30.94%
Energy Value (Kcal/100 gm)	359.766	313.266

The moisture content was observed 21.78% in leaves and 24.83% in stem respectively; The result was compared favorably with that of Oloyede *et al.* (2012) and Adebiyi el at. (2016) who worked on the *Nephrolepis furcans* and *Nephrolepis cordifolia* (L) in Nigeria respectively [20,21]. This indicates that the amount of moisture varies from species to species and from geographical locations. Low moisture content promotes shelf life of plant and hamper the growth of microorganism. The ash content gives an indication regarding the amount of minerals present in a particular sample, which are important in many biochemical reactions functioning as co-enzyme and aid physiological functioning of major metabolic processes in the body. It contains inorganic material of the plant because ashing destroys all the organic material present in the sample. High ash content in any food substance is an indication of high mineral content [22].

In contrast with other species, *Nephrolepis exaltata* do have higher protein level. The results of the protein analysis observed in this study; is almost similar what was previously reported for *Nephrolepis exaltata* by Johnson M *el at.* (2016). The relative positions of the protein bands were studied for *Nephrolepis exaltata* (L.) through SDS-PAGE and the plant showed maximum number of protein bands [23]. The Fiber content was found in small extent as it was observed in *Nephrolepis furcans* by Oloyede *et at.* 2012, which revealed the lower presence of Fat-free organic substances [20]. The result of the carbohydrate analyses of the plant is reported higher than the other species of plant which shows the food storage capability.

From the above results, it was shown that *Nephrolepis exaltata* has lesser amount of nutrients such as carbohydrates and crude fiber, but it had been found major amount of carbohydrate and protein in it, that determine nutritional value of the fern.

4. CONCLUSION

In conclusion the proximate analysis of *Nephrolepis exaltata* showed that the Sword fern is a good source of essential nutrients like protein, carbohydrate, fat, fibers. Protein analysis by Lowry method indicated that sword fern might be a rich source of isolatable protein. The plant has high ash content, thereby indicating the presence of minerals and Inorganic components. It can be inferred that due to the presence of primary constituents the several solvent extracts from Sword fern can be utilized in different sectors. The presence of different phytochemicals, anti-microbial, anti-fungal potential can also be stabilized. This study suggests that *Nephrolepis exaltata* can be a promising material for pharmaceutical application.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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