Proximate analysis of green tea *(Camellia sinensis)* leaf powder

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Abstract

Green tea *(Camellia sinensis)* is a prosperous source of polyphenols, especially catechins. Green tea extract has antioxidant, antibacterial, antiviral, anticarcinogenic, and antimutagenic effects. The green tea leaves were collected from Kodala Tea Estate, Rangunia Upazilla, Chattogram. Then, the sample was dried, ground, stored in small zipper bags, and analyzed. Chemical analyses of the samples were carried out for moisture, dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and total ash (TA), and nitrogen-free extract (NFE) determination at the Animal Nutrition Laboratory in the Department of Animal Science and Nutrition, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. The current study found DM 26.96%, CP 26.76%, EE 1.67%, CF 12.49%, Ash 6.89% and NFE 52.19%. Methanolic extract of the green tea leaves powder contains 95.45mg/Liter of total phenolic components. Therefore, green tea leaves powder can be a potential source of protein and phenolics containing feed additives in the poultry feed industry.

Keywords: Green tea leaf, proximate component, phenolics, Bangladesh

Chapter 1: Introduction

Green tea is manufactured from *Camellia sinensis* leaves and buds that have not been subjected to the same withering and oxidation processes as black tea. It originated in China, and its production and manufacture have since expanded to other East Asian countries (Wikipedia, 2017).

The leaves of the evergreen plant Camellia sinensis, which primarily grows in tropical and subtropical climes, are used to make green tea, a common beverage (Cao et al.,2005). It has been taken in unfermented (green tea), semi-fermented (oolong tea), and fermented (black tea) forms for centuries by ancient cultures for its therapeutic benefits. The extract has been shown to have antioxidant, antiviral, ant carcinogenic, and ant mutagenic properties in several investigations (Lin, Liu, & Mau, 2008). Tea leaves have antibacterial properties against a variety of pathogens. Toda et al., (1989) found that moderate daily consumption of green tea destroyed *Staphylococcus aureus* and *Vibrio cholera, Clostridium perfringens, Bacillus cereus*, *Pleisomonas shigelloides*, etc. It has also been shown in multiple studies to lower serum cholesterol levels and prevent hypertension (Muramatsu, Fukuyo, & Hara,1986). The edible component of plants or comparable carbohydrates resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine is referred to as dietary fiber. Green tea contains a class of polyphenol molecules known as catechins, which are polyphenols with potential health benefits for humans (Katiyar et al., 2001). In China, green tea was used 5000 years ago for its energizing and cleansing abilities in the elimination of alcohol and toxins, improving blood and urine, treating joint problems, and increasing resistance to diseases (AL-Rejaie, 2009). The cyclooxygenase (COX)-2, 5-, 12-, and 15-lipoxygenase activities in human colon mucosa cells and human colon cancer cells were reported to be inhibited by green tea polyphenols.

Overall, the extract has been found in numerous trials to help with weight loss, blood sugar regulation, disease prevention, and workout recovery. It can also aid in the maintenance of healthy skin and liver, as well as the reduction of blood fat levels, blood pressure regulation, and brain health. In contrast to topical applications of black tea polyphenols (Liu et al., 2008) and green tea polyphenols (Chen et al., 1998), feeding mice green tea polyphenols prevented increases in epidermal COX and lipoxygenase activity caused by UV radiation (Lou et al., 1999).

According to a report, the chemical components of green tea have antibiotic-like actions by non-selectively lowering the total counts of all microbes (Cao et al., 2005). The route of action of green tea fractions is thought to be the inhibition of phase I enzymes, which are involved in the activation of numerous carcinogens in animals, and the induction of phase II enzymes, which are linked to improved excretion of carcinogens (Duansak et al., 2003).

Additionally, a mixed silage preparation made from maize and green tea waste was successful in enhancing fermentation quality (Cai et al., 2003). Green tea waste was discovered to improve silage's lactic acid fermentation by (Kondo et al., 2004a, b, 2006). In studies with goats (Kondo et al., 2004c), sheep (Xu et al., 2003, 2004), and late-lactation Holstein cows, the feeding value of green tea waste silage has been examined (Eruden et al., 2003).

Objectives: The study was conducted for the following reasons:

1. To determine the proximate components of the green tea leaves powder available in the Chattogram region.
2. To measure the total phenolic components of green tea leaves powder.

Chapter 2: Materials and Methods

Study Site:

The sample collection was done from Kodala Tea Estate at Rangunia in Chattogram district, Bangladesh. The Kodala Tea Estate is in the Karnaphuli river basin,10 km south of Rangunia Upazilla Sadar in Chattogram. The laboratory analysis was performed at the Animal Nutrition laboratory of the Chattogram Veterinary and Animal Sciences University. The Chattogram district is one of the well-known hilly regions of Bangladesh. Chattogram lies at 22°20′06″N 91°49′57″E. It straddles the coastal foothills of the Chattogram Hill Tracts in southeastern Bangladesh. The Karnaphuli River runs along the southern banks of the city, including its central business district.

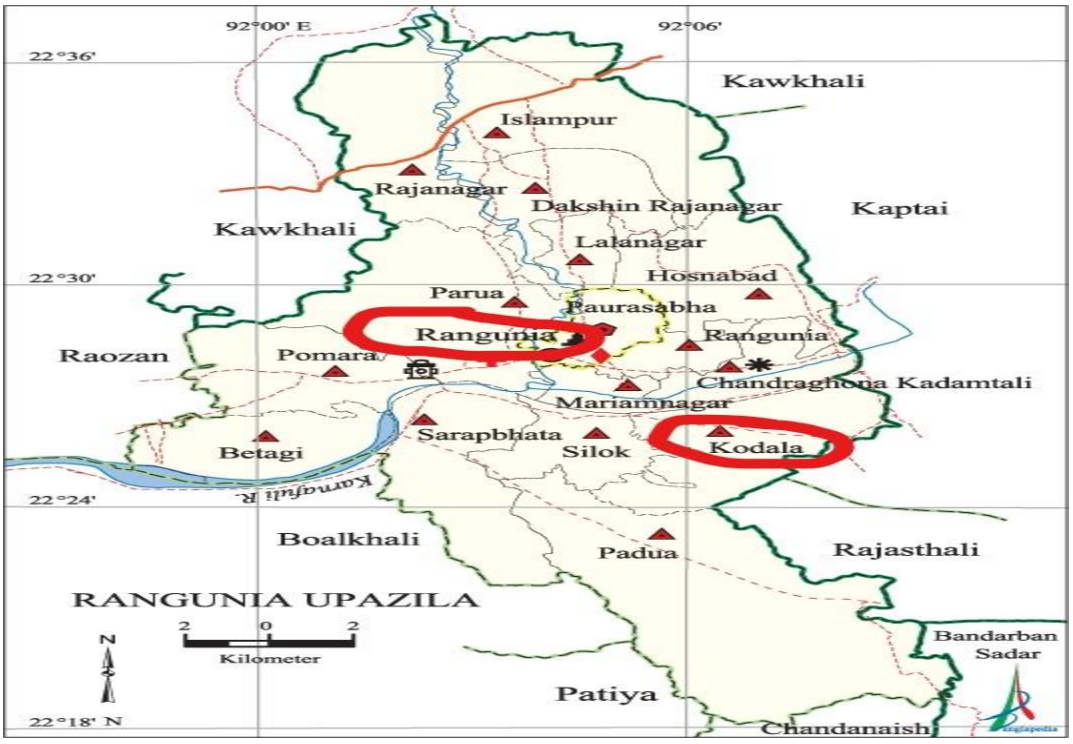


Figure 1: Study site

Table 1. Plants with scientific, family, and common names

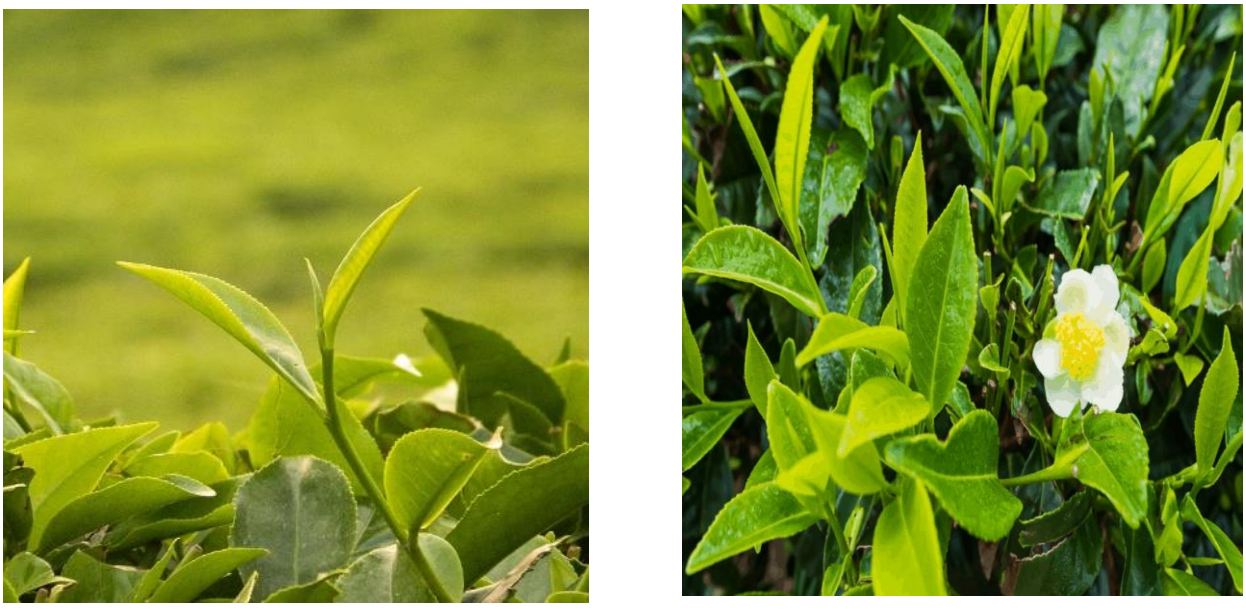
Plant species	Family	Common name	English name
Camellia sinensis	Theaceae	Tea plant, tea shrub	Green tea

Plant Sample Collection:

I collected fresh green tea leaves from Kodala Tea Estate for this experiment. Then, the sample was sun-dried, then ground the leaves in an electric grinder and make into a fine powder and sieve the powder and stored in small zipper bags until further chemical analysis.

Plant Sample Identification:

Identification of samples was done in the department of Animal Science and Nutrition, Chattogram Veterinary and Animal Sciences University. A table of these plants with scientific, family, and common names is given in Table 1.



(a) (b)

Picture 1 (a, b): Green tea *(Camelia sinensis)*

Proximate Analysis:

Proximate analysis procedures including the percentage of moisture, crude protein, ash contents, and crude fiber contents in the sample were determined by following AOAC (2006).

Determination of Moisture :

I accurately weighed the crucible added 6gm sample to the crucible, and reweighed. Then, I placed the crucible in a hot air oven at 105 ºC and dried it for 48-72 hours. After that, I removed the crucible from the oven, covered it in a desiccator, and weighed it. Finally, re-dried repeatedly until constant was achieved.

$$\%\text{Moisture} = \frac{\text{Weight of fresh sample -Weight of dried sample}}{\text{Weight of dried sample}} \times 100$$

Determination of Crude Protein (CP):

Crude protein was measured in three steps. In digestion, I measured 1gm of the sample and added 5 gm of digestion mixer and 20 ml concentrated sulphuric acid to it. Then, I placed the digestion flask the on Kjeldahl digestion set and gradually increased heat, and digestion up to clear residue. After that, removed the flask and cooled it. In Distillation, 20 ml of distilled water was added to the cooled clear residue, transferred the content to distillation, added 100 ml of 40% NaOH solution, and set the condenser. Then, I added 20 ml of 2% boric acid solution and mixed indicator in a conical flask and heated the distillation flask and collected 100 ml of distillate. Finally, I titrated the distillate against standard N/10 HCL solution.

$$\%\text{CP} = \frac{\text{Volume of standard N/10 HCL solution x Normality of standard HCL solution} \times 0.014 \text{ x } 6.25}{\text{Weight of the sample}} \times 100$$

Determination of Crude Fiber:

The crude fiber was measured with the help of the method of acid-base digestion. 1.25% of diluted sulfuric acid and 1.25% of sodium hydroxide used. Put the sample in a beaker and 200 ml of sulfuric acid was added. For half an hour boiled the sample and chilled the sample and filter them using filter paper. The material was washed three times using distilled water. The material was then transferred into the beaker and again digested using 200 ml of sodium hydroxide, boil it for 30 minutes, cooled, and then filtered to obtain residues of the sample, washed three times by using 25 ml of ethanol. This material was dried by putting it into the oven, cooled, and weight.

$$\%CF = \frac{\text{Weight of crucible, crude fiber, and ash} - \text{Weight of crucible and ash}}{\text{Weight of sample}} \times 100$$

Determination of Ether Extract (EE):

Around 2gm of grounded leaves powder was weighed and placed into a thimble. The Ether extraction beaker was carefully cleaned, dried in a hot air oven, and placed into a desiccator to cool down then recorded the weight of the beaker. Place the thimble into the extractor and close the top with cotton. Fit the extractor and pour ether up to siphoning. Again, pour ether up half of the previous amount. Switch on the heater and continue boiling at 40-60 ºC for 6-8 hrs. Place the flask in the hot air oven and heat at 100 ºC up to constant weight. Cool the flask in a desiccator and weight to measure ether extract.

$$\%EE = \frac{\text{Weight of the flask with ether extract} - \text{Weight of the flask}}{\text{Weight of the sample}} \times 100$$

Determination of Ash:

Around 5gm of grounded leaves powder was weighed and placed into a crucible and burnt the sample with crucible up to no smoke. Then, cooled the sample and transferred to muffle furnace at 550-600 ºC for 6-8 hours until white ash. After that, cooled at 150 ºC, transferred it to the desiccator and cooled the sample and weighted.

$$\%ASH = \frac{\text{Weight of crucible and ash} - \text{Weight of crucible}}{\text{Weight of sample}} \times 100$$

Determination of Nitrogen Free extracts (NFE):

Nitrogen free extract was calculated by subtracting %Moisture, %CP, %CF, %EE and %Ash from 100. The formula for NFE is as follow:

% NFE = 100 - (% Crude protein + % Crude fiber + % Ether extract + % Ash)

Determination of total phenolic contents:

First, I poured 30 ml methanol into a conical flask and measured 10 grams of green tea powder into it. Then, I wrapped the open part of the flask with aluminum foil and left it in the dark for 72 hours. After 72 hours, the flask was mixed and filtered with Whatman filter paper. The filtered liquid was placed in a rotary evaporator at 55 ºC,110 to 165 rpm for 4-5 minutes, and finally got 1ml methanol extract. After sample preparation,6 ml ethanol and 1.5 ml Folin -Ciocalteu (F.C) reagent were added to a 1 ml stock sample. Then, waited for 3 minutes and added 1.5 ml of sodium bicarbonate solution (standard 7.5 gm/100 ml distilled water) and incubated for 60 minutes at room temperature. Finally, read absorbance at 760 nm by using UV-spectrophotometer. Both standard and samples were run four times in the UV-Spectrophotometer.

Table 2: Concentration of the Standard reagents for total phenol contents determination

No.	ID	Type	Conc[mg/L]	Absorbance	760nm
1	STD-2	Standard	2.0	0.502	0.502
2	STD-3	Standard	3.0	0.555	0.555
3	STD-6	Standard	6.0	0.601	0.601
4	STD-7	Standard	7.0	0.673	0.673

Chapter 3: Results

3.1. Proximate component of green tea leaves powder:

The results of the chemical analysis are presented below in Table 1. Dry matter, Moisture, crude protein, ether extract, crude fiber, and ash are expressed in percentages. The current study found DM 26.96%, CP 26.76%, EE 1.67%, CF 12.49%, Ash 6.89% and NFE 52.19%.

Table 1: Proximate component of green tea leaf powder

Proximate content	Amount (%)
DM	26.96
Moisture	73.04
Crude Protein	26.76
Ether extract	1.67
Crude Fiber	12.49
Ash	6.89

3.2. Total phenolics component of green tea leaves extract:

The antioxidant activity of plant extracts lies in the presence of polyphenols that reduces damage caused by free radicals. It also prevents slow damage to cells and unstable molecules present in the body. The current study found phenolic concentration were 95.5 mg/l; 95.3 mg/l; 95.7 mg/l; 95.3 mg/l respectively. The average phenolic concentration was 95.45mg/l.

Table 2: Total phenolic contents of green tea leaf powder

No.	ID	Type	Conc[mg/L]	Absorbance	760nm
1	Sample 1	Unknown	95.5	3.194	3.194

2	Sample 2	Unknown	95.3	3.188	3.188
3	Sample 3	Unknown	95.7	3.199	3.199
4	Sample 4	Unknown	95.3	3.186	3.186
			Avg. 95.45		

Chapter 4: Discussion

The chemical composition of green tea is presented in table 2. In *Camelia Sinensis*, the Dry matter value was 26.96% which is lower than Pradhan and Dubey, (2020) findings who found 87.87% DM. The Crude protein value was 26.76 which is higher than Pradhan and Dubey, (2020) findings who found 22.86% Crude protein. The presence of 21–28% of crude protein in tea residues has also been reported on a dry weight basis (Shen et al, 2008). The crude protein present in a plant sample was appropriate according to the available ISO data. Moreover, tea protein mainly contains water soluble 82% glutelin and 13% (Wang et al, 2014). In perspective, the beneficial properties of tea are also due to the presence of bioactive compounds.

The Crude fiber value was 12.49% which is lower than Pradhan and Dubey, (2020) findings who found 16.05% crude fiber. The tea prepared from young leaves consists of very low fiber content while mechanically harvested tea consisted of a high amount of fiber due to the presence of stem (Œmiechowska and Dmowski, 2006). Crude fiber helps to stimulate the movement of the bowel and to prevent constipation (Mohammed and Sulaiman, 2009). Total Ash was 6.89% which is higher than Pradhan and Dubey, (2020) findings who found 5.94% Ash. The ash content in any plant sample is an indication of the presence of mineral content in a balanced amount Pradhan and Dubey, (2020).

The average of total phenolic contents of green tea leaf powder was 95.45 mg/l. The antioxidant activity helps the plants to prevent several diseases related to the cardiovascular system, liver, brain, kidney, and cancer and might also reduce aging (San et al., 2010; Leon et al., 2011). Most medicinal plants exhibit efficient antioxidant properties due to their phenolic constituents (Vani et al, 1997). The solvent extraction method plays a significant role in the extraction of phenolic compounds from food material in which the selection of extracting solvent is important for the recovery of phenolic compounds (Lim et al, 2019).

The glucose level rises in the blood circulation could give rise to diabetes and other metabolic disorders such as atherosclerosis, myocardial infarction, nephropathy, etc. (Mori et al, 2002). Due to the presence of polyphenol, tea could be considered a medicinal plant (Haidari et al, 2013). Traditional plants used as a medicine for diabetes also have a higher antioxidant activity Pradhan and Dubey, (2020). It may be possible that plant extracts may reduce the effect of

inflammatory cytokine release during diabetes which may be one of the causative reasons for insulin resistance (Sabu and Kuttan, 2002).

Chapter 4: Conclusion

Crude protein is an essential nutrient component for the livestock feed industry. Green tea leaves powder contains an impressive amount of protein and phenolic compounds. Therefore, green tea leaves powder can be a potential source of protein-rich feed additives in the livestock feed industry.

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Biography

The author is Md. Sohanur Rahman, son of Late. Maynal Hoque and Zahanara Khatun. He is the dweller of Rowmari, Kurigram. He completed S.S.C in 2013 from Komar Vangi High School, and Kurigram H.S.C in 2015 from Bangladesh Agricultural University College, Mymensingh. He got admitted to Chattogram Veterinary and Animal Sciences University for the degree of Doctor of Veterinary Medicine course in the 2016-2017 sessions. He is currently an intern student at the Faculty of Veterinary Medicine. He is very enthusiastic to be a researcher and is eager to be a skilled veterinarian in the future.