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Research article

Tea (*Camellia sinensis*) cultivated in three agro-ecological regions of Bangladesh: Unveiling the variability of methylxanthine, bioactive phenolic compound, and antioxidant activity

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ABSTRACT

Tea (*Camellia sinensis*) is a widely consumed beverage known for its numerous health benefits, largely attributed to its rich content of quality determining secondary metabolites such as methylxanthine compounds and bioactive phenolic compounds. The goal of this study was to find out variations of the levels of methylxanthines, bioactive phenolic compounds, and antioxidant activity in methanolic and hot water extracts of 129 tea samples grown in three different ecological regions of Bangladesh named Panchagar, Sylhet, and Chattogram. Methylxanthine and bioactive phenolic compounds were determined by using HPLC-DAD, and the antioxidant profile was analysed by UV–vis spectrophotometric methods for methanol and hot water extracts of tea leaves. The IC_{50} values showed the trend as Panchagar > Sylhet > Chattogram and Sylhet > Chattogram > Panchagar for water and methanol extract, respectively. The results revealed significant ($p < 0.05$) variations in the levels of methylxanthines content: Panchagar > Chattogram > Sylhet. Caffeine was significantly higher (103.02 ± 5.55 mg/g of dry extract) in the methanolic extract of tea leaves of Panchagar district and lower (53.33 ± 4.30 mg/g of dry extract) in the hot water extract of Sylhet district. Panchagar and Chattogram possessed significantly ($p < 0.05$) higher catechin content for methanol (57.01 ± 5.50 mg/g dry extract) and hot water (55.23 ± 4.11 mg/g dry extract) extracts, respectively. The utilization of canonical discriminant functions yielded highly favorable outcomes in the classification of tea from three distinct cultivation origins in Bangladesh, relying on their inherent features. This study demonstrated the potential effects of geographical variations on the bioactive compounds and antioxidant properties of tea, emphasizing the importance of regional differences in tea cultivation for optimizing its health benefits as well as dispersing tea cultivation across the country.

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1. Introduction

Tea (*Camellia sinensis*) belongs to family Theaceae which is an evergreen plant grows mainly in tropical and sub-tropical region. Tea, which is obtained from the leaves of the *Camellia sinensis* plant, is a widely consumed beverage globally due to its invigorating flavor and several advantageous effects on health. In 2016, 65.05 million kg of tea was produced from near about 167 tea gardens in Bangladesh and was increased to 93.83 million kg in 2022 [1]. Bangladesh is a vital tea producing country, being the world's tenth most immensely tea producer [2]. Generally, tea is grown in the northern and eastern part of the country include Sylhet, Maulvibazar, Habiganj, Brahmanbaria, Rangamati, Chattogram and Panchagar district [1]. These tea producing zones encompass with three agro-ecological zones and various tea cultivars are cultivated due to different climatic condition and geographical location [3]. Bangladeshi tea is cultivated in three distinct ecological zones, which are the Surma valley in greater Sylhet, the Halda valley in Chattogram, and the Karatoa valley in Panchagar district [4]. According to the available data, Sylhet has been identified as the region experiencing the lowest temperatures in Bangladesh, with an average high temperature of merely 25 °C [5]. Throughout several months of the year, the prevailing temperatures consistently exceed 25 °C, occasionally reaching as high as 28°. The period characterized by substantial rainfall and significant precipitation spans from the month of June to July. The annual average precipitation measures 4200 mm, while the humidity levels fluctuate between 55% and 89%. Chattogram exhibits a tropical climate. Chattogram experiences substantial precipitation throughout most of the months, accompanied by a brief period of reduced rainfall. According to the source, the mean temperature in Chattogram is recorded to be 25.3 °C [6]. July exhibits the highest relative humidity among the months, with a recorded value of 88.41%. February exhibits the lowest relative humidity, measuring at 67.90%. There is a fluctuation of 24 inches in rainfall between the month with the least precipitation and the month with the most precipitation. The period from May to September experiences the highest frequency of rainy days. The climatic conditions of Panchagar exhibit distinct characteristics between the wet and dry seasons. The rainy season is characterized by high temperatures, a sense of oppressiveness, and predominantly gloomy skies. Conversely, the dry season is marked by warm temperatures and bright skies. According to the climatic data for the Rangpur Division in Bangladesh for the given year, the temperature exhibits a range of fluctuations between 10 °C and 37 °C. It is infrequently observed to fall below 8 °C or surpass 41 °C [7]. The duration of the rainy season spans a period of eight months, commencing in March and concluding in November. During this time, there is a consistent and gradual increase in precipitation, with a minimum rainfall of at least 0.5 inches seen for a period of 31 consecutive days. In Panchagar, the month characterized by the highest precipitation is July, exhibiting an average rainfall of 13.5 inches. A review information on a long-term analysis of region wise weather and soil conditions was given in Supplementary Table S1.

Phytochemicals content of tea samples show a discrepancy based on climatic and geographical, plucking, shade, fertilizer, seasonal, processing, storage, and preparation variability [8]. Among all phytochemicals, polyphenols constitute are the most important bioactive molecules in tea and the predominant polyphenols are catechins, which metabolized into dimmers and oligomers with increasing degrees of oxidation of tea leaves [9–11].

Tea polyphenols, including flavonoids, and their resultant oxidative products have been associated with several pharmacotherapeutic benefits, including the potential to protect against cardiovascular illnesses [12] and cancer in humans [13]. The potential benefits of the intervention include immunosuppressive effects and the reduction of oxidative stress [14]. Additionally, it has been suggested that the intervention may have anti-diabetic properties, including the ability to lower hyperglycemia [15]. Furthermore, the intervention has been associated with the reduction of cholesterol and triglyceride levels, as well as the decrease in fat tissue accumulation [16]. Lastly, there is potential for the intervention to enhance cognitive learning abilities in specific populations [17]. In addition to this, tea catechins have been found to be accountable for the suppression of carcinogenesis across all three stages [18,19].

Tea contains various bioactive compounds like methylxanthines, including caffeine, theobromine, and theophylline, are naturally occurring compounds in tea that have been linked to various physiological effects. These compounds possess stimulant properties, improving cognitive function, promoting alertness, and boosting the central nervous system. Phenolic compounds, on the other hand, are renowned for their potent antioxidant properties. Caffeine undergoes demethylation, leading mostly to the liberation of paraxanthine, theobromine, and theophylline. These compounds are non-nutritive phytochemicals that possess protective or disease-preventive qualities [20].

Quality of harvest is a multi-attribute parameter that includes the nutritional, health, and sensory traits of crops due to the concentrations of primary and secondary metabolites, minerals, and phytochemicals, in association with organoleptic properties, biological activity, shelf life as well [21,22]. While the literature stipulates considerable demonstration on the influence of environmental variation on crop yields [23–25], not many research have focused on the effects of environmental distinction on crop quality [22,26]. Distinction in geographical factors have resulted in upsurge or decline of numerous secondary metabolites in a wide variety of food and beverage crops including tea [27,28]. Until present, several studies have looked into the amount of methylxanthines and polyphenols in black and green tea from well-known commercial brands in Bangladesh [29–31]. However, the quantity of secondary metabolites in tea produced in Bangladesh's various agro-ecological zones as well as the consistency of environmental elements like temperature, precipitation, elevation, and sun radiation have not yet been discussed.

In this study, with a fresh insight the variability of secondary metabolites such as methylxanthine, bioactive phenolic compounds particularly quality definitive catechin and antioxidant activity in tea (*Camellia sinensis*) across three distinct agro-ecological regions of Bangladesh in conjunction with environmental factors have been explored. By conducting a comprehensive comparative analysis, it unveils the unique regional profiles of these key secondary metabolites, highlighting the influence of diverse environmental conditions on tea quality. The investigation encompasses not only the total phenolic content but also specific bioactive phenolic compounds, providing valuable insights into the phytochemical diversity of different tea varieties. Additionally, the evaluation of antioxidant activity contributes to a better understanding of the variations in the tea's overall antioxidant potential. These findings have practical

implications for quality control, market differentiation, and site-specific cultivation strategies, while also offering a scientific basis for potential tea geographical indication, ensuring the protection and promotion of tea from specific agro-ecological regions in Bangladesh.

2. Materials and methods

2.1. Sample collection

Young and light green top five leaves of 40–45 days matured of *Camellia sinensis* were collected from three distinct ecological tea growing region of Bangladesh named Panchagar (n = 24), Chattogram (n = 56) and Sylhet (n = 49) during the period of march to April. The locations of samples are J.K. Tea Garden, Baniapara, Panchagar (Latitude: 26.33° N, Longitude: 88.56°E); Bangladesh Tea Research Institute (BTRI) sub-station, Fatikchari, Chattogram (Latitude: 22.69° N, Longitude: 91.79° E) and Bangladesh Tea Board (BTB), Lackatoorah, Sylhet (Latitude: 24.9° N, Longitude: 91.87° E) (Fig. 1 and Table S2).

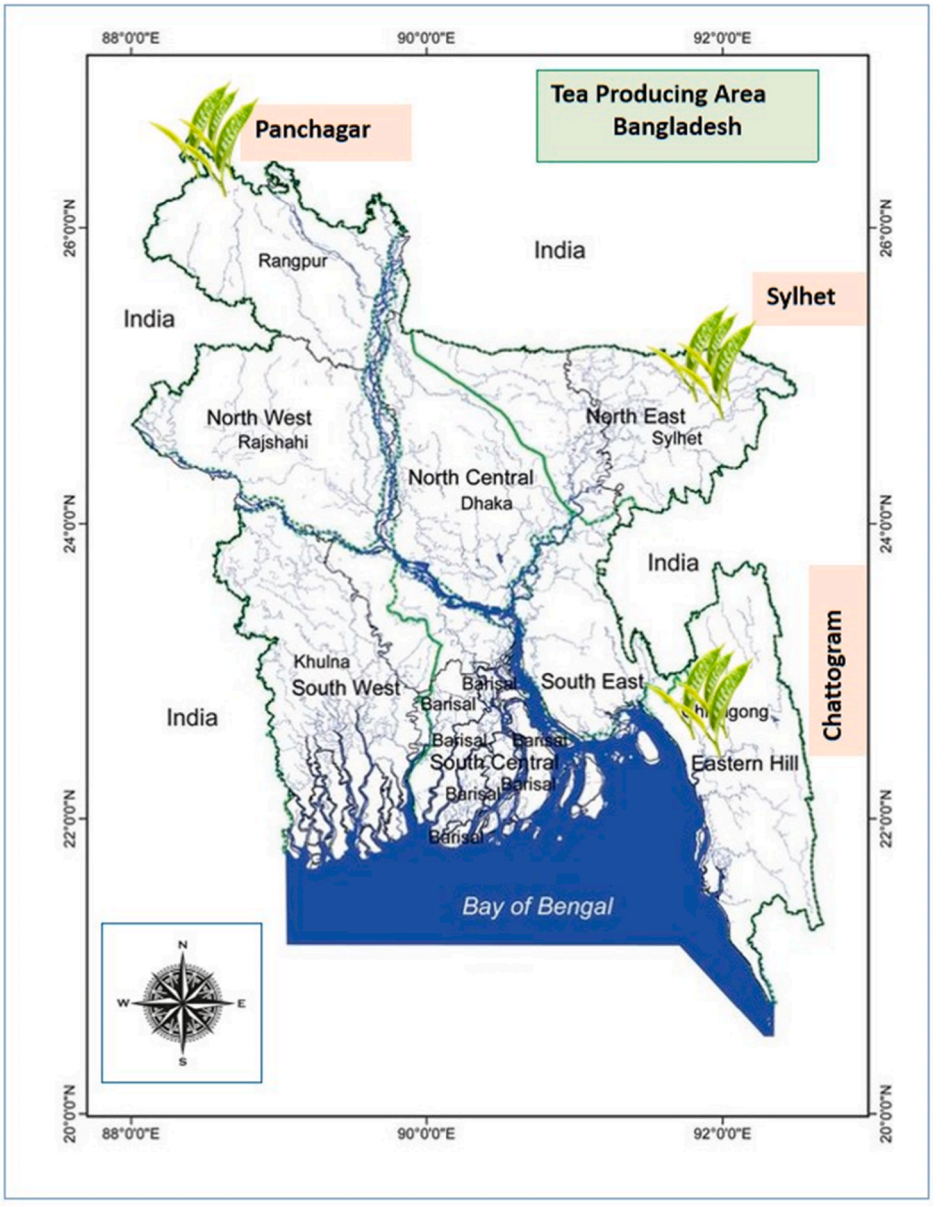

Fig. 1. Three distinct ecological tea growing region of Bangladesh: Panchagar (Latitude: 26.33° N, Longitude: 88.56°E); Chattogram (Latitude: 22.69° N, Longitude: 91.79° E) and Sylhet (Latitude: 24.9° N, Longitude: 91.87° E).

2.2. Sample extraction

Tea leaves were freeze dried at −42 °C (Thermo Fisher Modulyod-230) and powdered by a grinder and then stored at 4 °C in plastic bags. In case of hot water extraction, the 10 g powdered sample was taken into a round bottom flask with 100 mL water and boiled at 100 °C for 30 min. After cooling, the sample was centrifuged and filtered. The filtrate was then dried by freeze drier. On the other hand, 10 g dried sample was dissolved in 100 mL methanol and mixed by a shaker (GFL orbital shaker 3005) for 48 h at room temperature (23.4 °C). Then the sample was centrifuged at 5000 rpm and filtered. The filtrate was then concentrated by vacuum rotary evaporator (IKA RV10 D S99). Both extracts were stored at −18 °C for further analysis.

2.3. Chemicals and reagents

The standard chemicals obtained from Sigma (St. Louis, MO, USA) included caffeine, theobromine, theophylline, catechin hydrate, epicatechin, rutin hydrate, gallic acid, syringic acid, arabinonic acid, *p*-coumaric acid and vitamin C. The solvents used for extraction and analysis are methanol, acetonitrile, acetic acid, and IPA. The Folin–ciocalteu reagent is employed for the determination of total phenolic content. The chemicals 1, 1-diphenyl-2-picryl hydrazyl (DPPH), sodium acetate, tri-sodium hydrogen phosphate, sodium carbonate, aluminum chloride, ammonium molybdate, sulfuric acid, and potassium di-hydrogen phosphate were procured from Sigma (St. Louis, Missouri, USA). The water underwent purification using a Milli-Q system (Millipore, Bedford, MA, USA) to achieve a purity of 18 MΩ cm^{-1}.

2.4. Yield determination

The yield percent was computed to monitor the impact of the solvent system on the extraction process. The following formula was used to get the percentage yield:

$$\text{Yield } (\%) = 100*(\text{A-B})/\text{W}.$$

The weight of the extract-containing flask after evaporation is represented by A in this equation, the weight of the empty flask after drying is represented by B, and the weight of the dried sample is represented by W [32].

2.5. Determination of antioxidant profile

2.5.1. Total flavonoid content (TFC)

The determination of flavonoids was conducted using the aluminum chloride colorimetric method [32,33]. Initially, the reagent was prepared by combining 0.3325 g of crystalline aluminum chloride ($AlCl_3$) with 1 g of crystalline sodium acetate in 100 mL of deionized water. The sample was allowed to thaw at ambient temperature, followed by the addition of 4.8 mL of water. An additional 2.5 mL of reagent was introduced into the mixture, which was thereafter allowed to incubate for a duration of 5–6 min. The optical density (OD) was measured at a wavelength of 430 nm using the Thermo Scientific UV-VIS spectrophotometer (Model, Evolution 300), which operates on a double beam principle.

To generate the calibration curve, a solution of quercetin weighing 0.01 g was dissolved in 100 mL of methanol using both an ultrasonic bath and vortexing techniques. This resulted in the preparation of stock solutions with a concentration of 100 μg/mL. Subsequently, a series of solutions with concentrations of 80 μg/mL, 60 μg/mL, 40 μg/mL, and 20 μg/mL were generated through the process of serial dilution, utilizing the standard solution derived from the stock solution. Following this, a calibration curve was established. The quantification of flavonoids was reported as milligrams of quercetin equivalent per gram of dry extract.

2.5.2. Total tannin content (TTC)

The tannin content was quantified with the Folin-ciocalteu phenol reagent [32,34]. A 0.5 mL aliquot of a 10000 μg/mL extract was combined with 8.5 mL of distilled water and 0.5 mL of Folin-ciocalteu phenol reagent. The mixture was then allowed to incubate at room temperature for a duration of 5 min. Subsequently, a volume of 1 mL of a sodium carbonate solution with a concentration of 35% was introduced. The solution was vigorously agitated, allowed to equilibrate at ambient temperature for a duration of 20 min, and thereafter subjected to spectrophotometric analysis at a wavelength of 725 nm. A series of standardized solutions containing tannic acid were measured using a blank as a reference. The tannin findings were quantified in milligrams of tannic acid per gram of dry extract.

2.5.3. Total phenolic content (TPC)

The quantification of total phenolics was conducted with the Folin-ciocalteu phenol reagent [32,35] A 0.5 mL aliquot of a 10000 μg extract per mL sample was combined with 8.5 mL of distilled water and 0.5 mL of Folin-ciocalteu phenol reagent. The mixture was then incubated at room temperature for a duration of 30 min. Next, a volume of 1 mL of a sodium carbonate solution with a concentration of 35% was introduced. The solution was vigorously agitated, allowed to equilibrate at ambient temperature for a duration of 20 min, and the optical density was afterwards determined at a wavelength of 765 nm. The phenolic data were quantified and reported as the amount of gallic acid in milligrams per gram of dry extract.

2.5.4. Total antioxidant activity (TAA)

The assessment of the extract's overall antioxidant activity was conducted using the phosphomolybdenum assay method. This method relies on the extract's ability to reduce Mo (VI) to Mo (V), resulting in the formation of a green phosphate-Mo (V) complex under acidic conditions [32,36,37]. The sample was combined with a reagent solution consisting of 3.0 mL of a mixture containing 0.6 M H_2SO_4, 28 mM Na_3PO_4, and 4 mM ammonium molybdate. The resulting reaction mixture was then subjected to incubation at a temperature of 95 °C for a duration of 90 min. Following the process of cooling to room temperature, the absorbance of the solution was quantified at a wavelength of 695 nm. The quantification of antioxidant activity was determined by measuring the amount of milligram equivalents of ascorbic acid.

2.5.5. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity

The DPPH radical functions as the oxidizing radical that undergoes reduction by the antioxidant. The measurement of scavenging activity was conducted using the modified approach outlined by Chang et al. [38]. In this experimental procedure, a volume of 2 mL of methanol DPPH solution was combined with 2 mL of extract solution at several concentrations. The resulting mixture was subjected to vigorous stirring for a duration of 15 s. Subsequently, the solutions were let to remain undisturbed in a dimly lit location at ambient temperature for a duration of 10 min to facilitate the occurrence of the desired chemical reaction. Absorbance at a wavelength of 517 nm was measured against a blank after a duration of 10 min, as described in the studies conducted by Chang et al. [38,39].

The equation used to determine the activity of DPPH radical-scavenging is expressed as follows: DPPH radical-scavenging activity (%) = {(A0-A)/A0}X100. In this equation, A0 represents the absorbance of the control solution, which contains all reagents except for the plant extracts. On the other hand, A represents the absorbance of the DPPH solution including the plant extract. The percentage of DPPH radical-scavenging activity was graphed against the concentration of the sample extract in order to ascertain the concentration required to reduce DPPH radical-scavenging by 50%, commonly referred to as IC_{50}. The slope for the nonlinear regression analysis was determined by constructing a graph that plotted the percentage of DPPH radical inhibition against the logarithmic concentration values of DPPH.

2.6. Determination of methylxanthines (caffeine, theobromine, theophylline)

2.6.1. Standard preparation

Theobromine, theophylline, and caffeine were dissolved in water to create individual stock standard solutions with concentrations of 400 μg/mL, 1000 μg/mL, and 1000 μg/mL, respectively.

2.6.2. Sample preparation

Hot water and Methanol extracts (0.1 gm) were dissolved into water (10 mL) and methanol (10 mL) respectively. Solutions were vortex and sonicated, which were used for chromatographic analysis.

2.6.3. HPLC system for methylxanthines analysis

The Thermo Scientific Dionex Ultimate 3000 Rapid Separation LC (RSLC) system, manufactured by Thermo Fisher Scientific Inc. in Massachusetts, USA, was utilized for conducting chromatographic analyses. This system was equipped with many components, including a quaternary pump (LPG-3400RS), an Ultimate 3000RS autosampler (WPS-3000), and a diode array detector (DAD-3000RS). The separation of caffeine, theobromine, and theophylline was conducted using an Acclaim® C18 column (4.6 × 250 mm; 5 μm) manufactured by Dionix, USA. The processes of data collecting, peak integration, and calibrations were executed using Dionix Chromeleon software (Version 6.80 RS 10).

2.6.4. Chromatographic conditions

The separation and quantification of theobromine, theophylline, and caffeine in tea leaves were performed using high-performance liquid chromatography (HPLC), as outlined in the studies conducted by del Rosario Brunetto et al. and Bispo et al. with certain adjustments made [40,41]. The mobile phase employed for the transfer and separation of analytes consisted of a 40% methanol solution in deionized water (v/v) at a constant flow rate of 1.0 mL/min. The injection volume utilized was 20 μL. In order to identify PDA, the wavelength program was optimized, and peak purity match was taken into account for monitoring methylxanthine compounds at their respective maximum absorbance wavelength of λ 276 nm. The whole duration of the analysis was 10 min.

2.6.5. Peak characterization and quantification

Methylxanthines were identified through a comparison with the chromatogram of standards, utilizing the retention duration and the absorbance spectrum profile. Calibration curves were created using a linear model, where the peak area was plotted against the concentration of standards. The coefficient of determination (r^2) for these calibration curves exceeded 0.995, indicating a strong correlation between the peak area and concentration. The data were presented in the form of mean values accompanied by their corresponding standard deviations, which were obtained through three separate and independent investigations.

2.7. Identification of bioactive polyphenolic compound

2.7.1. Standard solution for polyphenolic compounds

A standard solution of each phenolic component was made in methanol at a concentration of 100 μg/mL. The mixed standard

solution was then created by diluting the mixed stock standard solutions in methanol, resulting in a concentration of 5 μg/mL for each polyphenol. The calibration curves for the standards were generated by the process of diluting the stock standards using methanol. This involved creating five sets of standard dilutions, resulting in concentrations ranging from 1.0 to 5.0 μg/mL. The calibration curves were generated by plotting peak area against the concentration of the standard. The identification of the compound was determined by assessing the match of peak purity.

2.7.2. HPLC system for polyphenolic analysis

The same HPLC system described in section 2.6.3 was used. The separation of phenolic compounds was conducted using an Acclaim® Polar Advantage II (PAII) C18 column (4.6 × 250 mm; 5 μm; 120 A°) manufactured by Dionix, USA. The column was maintained at a controlled temperature of 30 °C using a temperature-controlled column compartment (TCC-3000). The process of data collecting, peak integration, and calibrations was executed using Dionix Chromeleon software, namely Version 6.80 RS 10.

2.7.3. Chromatographic condition

The mobile phase was composed of four solvents: acetonitrile (solvent A), acetic acid solution with a pH of 3.0 (solvent B), methanol (solvent C), and a 5% solution of IPA (solvent D). A 5-min post-run was conducted under initial circumstances to achieve column equilibration. The flow rate was maintained at a constant value of 1 mL/min throughout the study, while the injection volume was set to 20 μL. To detect PDA, the wavelength program was tuned to observe phenolic chemicals at a wavelength of 280 nm [32,42,43].

2.8. Canonical discriminant functions (CDF)

CDF have been used to classify the tea samples to three groups of their origins based on their antioxidant profile, polyphenol and methylxanthine parameters.

2.9. Statistical analysis

Initially, the descriptive statistics of several test parameters of tea from three distinct sources in Bangladesh, namely Panchagar, Chattogram, and Sylhet, were calculated. These data included the mean and standard deviation (SD). An Analysis of Variance (ANOVA) was conducted to assess the equality of the parameters found in tea samples from three distinct areas in Bangladesh. The duncan multiple rank test (DMRT) of Post Hoc test was conducted for the parameters that exhibited significant variation ($p < 0.05$). Data analysis was conducted using version 22.0 of the statistical software SPSS. The data were visualized using RStudio version

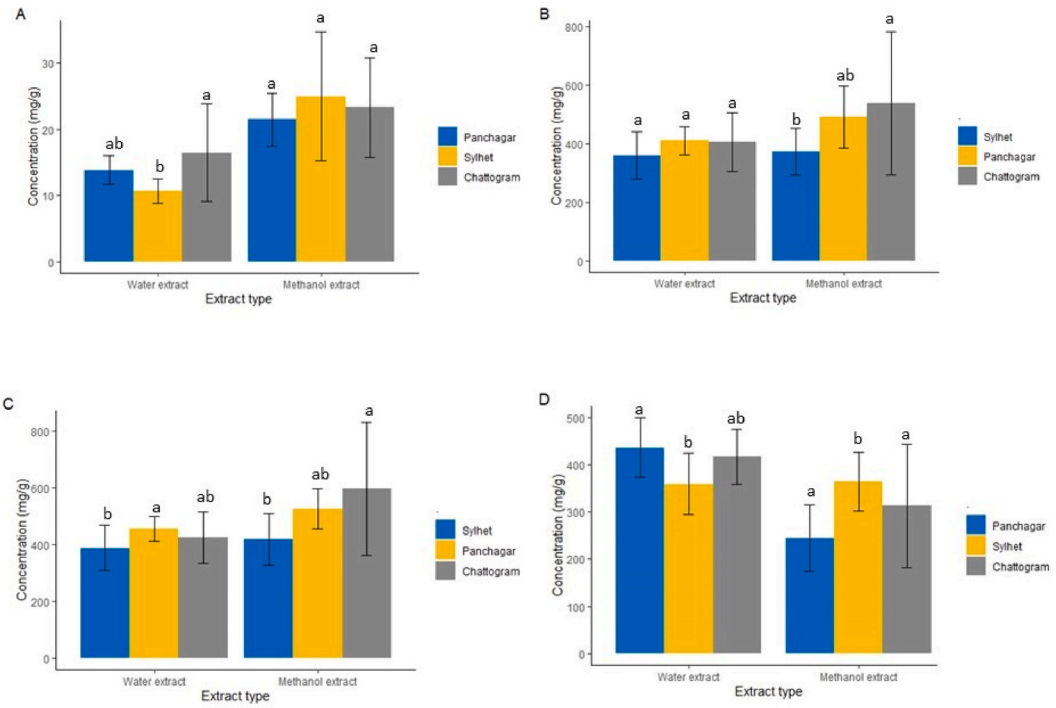

Fig. 2. Antioxidant profile of tea extracts. A: total flavonoid content (TFC), B: total tannin content (TTC), C: total phenolic content (TPC), D: total antioxidant activity (TAA).

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3. Results and discussion

3.1. Yield extract (%)

The biochemical composition of the raw material affects the yield at which active components can be extracted from plant sources. Enhancing the recovery of phenolic and flavonoid chemicals is largely dependent on the extractant's polarity [44]. The yield of methanol and hot water extract from different tea leaf were 19.60 ± 5.6 % and 20.07 ± 4.01% respectively, (Supplementary Table S3). El Mannoubi states that the polarity and content of the plant material components are important considerations for selecting an adequate extraction solvent [45].

3.2. Quantification of antioxidant profile

3.2.1. Total flavonoid content (TFC)

The Flavonoid content in methanolic extract of tea leaf of Panchagar, Chattogram and Sylhet were 21.49 ± 3.04, 23.32 ± 2.45 and 24.97 ± 3.25 (mg QAE/g of dry extract), respectively. No significant difference was observed in Flavonoid content in tea among the three regions (Fig. 2A). The Flavonoid content in hot water extract of tea leaf of Panchagar, Chattogram and Sylhet were 13.86 ± 2.10, 16.48 ± 3.45 and 10.70 ± 1.8 mg QAE/g of dry extract, respectively. The minimum and maximum value were mentioned in Table S3 for all parameter of antioxidant profile. Jakubczyk et al. reported that traditional extraction of tea at 90 °C in water possessed 1460 mg flavonoids/L [46]. Climate, soil, and geographic location, in addition to species, all influence natural ecosystem production. Each of these variables can have a substantial impact on the quantity and quality of plant performance. Many factors, including water, air, soil, elevation, species differences, extraction processes, and antioxidant measurements, are hypothesized to influence the amount of secondary metabolites, such as flavonoids and phenol, in plants [47].

3.2.2. Total tannin content (TTC)

The tannin content in methanolic extract of tea leaf of Panchagar, Chattogram and Sylhet were 490.67 ± 10.7, 537 ± 10.16 and 372 ± 28.65 mg TAE/g of dry extract, respectively (Fig. 2B). The tea from Chattogram contained significantly higher amount of tannin compared to the remaining two. The Tannin content in hot water extract of tea leaf of Panchagar, Chattogram and Sylhet were 410.24 ± 15.79, 405.64 ± 15.50 and 359.40 ± 21.50 mg TAE/g of dry extract, accordingly. However, a study in Turkey expressed that tannin content in the water extract of black and white tea were 34 mg/g and 211 mg/g, respectively. On the other hand, tannin content of ethanol extract of green tea was 266 mg/g in the same study [48].

3.2.3. Total phenolic content (TPC)

The TPC in tea varied according to extraction solvent and growing area (Fig. 2C). Methanolic extracts had a higher TPC than hot water extracts. The TPC of methanol extracts of Panchagar, Chattogram, and Sylhet had 525 ± 21.05, 596.82 ± 20.45 and 417.75 ± 25.55 mg QAE/g of dry extract. Previous studies reported that the phenolic content was 170 mg GAE/g in the methanol extract [49]. The TPC in hot water extracts of Panchagar, Chattogram, and Sylhet were 455.74 ± 25.15, 425.14 ± 30.75 and 388.41 ± 30.56 mg/g

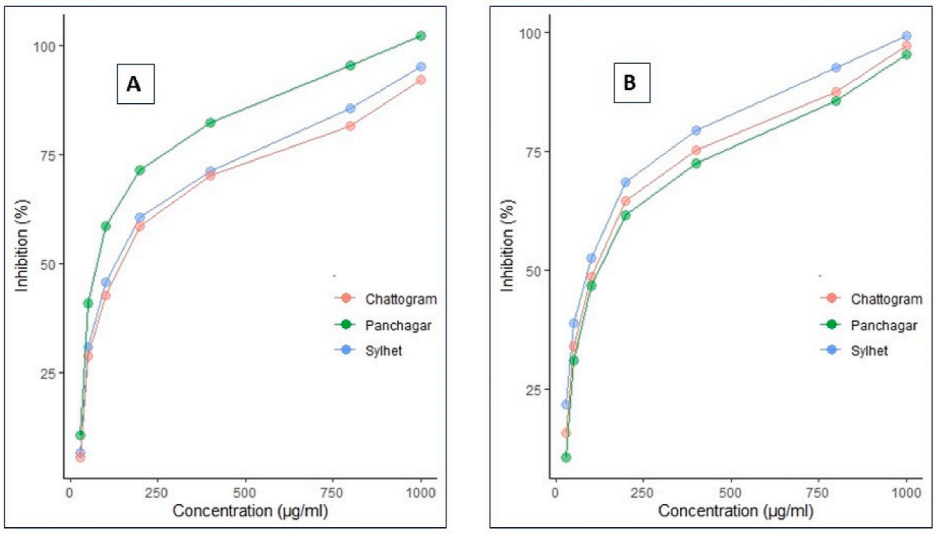

Fig. 3. DPPH scavenging activity of tea extracts. A: methanol extract, B: hot water extract.

of dry extract, respectively. A report by Jakubczyk et al. expressed that 925 μg/g of TPC was present in the water extract of tea [46]. Orak and his co-workers found 245 mg/g of TPC in the water extract of white tea [48].

3.2.4. Total antioxidant activity (TAA)

It was found that the water extracts had a greater TAA than the methanol extracts (Fig. 2D). Regarding water extract, Panchagar exhibited the highest TAA concentration (436.24 ± 12.15 mg/g). Conversely, Sylhet had the highest TAA (359.20 ± 26.55 mg/g of TAA) in the methanol extract. Jakubczyk et al. reported 213 μg/g of TAA in water extract of tea [46]. TAA showed slight negative correlation with TFC and positive weak correlation with gallic acid. Moreover, it had negative weak correlation with rutin hydrate and positive weak correlation with epicatechine, theobromine and theophylline.

3.2.5. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

The IC_{50} values reported were based on the actual experimental work with extrapolation of the data obtained from percent DPPH free radical scavenging activity. The IC_{50} of methanol extract of Panchagar, Sylhet, and Chattogramtea leaf was 82 ± 2.45, 115 ± 1.25 and 124 ± 0.25 μg/mL of dry extract, respectively (Fig. 3A). In comparison to the other two regions, Panchagar tea had significantly higher DPPH scavenging activity. The IC_{50} in hot water extract of tea leaf from Sylhet, Chattogram, and Panchagar, was 160 ± 1.45, 178 ± 2.75 and 189 ± 1.45 μg/mL of dry extract, respectively (Fig. 3B). According to Turkmen et al. solvent that has higher polarity is more efficient to scavenge free radicals than less polar solvent may be the possible reason of higher antioxidant profile of methanol extract than water extract [50]. Kodama et al.conducted a study in which they quantified the DPPH scavenging activity of green tea, expressing the results as a range of 23–131 mmol of Trolox Equivalents (TE) per 200 mL [51]. In another studyshowed IC_{50} ranged from 40 to 70 μg/mL in different variety of green tea in India [52]. Additionally, Gawron-Gzella et al. had reported that the IC_{50} was 263–329 μg/mL in tea leaves of Poland [53].

3.3. Methylxanthine

Methylxanthines found in tea, which are a group of compounds including caffeine, theobromine, and theophylline. Methylxanthines content of tea leaves extract were identified against chromatogram of standards considering retention time of the compounds (Fig. S1 and Table S4) and the UV spectrum was provided in Figs. S2(A–C).

3.3.1. Caffeine

The caffeine content in hot water extract of tea leaf of Panchagar, Chattogram and Sylhet were 63.15 ± 3.55, 66.06 ± 5.25and 53.33 ± 4.30 mg/g of dry extract, respectively (Table 1). The caffeine content in water extract of tea from Chattogram region is significantly higher than the Sylhet region ($p < 0.05$). There was no statistically significant difference between the Panchagar and Chattogram region. Whereas, the caffeine content in methanolic extract of tea leaf of Panchagar, Chattogram and Sylhet were 103.02 ± 5.55, 88.35 ± 4.25 and 70.33 ± 6.65 mg/g of dry extract, respectively (Table 1). The tea from Panchagar contained significantly higher amount of caffeine than other two regions ($p < 0.05$) in methanolic extract. Wu et al. showed that, 15.66–23.50 and 24 mg/g of caffeine was present in green and black tea. Nadiah and Uthumpornalso expressed that caffeine content was 41 and 139 mg/g in green and black tea [49]. The study findings showed that methylxanthines content was found to be higher in methanol extract than water extract, which may be due to the caffeine content of tea, which was also supported by other studies [54,55].

3.3.2. Theobromine

The Theobromine content in methanolic extract of tea leaf of Panchagar, Chattogram and Sylhet were 4.25 ± 0.45, 6.07 ± 1.25 and 3.89 ± 0.25 mg/g of dry extract (Table 1) respectively. The tea from Chattogram contained significantly higher amount of theobromine compared to other two regions in both solvent ($p < 0.05$). Theobromine content in hot water extract of tea leaf of Panchagar, Chattogram and Sylhet were 6.63 ± 1.45, 12.04 ± 2.75 and 6.03 ± 1.45 mg/g of dry extract, accordingly. Theobromine levels in green and black tea were reported to be significantly higher than those observed in the study by Wu et al. and Khanchi et al. who stated that these levels ranged from 0.85 to 1 mg/g and 0.3–0.45 mg/g, respectively [56,57]. On the other hand, the amount of theobromine levels that was determined in this investigation was comparable to the findings that was reported by Sharma et al. [54].

3.3.3. Theophylline

Theophylline content in methanolic extract of tea leaf of Panchagar, Chattogram and Sylhet were 5.03 ± 0.62, 3.76 ± 0.25 and 3.35 ± 0.70 mg/g of dry extract (Table 1), respectively. The tea from Panchagar contained significantly higher amount of theophylline

Table 1
Methylxanthine (mg/g of dry extract) in hot water and methanol extract.

Ecological region	Hot Water Extract			Methanol Extract		
	Caffeine	Theobromine	Theophylline	Caffeine	Theobromine	Theophylline
Panchagar	63.15 ± 3.55^{a}	6.63 ± 1.45^{a}	5.12 ± 1.15^{a}	103.02 ± 5.55^{a}	4.25 ± 0.45^{a}	5.03 ± 0.62^{a}
Chattogram	66.06 ± 5.25^{a}	12.04 ± 2.75^{b}	4.12 ± 2.25^{ab}	88.35 ± 4.25^{b}	6.07 ± 1.25^{b}	3.76 ± 0.25^{b}
Sylhet	53.33 ± 4.30^{b}	6.03 ± 1.45^{a}	2.37 ± 0.55^{b}	70.33 ± 6.65^{b}	3.89 ± 0.25^{a}	3.35 ± 0.70^{b}

Results are presented as Mean ± SD.means containing the same letter (s) in the column did not differ significantly ($p < 0.05$).

compared to others. However, theophylline content in hot water extract of tea leaf of Panchagar, Chattogram and Sylhet were 5.12 ± 1.15, 4.12 ± 2.25 and 2.37 ± 0.55 mg/g of dry extract, respectively. This study showed that tea samples contained much lower theophylline than caffeine. Jeszka-Skowron et al. also reported lower amount theophylline than caffeine [58].

3.4. Bioactive polyphenolic compound

Five polyphenolic compounds were identified in tea extracts (Table 2) comparing the chromatogram of seven polyphenolic standards (Fig. S3 and Table S4) and the UV spectrum were presented in Figs. S4(A–G). Those were Gallic acid (GA), Catechin (CA), Rutin hydrate (RH), Epicatechin (EC) and Syringic Acid (SA). Phytochemical content of methanol and water extracts was found higher in Panchagar region with significant difference ($p < 005$).

Gallic acid was available in both methanol and water extracts. The amount of gallic acid was 5250 ± 1.81, 4040 ± 1.92 and 3730 ± 2.06 μg/g dry extract in water of Panchagar, Chattogram and Sylhet, respectively. A report by Bae et al. stated 4970 μg/g and 6550 μg/g of gallic acid in ethanolic extracts of green and black tea [59]. The level of gallic acid in methanol extract was 640 ± 0.10, 590 ± 0.15 and 510 ± 0.04 μg/g in Panchagar, Chattogram and Sylhet, accordingly. Pinto et al. expressed, 582 μg/g and 160 μg/g of gallic acid in the aqueous extract of green and black tea [60].

The level of catechin was 50.62 ± 3.60, 55.23 ± 4.11 and 39.96 ± 3.25 mg/g in water extract of Panchagar, Chattogram and Sylhet, respectively. A study by Nadiah and Uthumporn expressed 35 mg/g of catechin in the ethanolic (50%) extract of green tea [49].

Epicatechin levels in hot water extract from Panchagar, Chattogram, and Sylhet were 14.82 ± 2.2, 12.33 ± 1.85 and 8.63 ± 1.15 mg/g, respectively. Methanolic extracts had 20.07 ± 2.45, 12.09 ± 2.90 and 11.38 ± 1.25 mg/g of epicatechin in the region of Panchagar, Chattogram and Sylhet, accordingly. A report by Wu et al. stated 6–9 mg epicatechin/g of extract in green and black tea [56]. Bae et al. also stated the similar level of epicatechin in ethanolic extract [59].

Amount of Rutin hydrate were 290 ± 0.02, 160 ± 0.01 and 120 ± 0.04 μg/g water extract of Panchagar, Chattogram and Sylhet, respectively. Methanolic extracts had 430 ± 0.02, 170 ± 0.04 and 130 ± 0.05 μg/g of Rutin hydrate in the region of Panchagar, Chattogram and Sylhet, respectively. Bae et al. had found 670 and 2180 μg/g of rutin hydrate in green and black tea [59]. Vu and Alvarezexpressed the level of rutin hydrate in the range of 388–419 μg/g green tea [61].

Syringic Acid was only detected in Methanol extract of tea. Panchagar, Chattogram and Sylhet had 1320 ± 0.05, 780 ± 0.02 and 330 ± 0.02 μg/g of Syringic Acid, respectively. According to Jeszka-Skowron et al., different varieties of green tea and black tea had 2.07–5.67 and 3.92–15.12 μg/g of syringic acid [58].

3.5. Correlation and classification by canonical discriminant function

Catechin had positive association with Gallic acid (r = 0.743) and caffeine (r = 0.766). Besides this, epicatechin exhibited strong positive correlation with syringic acid (r = 0.875). All phytochemical compounds except epicatechin varied among three different tea growing zone. According to Astill et al. the observed variance in tea may be attributed to factors such as the diverse range of tea varieties, geographical origins, environmental conditions, and agronomic circumstances [62].

ANOVA test demonstrated that except TTC and IC_{50}, all antioxidant profile parameters of tea were significantly different in water extract. However, in case of methanol extract, except flavonoid all the others were significantly different ($p < 0.05$). In case of polyphenols of tea, all parameters except gallic acid (catechin, rutin hydrate, epicatechin, arabinoic acid, *p*-coumaric acid and syringic acid) were significantly different among their sources; Panchagar, Chattogram and Sylhet ($p < 0.05$) in both in water and methanol extracts. ANOVA test results confirm that all Methylxanthine parameters (Theobromine, Paraxanthine, Theophylline and Caffeine) of tea are significantly different according to their production region (Panchagar, Chattogram and Sylhet) both in water and methanol extract at 5 percent level of significance ($p < 0.05$).

Two canonical discriminant functions namely function-1 and function-2 had been developed on the bases of which the tea were

Table 2
Phytochemicals in hot water and methanol extract.

Extract Type	Polyphenolic compound	Ecological region		
		Panchagar	Chattogram	Sylhet
Hot water	Gallic acid[1]	5250 ± 1.81[a]	4040 ± 1.92[ab]	3730 ± 2.06[2]
	Catechin[2]	50.62 ± 3.60[a]	55.23 ± 4.11[a]	39.96 ± 3.25[2]
	Rutin hydrate[1]	290 ± 0.02[a]	160 ± 0.01[b]	120 ± 0.04[2]
	Epicatechin[2]	14.82 ± 2.20[a]	12.33 ± 1.85[a]	8.63 ± 1.15[2]
	Syringic Acid[1]	nd	nd	nd
Methanol	Gallic acid[1]	640 ± 0.10[a]	590 ± 0.15[ab]	510 ± 0.25[2]
	Catechin[2]	57.01 ± 5.50[a]	51.01 ± 2.65[ab]	38.78 ± 1.85[2]
	Rutin hydrate[1]	430 ± 0.12[a]	170 ± 0.24[b]	130 ± 0.15[2]
	Epicatechin[2]	20.07 ± 2.45[a]	12.09 ± 2.90[b]	11.38 ± 1.25[2]
	Syringic Acid[1]	1320 ± 0.25[a]	780 ± 0.42[ab]	330 ± 0.12[2]

Results are presented as mean ± SD. Means containing the same letter (s) in the row did not differ significantly ($p < 0.05$).
[1] μg/g dry extract.
[2] mg/g dry extract, nd = not detected.

classified as they were from Panchagar, Chattagram or Sylhet (Table S5). The result of canonical discriminant functions for classification was shown in Table 3 and Fig. 4. The findings of this study indicate that 90.7 percent of the initial grouped cases were accurately identified. Cross-validation was exclusively performed for the selected cases in the analysis. During the process of cross-validation, the classification of each individual instance was determined based on the functions that were obtained from all other examples, excluding the case in question. Approximately 88.4% of the cross-validated grouped cases exhibited accurate classification. The utilization of canonical discriminant functions yielded favorable outcomes in the classification of tea from three distinct cultivation origins in Bangladesh, relying on their respective features.

3.6. Comprehend tea quality with geographical distinction

The present study emphasized on the knowledge gap to recognize the geographical distinction related to environmental variation effects on tea quality, as it was firmly set that the successful growth and productivity of tea crop was dependent on climatic conditions including rainfall, temperature, intensity of sunlight, and humidity [63]. Conversely, lack of investigations to examine the effects of environmental distinction on tea quality in different agro-ecological regions of Bangladesh. Bioactive polyphenolic content in tea grown in three distinct regions exhibited that catechin found the highest in tea of Chattogram and rest four namely gallic acid, rutin hydrate, epicatechin and syringic acid shown maximum in tea grown in Panchagar. Previous studies shown that higher elevation with cooler temperature was usually accompanying with higher tea quality in terms of higher level of catechins [64]. While, caffeine and theobromine content was more in tea harvested in Chattogram, though theophylline is the highest in tea of Panchagar. Remarkably, tea cultivation in Sylhet was showed comparatively less phenolic compounds & methylxanthines which could be allied that apparently precipitation, number of rain days and pH of soil had a critical role in the quality of the tea. Information indicated in Table S1 that there were minute differences in temperature pattern of these three regions or a moderate variance in climate zone, and sunlight had little effect on tea quality for these three agro-ecological regions. Furthermore, it was evident that tea could be harvested within a wide range of height and slope. The tea growing territory could be extended from sea level such as Japan to Kenya and Rwanda where the altitude was 2700 m above the mean sea level. Though the effect of the altitude of tea cultivation area and geography on tea yield had been widely reviewed, whereas till now its impacts on the quality of tea was least understood [65].

4. Conclusion

In conclusion, this study provided significant insights into the variability of methylxanthine, bioactive phenolic compounds, and antioxidant activity in tea (*Camellia sinensis*) cultivated across three agro-ecological regions of Bangladesh. The comprehensive comparative analysis revealed distinct regional profiles, emphasizing the influence of environmental factors on tea composition. The examination of specific bioactive phenolic compounds enhanced our understanding of the phytochemical diversity and potential health benefits associated with different tea varieties. Furthermore, the evaluation of antioxidant activity provided valuable information on the overall antioxidant potential of teas from each region. These findings will have practical implications for quality control, market differentiation, and site-specific cultivation strategies, enabling stakeholders to optimize tea production and meet consumer demands. Additionally, the scientific basis established in this study will support the potential for tea geographical indication, facilitating the protection and promotion of teas from specific agro-ecological regions in Bangladesh. Ultimately, this research will contribute to the advancement of tea science and provides a foundation for further studies exploring the relationship between agro-ecological factors and tea quality.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Abu Tareq Mohammad Abdullah: Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Supervision, Visualization, Writing – original draft. **Mahbuba Ibrahim Sayka:** Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohammad Mahfuzur Rahman:** Writing – review & editing, Validation, Resources, Project

Table 3
Classification results of Canonical Discriminant Functions.

Dataset	District	Predicted Group Membership			Total
		Panchagar	Chattogram	Sylhet	
Original	Panchagar	22 (91.7)	0 (0.0)	2 (8.3)	24 (100)
	Chattogram	2 (3.6)	49 (87.5)	5 (8.9)	56 (100)
	Sylhet	0 (0.0)	3 (6.1)	46 (93.9)	49 (100)
Cross-validated	Panchagar	22 (91.7)	0 (0.0)	2 (8.3)	24 (100)
	Chattogram	3 (5.4)	46 (82.1)	7 (12.5)	56 (100)
	Sylhet	0 (0.0)	3 (6.1)	46 (93.9)	49 (100)

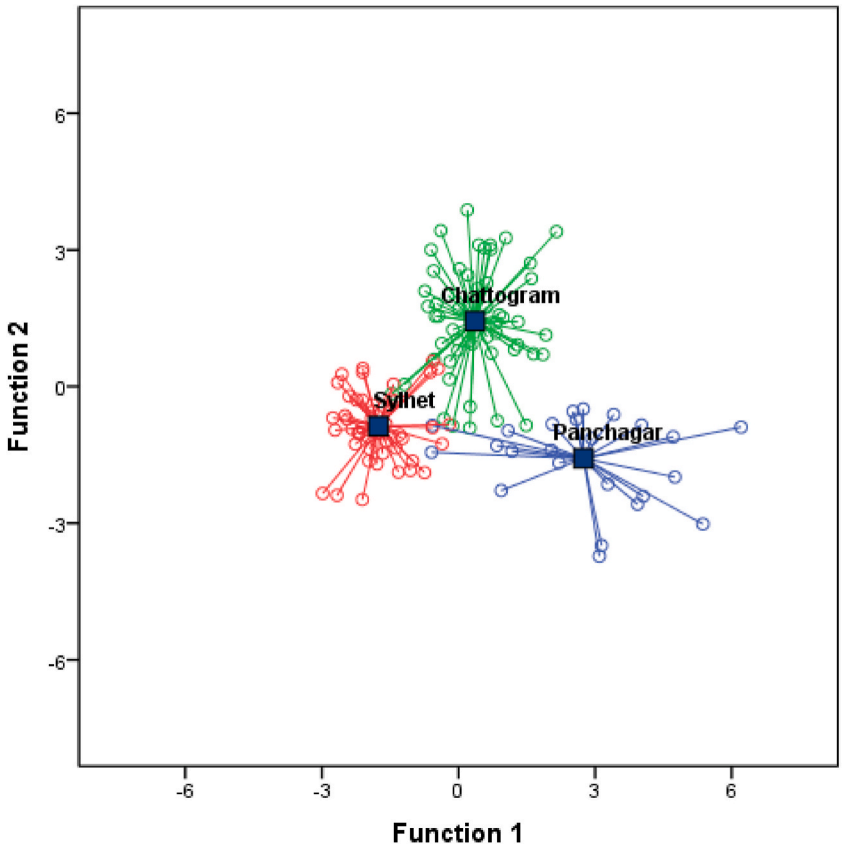

Fig. 4. Classification by canonical discriminant functions.

administration, Investigation, Formal analysis. **Miskat Sharif:** Visualization, Validation, Methodology, Investigation, Formal analysis. **Tanzir ahmed Khan:** Validation, Methodology, Investigation, Formal analysis. **Sharmin Jahan:** Visualization, Software, Resources, Investigation, Formal analysis. **Reaz Mohammad Mazumdar:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Mohammad Nashir Uddin:** Data curation, Methodology, Software, Visualization, Writing – review & editing. **Md. Mozammel Hoque:** Writing – review & editing, Resources, Project administration, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used QuillBot in order to imporove language. After using this tool, the author(s) reviewed and edited the content as needed and took full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28760.

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