DETERMINATION OF TOTAL POLYPHENOL IN COMMERCIAL TEA BRANDS AVAILABLE IN LOCAL MARKET OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled, **"DETERMINATION OF TOTAL POLYPHENOL IN COMMERCIAL TEA BRANDS AVAILABLE IN LOCAL MARKET OF BANGLADESH"** *submitted to the Department of Agricultural Chemistry, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirement for the degree of* **MASTER OF SCIENCE IN AGRICULTURAL CHEMISTRY** *embodies the results of a piece of bona fide research work carried out by* ***ARNAB ROY,*** *bearing Registration No.* **19-10322** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma, elsewhere in the country or abroad.*

I further certify that such help or sources of information, as have been availed of during the course of this investigation has duly been acknowledged.

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Dedicated To My Beloved Parents and Respected Teachers

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Full Word	Abbreviation
2,2-diphenyl-1-picryl-hydrazyl-hydrate	DPPH
Antioxidant activity	AA
Briskness index	BI
Broken Orange Pekoe	BOP
Caffeic acid	CA
Catechin	C
Colour index	CI
Differential pulse voltammetry	DPV
Epicatechin	EC
Epicatechin gallate	ECG
Epigallocatechin	EGC
Epigallocatechin gallate	EGCG
Ethyl acetate	EA
Ferric ion reducing antioxidant power	FRAP
Ferric thiocyanate	FTC
Flowery Broken Orange Pekoe	FBOP
Folin ciocalteu	FRC
Gallic acid equivalents	GAE
Gallocatechin	GC
Half maximal effective concentration	EC50
Highly polymerized substances	HPS
Kilogram	Kg
Liquid chromatography Diode-Array Detection Mass spectrometry	LC-DAD-MS
Low density lipoprotein	LDL
Milligram	mg
Optical density	OD
Oxygen radical absorbance capacity	ORAC
Percentage	%
Partial least squares	PLS
Reactive oxygen species	ROS
Root means square error of prediction	RMSEP

Standard deviation	SD
Systolic blood pressure	SBP
Theabrownin	TB
Theaflavin	TF
Thearubigin	TR
Thin-layer chromatography	TLC
Total catechin	TC
Total liquor colour	TLC
Total polyphenol content	TPC

DETERMINATION OF TOTAL POLYPHENOL IN COMMERCIAL TEA BRANDS AVAILABLE IN LOCAL MARKET OF BANGLADESH

ABSTRACT

Tea is the most widely consumed beverage in the world. Tea leaves are a source of polyphenols which have antioxidant capacity. Fourteen marketed brands of black tea, green tea and white tea of Bangladesh (viz. Matcha green tea, Kazi and Kazi green tea,Taaza (Brooke bond), Bengal classic tea, Jafflong tea, Kazi & Kazi black tea, Ispahani premium green tea, Fresh premium tea, Jafflong green tea, Ispahani mirzapur tea , Lipton green tea, Fresh premium green tea, Twinings of Londan Black tea and Silver Needle white tea) were randomly collected from the local supermarkets to observe the amount of polyphenol in tea using UV-spectrophotometer as a tool. The research was carried out during the time from December 2020 to March 2021 at the Agro-environmental chemistry laboratory of Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka. The contents of methanol soluble polyphenol and hot water-soluble polyphenol were determined in fourteen tea brands. Hot water extraction showed the highest polyphenol content. The contents of hot water-soluble polyphenols ranged from 32.85± 1.6 to 144.50± 18.21mg FAE/g of tea. The contents of methanol soluble or free phenolics was estimated among the fourteen tea brands of tea ranging from 9.86 ± 3.95 to 138.25 ± 15.95 mg/g of tea dry weight basis. Green teas showed the highest amount of polyphenol content. Black tea has lower polyphenol content. In relation with polyphenol green tea seems to be healthier to drink.

CHAPTER I

INTRODUCTION

Tea is an evergreen shrub or tree from Theaceae family, species Camelia. Its leaves are dark green and shiny, opposite and round, flowers are large, white, pink or red and fruits small and brown. Two basic botanical varieties include: Chinese tea's shrub (*Camelia sinensis*) as well as Indian tea plant (*Camelia assamica*) (Balentine *et al*., 1997; Chu and Juneja, 1997). Tea is grown in a number of countries, however mainly in China, India, Japan and Srilanka (Fernandez *et al.*, 2002). Harvesting lasts throughout the year yet its time influences the quality of tea leaves. The best teas are from the spring collection as then they are delicate and the most aromatic, top-grade teas, however, are gained from undeveloped leaves and young leaflets of top twigs, which, further prepared, show suitable gustatory features and special aroma (Chu and Juneja, 1997). According to Chinese mythology, a tea plant was discovered thousands of years ago in South-East Asia. Initially tea leave infusion has been considered for medicine, later on it became the most popular beverage all over the world (Fernandez *et al.,* 2002). Tea market is very rich. Over three hundred tea variety are produced. The basic classification includes three categories of teas: green, oolong and black (Harbowy and Balentine, 1997). According to FAO world tea production in 2021 increased to an estimated 6.5 million tons, from 6.3 million tons in 2020. The average tea production of Bangladesh was 85.05 M kg in 2016 which was 67.38 M kg in 2015 and the rate of increase was 26.22 percent (BBS, 2017). In the annual output about 20% is constituted by green tea, and over 70% by black tea. At present the average tea consumption reaches about 120 mL/day/person. (Mukhtar and Ahmad 1999). The preferences in tea consumption are different in various regions of the world. Most Americans and Europeans prefer black tea; the Japanese and Northern China inhabitants prefer green tea. Oolong tea is mainly consumed in Taiwan and Southern China. (David, 2003).

Since ancient times, tea has been used as a health product or medicine to prevent and treat various diseases. Previous studies have shown the numerous benefits of tea, such as antioxidant, bacteriostatic, and anti-cancer activities and regulation of lipid metabolism. (Yan, *et al.*, 2020) Processed tea leaves are known to contain a wide variety of phytochemicals, of which polyphenols constitute the major portion. Polyphenols are act as antioxidants in tea. Antioxidants are capable of stabilising or deactivating reactive oxygen species (ROS) such as hydroxyl radical, ferryl ion, superoxide radical anion, peroxyl radical and hydrogen peroxide, which are induced by oxidative stress, before they attack cells and biological targets. Antioxidants are therefore believed to be crucial for maintaining optimal cellular and systemic health and well-being (Rahman, 2007; Dufresne and Farnworth, 2001). During the last decade, the effects of tea and tea polyphenols were extensively investigated and studies showed that tea is capable of lowering the risk of cardiovascular diseases and cancers (Chen *et al*., 2008; Mukamal *et al.*, 2007), reducing body fat, systolic blood pressure (SBP), and low-density lipoprotein (LDL) cholesterol (Nagao *et al.*,2007). Among age-associated pathologies and neurodegenerative diseases, green tea was shown to confer significant protection against Parkinson’s disease and Alzheimer’s disease (Hu *et al*., 2007; Rezai-Zadeh *et al.*, 2005). On the other hand, persistent tea consumption by mothers during pregnancy might be associated with an increased risk of preeclampsia, especially severe preeclampsia (Wei *et al.*, 2009). Furthermore, phenolics might modulate the activity of a wide range of enzymes and cell receptors. In this way, in addition to having antioxidant properties, phenolics have several other specific biological actions that are yet poorly understood (Manach *et al.*, 2004).

Phenolics are a group of compounds with phenolic structural features abandon in nature. The term polyphenol is also used as a collective term for several sub-groups of phenolic compounds. More than 8,000 polyphenolic compounds have been identified in various plant species (Pandey *et al.*, 2009). All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid (Pandey *et al.*, 2009; Tsao, 2010). Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linked with other phenol may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another (Tsao, 2010).

Tea is a very important cash crop of Bangladesh. It plays vital role in the national economy. A large number of tea estates have been established in Bangladesh depending on the tea as raw material. Tea uses as a common beverage in Bangladesh. However, there was increasing annual demand for tea hereafter increasing production of tea was observed in 2020-2021 (BTB, 2022).

Several Tea brand company supply teas in local market manufactured by different estates. Consumption of tea have health benefits for the presence of bioactive polyphenols. Little information is reported in the literature regarding the total phenolics of different brands of teas in Bangladesh. Therefore, the present research aims to investigate total phenolic content of Tea. In order to fulfil the above mentioned aim the following objectives have been undertaken:

1) To compare the phenolic content in different commercial brands available in Bangladesh.
2) To compare the solubility of polyphenol by different extraction methods.

CHAPTER II

REVIEW OF LITERATURE

Tea is consumed all over the world as a common beverage. Different types of tea produced in the world. Depending upon demands many countries produce many types tea. Green tea, black tea, white tea has different types of nutrients components. Due to different types of fermentation process different compound formed. The most common compound found in tea is polyphenols.

The main classes of polyphenols might include phenolic acids, flavonoids, stilbenes and lignans. Figure 1 illustrates the different groups of polyphenols with examples and their chemical structures.

Phenolic acids are non-flavonoid polyphenolic compounds. Phenolic acids might further be classified into two types, Benzoic acid and cinnamic acid derivative based on C1-C6 and C3-C6 backbones. Gallic acid, Vanillic acid are some example of benzoic acids, and ferulic acid and Sinapinic acid are some examples of cinnamic acids (Tsao, 2010).

Flavonoids, a group of polyphenols are the most abundant in nature which has more than 5000 different types. The main subclasses of flavonoids include the flavones, flavonols, flavanones, catechins or flavanols, anthocyanidins and isoflavones (Figure 2) illustrates basic skeleton of flavonoids and its main subclasses with structures.

The chemical structure of flavonoids varies according to the hydroxylation pattern, conjugation between aromatic rings, glycosidic moieties, methoxy groups and other substituents (Santos *et al.*, 2017). Flavonoids contain C_6-C_3-C_6 basic structure where it has two C_6 units (ring A and ring B) are of phenolic nature. The wide majority of the flavonoids have the ring B attached to the C_2 position of ring C. In few flavonoids such as isoflavones and neo flavonoids, ring B is attached at the C_3 and C_4 position of ring C (Rong *et al.,* 2010). Biological activities may vary on the basis of structural differences and the glycosylation pattern. Flavonoids has the widest colour range from pale-yellow to blue pigments that are responsible for colouring of most flowers, fruits and seeds.

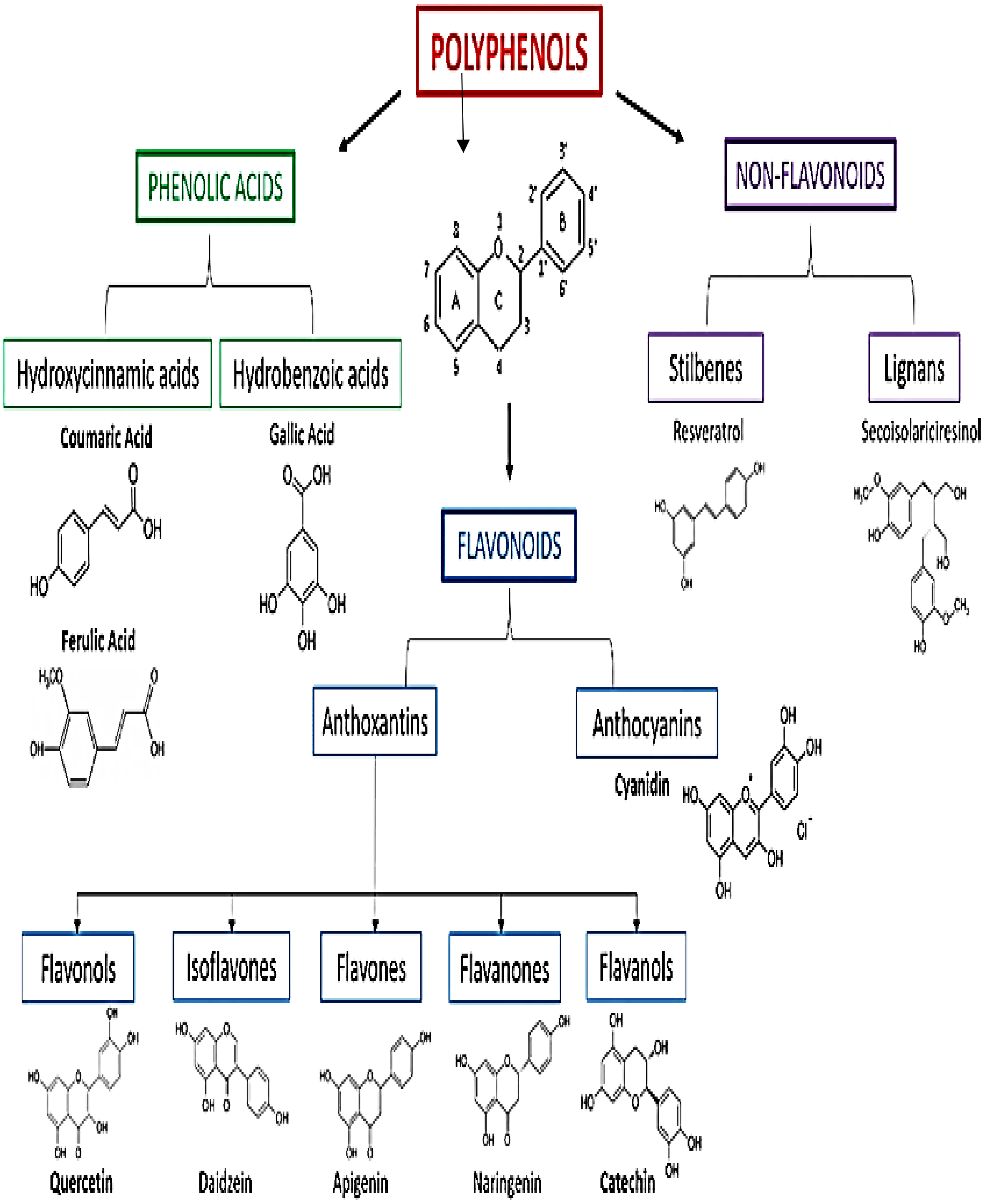

Figure 1: Classification of phenolics (Grzesik *et al.*,2018)

Flavonols are the most universal flavonoids of nutritional interest in foods and among them, quercetin and kaempferol are abandon in nature (Rong *et al.*, 2010; Santos *et al.*, 2017). The major types of flavonoids identified in tea are flavan-3-ols (or catechins), oligimeric flavonoids (including thearubigins and theaflavins generated during fermentation), and flavonols (eg, quercetin).

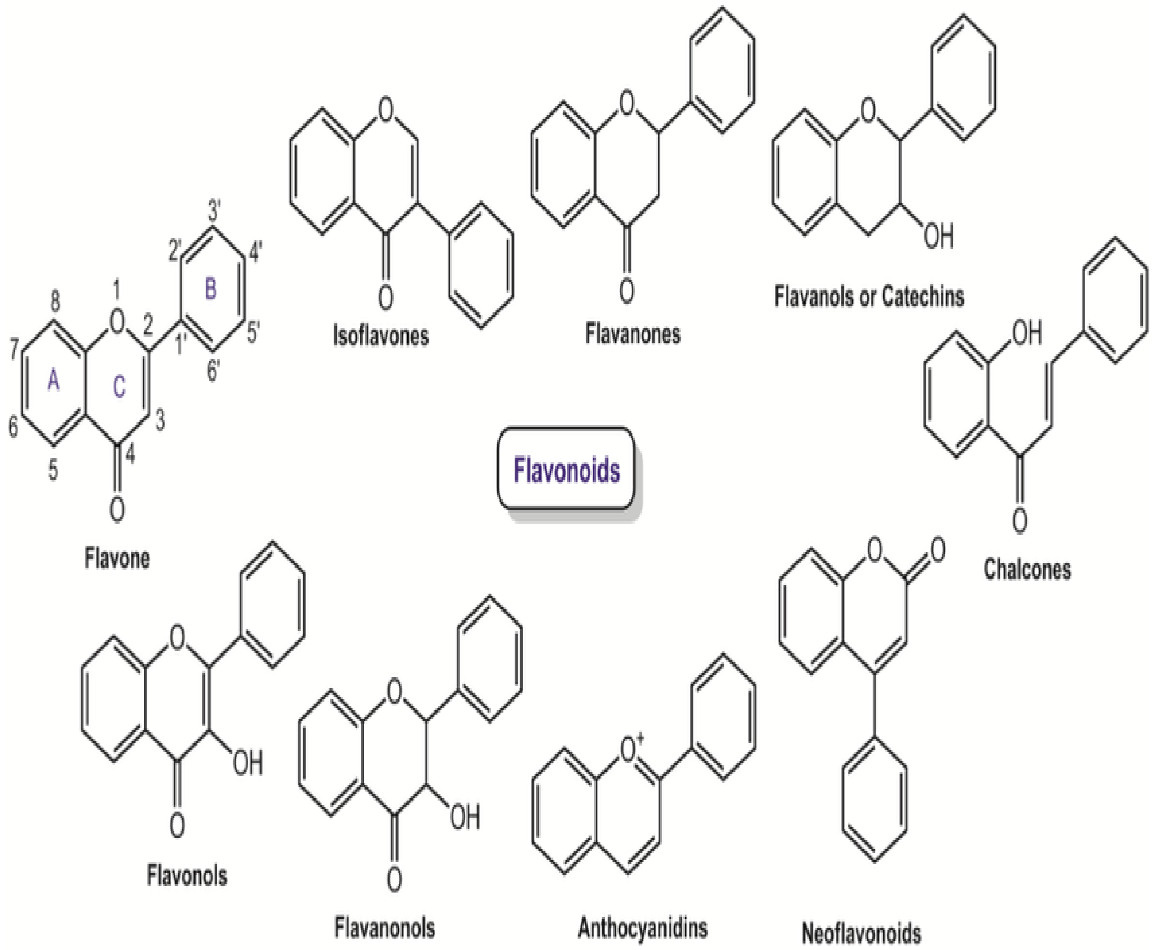

Figure 2. Different types of flavonoids found in tea (Habila *et al.*,2020)

Tea leaves are, apart from wine, fruit and vegetables, a very good source of polyphenolic compounds (Ho *et al.*,1997). Green tea leaves consist of flavonoids as well as phenolic acids which can make up to 30% of fresh leaves dry weight and only 10% of dry weight of black tea (Wang *et al.*, 2000). Those constituents are referred to as bioflavonoids or vitamin P, as they show a wide spectrum of biological effects in living organisms (Dreosti, 1996). The most important polyphenol groups tea leaves are substances whose nomenclature is still a divergence. These compounds are often referred to as flavan-3-ols or catechins (Balentine,1997). Tea leaves have been reported to be the only food product containing epigallocatechin gallate (EGCG) (Chu & Juneja, 1997).

In tea leaves three basic polyphenol groups can be distinguished: catechin, theaflavins and thearubigenes (Yanishlieva-Maslarowa & Heinonen, 2001). Both green and black tea contains a similar quantity of flavonoids, differing in respect of their chemical structures. Green tea is characterised by a higher content of simple flavonoids – catechins which become transformed to more complex compounds (theaflavins and thearubigens) during the fermentation of tea leaves (Balentine *et al.,* 1997). Other products of polyphenol oxidation are tannins. Tannins were divided into two groups: hydrolysable, water-soluble, including simple phenolic acids, gallic acid esterified into the polyols, and nonhydrolysable tannins (condensed), the polymers of elementary flavonoids particles (Chung *et al.,* 1998). Catechins are flavan derivatives and in a group of flavonoids they are distinguished by the highest oxidation degree of heterocyclic ring and by good solubility in water. In tea leaves, they appear mainly in the form of gallic acid esters. The main catechins of green tea are: (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) (Ninomiya et al., 1997).

The structural formula of catechin was introduced in Figure 3. The presence of methylated catechins was reported by Saijo *et al.* (1982) and Sano *et al.* (1999). According to mass spectrometry and nuclear magnetic resonance two catechins derivatives were identified as (-)-epigallocatechin–3-(3-O-methylgallate) and as (-)-epigallocatechin-3-(4-O-methylgallate) (Amarowicz & Shahidi, 2003).

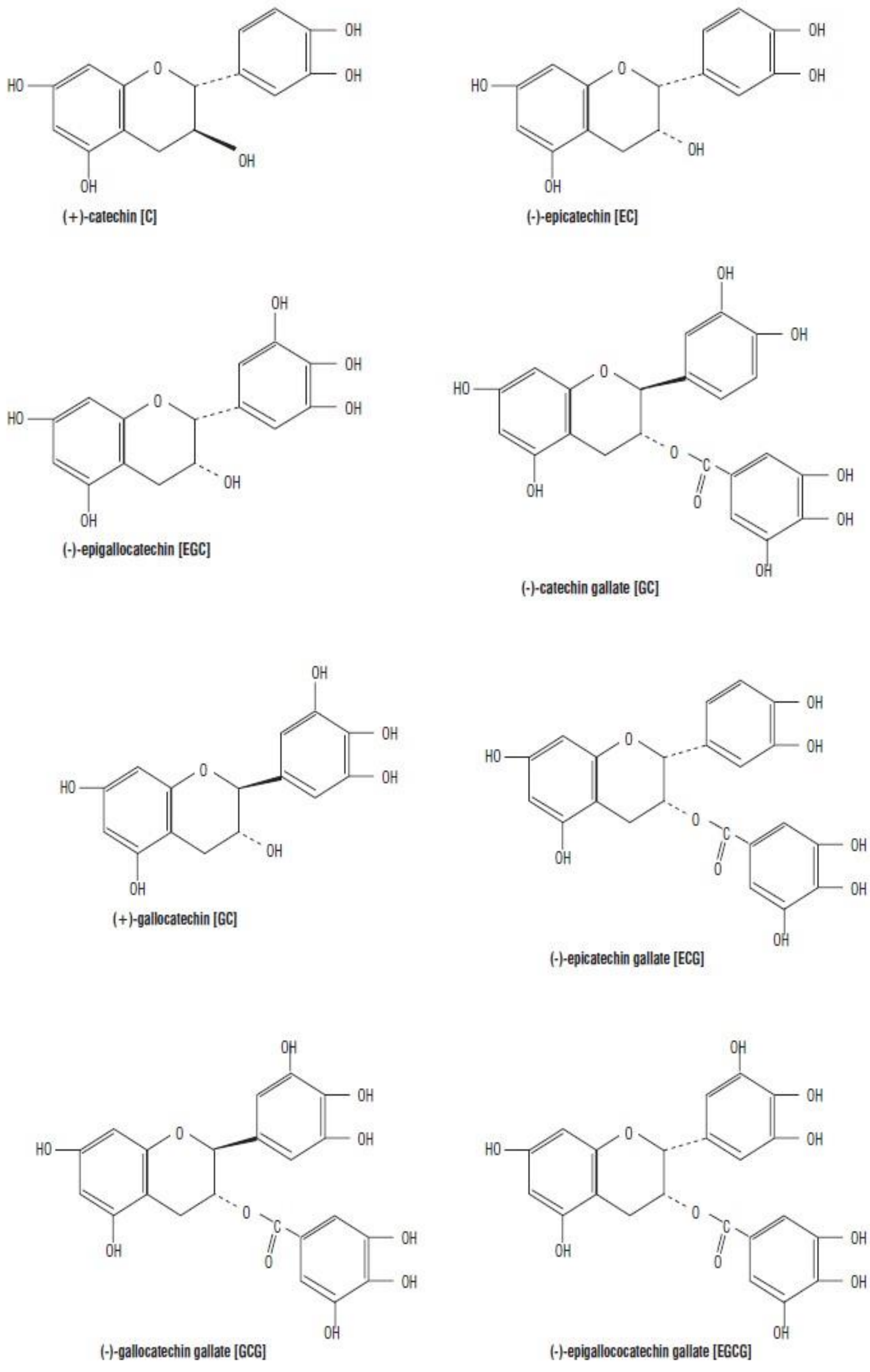

Figure 3: Different structural formula of catechin (Ninomiya *et al.*, 1997)

TLC separation of methylated EGCG was reported by (Amarowicz *et al*.,2005). The content of proanthocyanidins in green tea is at least 10 times lower than catechins (Hashimoto *et al*., 1992). Four dimeric proanthocyanidin gallates (prodelphidin B-2 3'-O-gallate, procyanidins B-2 3,3'-di-O-gallate, B-2 3'-O-gallate, and B-4 3'-O-gallate) and two dimeric flavan-3-ol gallates in which two flavanol units are linked at the B-ring have been reported by (*Nonaka et al.*1984). At least 12 proanthocyanidins were identified in green tea samples by means of HPLC-MS and HPLC-DAD (Kiehne *et al.,* 1997).

Among them EGC-EGCG, EC-EC-EC, EC-EGC, EGC-EGC, EGCG-EGCG, EC-ECG, EGCG-ECG, ECG-EGCG, ECG-ECG were present. EGCG is an extremely active compound with eight -OH groups determining its high antioxidant activity. Investigations have shown that green tea contains a larger quantity of catechins than the black one (Khokhar & Magnusdottir, 2002). In contrast, black teas contain a larger quantity of gallic acid, which is likely to be released during fermentation from catechin gallates (Halder *et al.,* 1998). The content of catechins in tea leaves is also a result of multiple factors, leaf age, because tea produced from young leaves contains considerably larger quantities of these compounds. The content of EC, EGC, EGCG, and ECG in eight commercial green teas determined by (Price and Spitzer 1993) reached 0.87–2.0%, 2.75–5.44%, 2.19–3.95%, 0.56–0.91%, respectively (results in % w/w). Mukhtar and Ahmad (1999) studied the polyphenolic composition of tea leaves infusion. They showed that the cup of green tea infusion contained about 400 mg of polyphenolic antioxidants, of which 200 mg was EGCG. On the contrary, while (Graham, 1992) reported, that a cup of green tea infusion might contain about 90 mg of EGCG. On the grounds of the above-mentioned data, it is possible to believe that many factors influence the composition of tea leaves polyphenols as well as prepared infusion. It was observed that the content of catechins in tea leaves is closely correlated with the quality of infusion. The highest content of catechins was noted in teas produced from young tea leaves (Thanaraj and Seshardi, 1990). Phenolics nutrients constitutes identified in different tea shown in Table 1.

Table 1. Phenolics compound identified in different types of tea

Polyphenol	Tea type	References
Flavanols(flavan-3-ols)	Green tea	Balentine *et al.,* (1997)
(-)-epicatechin, (-)-epicatechin gallate, epigallocatechin, (-)-epigallocatechin		
Flavonols	Green tea	Balentine *et al.*, (1997); Graham, (1992)
Quercetin, Kaemferol, Myricetin, Glycosides flavonols		
Flavones	Green tea	Balentine *et al.*, (1997); Graham, (1992)
Apigenin glycosides		
Catechins	Black tea	Balentine *et al.,* (1997)
Theaflavins, Theaflagallins, Theasinensins, Theacitrins		

Chowdhury *et al.* (2018) carried out a study for qualitative and quantitative evaluation to screen out their status of caffeine, phenolic compounds [Total Polyphenol (TP), Theaflavin (TF), Thearubigin (TR), Highly Polymerized Substances (HPS), Total Liquor Colour (TLC), Briskness Index (BI) and Colour Index (CI)] and nutrient elements (N, P, K, Ca and Na) of six marketed brands of Kazi and Kazi tea produced in Bangladesh viz; Black tea, Ginger tea, Tulsi tea, Green tea, Orthodox black tea and Orthodox green tea. The values of caffeine and TP were found to be varied from 2.24% (Orthodox green tea) to 2.65% (Black tea) and 18.84% (Black tea) to 21.88% (Orthodox green tea) respectively. Likewise, the estimated amounts of TF, TR, TLC, CI and BI were determined to be the highest in Black tea but maximum HPS was in Orthodox green tea whereas the lowest TF, TLC and CI were determined in Orthodox green tea. On the other hand, TR, and BI were detected to be minimum in Ginger tea and HPS was detected to be minimum in Black tea. On the contrary, the total amount of studied nutrient contents (N, P, K, Ca and Na) was estimated to be the highest in Orthodox black tea (9.99%) and the lowest in Ginger tea (7.55%). They concluded that the Orthodox green tea was superior over the other brands in relation to total qualitative status and consequently, all the studied brands of Kazi and Kazi tea may be ranked as Orthodox green tea>Green tea>Orthodox black tea>Ginger tea>Black tea>Tulsi tea.

Rahman *et al.* (2021) conducted an experiment to create new knowledge as well as awareness about the consumption of green tea and compare the black and green tea available in Bangladesh based on their bioactive compounds and antioxidant activity. In this experiment, total phenolics, total tannin, total flavonoids, and caffeine content were measured as bioactive compounds, and antioxidant activity was evaluated by using two different methods such as DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity and ABTS+ radical scavenging activity of methanol extracts of black and green tea. Every bioactive compound in black and green tea was found to be significantly different ($P < 0.05$). The total phenolic content, on average, was measured at 242.46 mg GAE/g dry extract and 763.41 mg GAE/g dry extract in black and green tea, respectively. Black tea contained 6.47 mg TAE/g dry extract tannin, whereas green tea had much more tannin content, 14.51 mg TAE/g dry extract, which is more than double in amount. On the other hand, the total flavonoid content was almost double in black tea (61.82 mg QE/g dry extract) compared to green tea (31.85 mg QE/g dry extract). Antioxidant activities were determined at different concentrations of tea samples. At every concentration, green tea presented higher ABTS+ and DPPH radical scavenging activity than black tea. The highest percentage of inhibition was observed at 20 ppm both in black and green tea, finding 98.50 % and 99.07 % inhibition, respectively. Overall, significantly ($P < 0.05$) higher number of phenolic compounds as well as antioxidant activity were observed in green tea.

Kaur *et al.* (2015) assessed to compare the total polyphenol content (TPC), antioxidant activity in black and green tea. The total polyphenol content in green tea and black tea was determined according to the International Organization for Standardization method (ISO) 14502-1. The antioxidant capacity was determined by Ferric Ion Reducing Antioxidant Power (FRAP) and Polymolybdenum method. The green tea showed a higher polyphenol content and antioxidant levels than black tea ($p < 0.05$). The antioxidant activities were well correlated with the total polyphenol content ($r^2 = 0.9571$ for FRAP method and $r^2 = 0.9287$ for Polymolybdenum assay). The Kangra green tea (North India) had higher TPC and antioxidant levels than Darjeeling green tea (Northeast India).

Nibir *et al*. (2017) studied a comparative assessment of total polyphenols, antioxidant and antimicrobial activity of different tea varieties of Bangladesh. In this study, extracts of four different brands of Bangladeshi tea (FBOP, BOP, red dust and green tea) were analyzed for their polyphenol content, antioxidant property and antibacterial activity. Total polyphenol content, antioxidant property and antibacterial activity were higher in green tea extract compared to other tea varieties. They also determined that total phenolic and flavonoid contents were significantly higher ($P<0.05$) in green tea compared to other three black tea varieties. The green tea also showed a higher free radical scavenging and antioxidant activities than all the other tea varieties tested ($P < 0.05$). In addition, the extracts of all four tea varieties showed inhibitory activity against several pathogenic bacteria and also the same trend of higher antimicrobial activity of green tea than other tea varieties were observed.

Jayasekera *et al*. (2010) demonstrated considerable variation among both fermented and unfermented Sri Lankan tea samples for TPC and antioxidant properties as determined by FRAP and DPPH and showed that there was considerable potential for using selected combinations of plantations and season to select high antioxidant teas. There was also evidence for an interaction effect for TPC, FRAP and DPPH among season and plantation, such that differences seen among plantations differed with season. Fermentation during the monsoon season produced a statistically significant increase in TPC but a decrease in FRAP and TPC was largely limited to the monsoon season teas. The results in this experiment highlighted significant ($P < 0.05$) variations in antioxidant activity across the six plantations. FRAP and DPPH for both fermented and unfermented teas from the four highland plantations showed a significant ($P < 0.05$) interaction between season and plantation. A similar interaction between season and plantation was observed for total phenolics in unfermented teas from the four highland plantations. The variability of the total phenolics for fermented teas, however, was independent of seasonal variations. A significant correlation ($r = 0.5$, $P < 0.05$) was observed between FRAP and total phenolics.

Kerio *et al*. (2013) showed that TPC, catechin profiles and antioxidant activities were significantly ($P<0.05$) higher in unaerated than in aerated teas. Tea products from the purple leaf coloured tea cultivars had levels of TPC, total catechin (TC) and antioxidant activities similar to those from the green leaf-coloured cultivars, except for teas from the Japanese cultivars that were very low in the assayed parameters. Caffeine content was significantly ($P< 0.05$) lower in products from the purple leaf-coloured cultivars than in those from the green leaf coloured tea cultivars. Antioxidant activity (%) was higher in tea products from the Kenyan germplasm than in those from the Japanese cultivars. Antioxidant potency of tea products was significantly ($r = 0.789$, $P< 0.01$) influenced by the total anthocyanin content of the purple leaf-coloured cultivars. Cyanidin-3-O-glucoside was the anthocyanin most highly correlated with AA% ($r = 0.843$, $P< 0.01$ in unaerated tea). Total catechins in the unaerated products from the green leaf coloured tea cultivars were also significantly correlated with antioxidant capacity ($r = 0.818$, $P< 0.01$).

Hashish *et al.* (2018) executed a study for evaluation and comparison of total phenolic compounds and flavonoid contents and the antioxidant activity of black and green tea drink among some available brands in the Egyptian Market. They showed that, there were significantly differences ($P <0.05$) in the total polyphenols content and antioxidant activity of the commercial tea samples. Koshary black tea and boiled green tea drinks had the highest levels of the total phenolic compounds while, the Koshary black and green tea drinks had the highest flavonoid content and antioxidant activity as well. There was also significant moderate correlation between the total phenolic and flavonoid contents and the antioxidant activity for boiled tea drinks. This study showed the presence of antioxidant compounds (phenolic acids and flavonoids) and demonstrated level of antioxidant activity in different types of black and green tea (Camellia sinensis L.) and that not exclusive to the global and famous brands but also found in the economic commercial brands. It was concluded that high total phenols and flavonoid content and antioxidant activity were observed in black and green tea prepared by infusion (Koshary). There was significant correlation between the total flavonoid content (higher than the phenolic) and the antioxidant activity of the tea extracts. Moreover, Koshary tea is the best method to maintain the high levels of polyphenols and antioxidants activity.

Javad *et al.* (2020) determined the comparative assessment of phenolic compounds and antioxidant properties of green tea made by microwave assisted and stove heating. They showed that microwave extracts had significantly higher quantities of phenolics and flavonoids from all tea samples as compared to conventional stove heating. Moreover, microwave assisted extraction showed highest amount (2.19%) of gallic acid in Lipton green tea while the Quercetin content (1.23%) was found invariable in the samples.

Stephen Thanaraj and Seshadri (1989) reviewed the influence of polyphenol oxidase activity and polyphenol content of tea shoot on quality of black tea. They studied the Variation in polyphenol oxidase activity and levels of total polyphenols and catechins with respect to different clones and shoot components, and its effect on quality of black tea (*Camellia sinensis* (L) O Kuntze. There was a wide variation in polyphenol oxidase activity of the different clones tested. The optimum fermentation time and polyphenol oxidase activity of different clones exhibited a hyperbolic relationship, viz $y = 2.36 + 1129/x$, where y = optimum fermentation time in minutes and x = polyphenol oxidase activity in µm catechol oxidized g^{-1} acetone powder min^{-1}, with an r value of 0.98, which is significant at $P \leq 0.001$. A good non-linear relationship was found between polyphenol oxidase activity of fresh tea shoots of different clones and the theaflavins content of corresponding black teas. Among different shoot components, bud and first leaf had higher levels of polyphenols and catechins than internodes. However, the polyphenol oxidase activity showed a reverse trend: the internodes exhibited a higher enzyme activity compared with other components. Formation of theaflavins during fermentation of different shoot components was in good agreement with polyphenol oxidase activity. High performance liquid chromatographic analysis of the theaflavins fraction in tea brew of black teas made from different components of tea shoot showed that buds resulted in black tea with the highest amount of theaflavin gallates, whereas teas produced from internodes had the lowest amount of theaflavin gallates.

Ali *et al.* (2005) studied polyphenolic content, antioxidant activity and phenolic profile of Tea and herbal infusions. The total phenolics recovered by ethyl acetate from the water extract, were determined by the Folin–Ciocalteu procedure and ranged from 88.1± 0.42 (Greek mountain tea) to 1216 ± 32.0 mg (Chinese green tea) GAE (Gallic acid equivalents)/cup. The antioxidant activity was evaluated by two methods, DPPH (2,2-diphenyl-1-picryhydrazyl radicals), and chemiluminescence assays, using Trolox and quercetin as standards. The EC50 of herbal extracts ranged from 0.151 ± 0.002 mg extract/mg DPPH (0.38 quercetin equivalents and 0.57 Trolox equivalents), for Chinese green tea, to 0.77± 0.012 mg extract/mg DPPH (0.08 quercetin equivalents and 0.13 Trolox equivalents), for Greek mountain tea. Chemiluminescence assay results showed that the IC50 ranged from 0.17 $\pm 3.4 \times 10^{-3}$ μL extract/ml of the final solution in the measuring cell (1.89 quercetin and 5.89 Trolox equivalents) for Chinese green tea, to 1.10 $\pm 1.86 \times 10^{-2}$ g extract/ml of the final solution in the measuring cell (0.29 quercetin and 0.90 Trolox equivalents) for Greek mountain tea. The phenolic profile in the herbal infusions were investigated by LC-DAD-MS in the positive electrospray ionization (ESI+) mode.

Alam *et al.* (2018) used seven different marketed brands of Shaw Wallace tea available in the local markets of Bangladesh for screening active components. They studied the seven marketed brands before their expiry date to screen out their qualitative status of [viz; Caffeine, total Polyphenol (TP), antioxidant activity (AA), theaflavin (TF), thearubigin (TR), theabrownin (TB), highly polymerized substances (HPS), total liquor color (TLC), the briskness index (BI), color index (CI), total catechin (TC) and nutrient contents N, P, K, Ca, Na and Fe]. The values of Caffeine and TP were found to be varied from 3.98 % (Premium Tea bag) to 5.5% (BOP) and 37.50% (Danadar) to 32.09% (Fine Dust) respectively. Similarly, the estimated values of TF, TR, TB, TC and TLC were detected to be maximum (1.18%, 15.76%, 12.91%, 5.17% and 5.4) in Premium tea and Danadar tea but HPS and BI contents were found to be (5.27% and 21.40) maximum in Hotel special and BOP respectively. In case of Antioxidant activity (AA), the maximum value (88.80) was gained in Golden leaf and minimum value (86.67%) in Premium tea bag. On the contrary, CI was found to be the highest (7.12) in Golden Leaf and the lowest (4.89) in Fine Dust. Considering the total amount of Nutrient Contents (sum of N, P, K, Ca, Na and Fe), the highest value was also estimated to be 9.045% in Danadar tea and the lowest value was 7.28% in Fine Dust tea. The highest average value of N and K contents were found to be 5.14% and 2.05% in Danadar tea whilst the lowest average value of N and K contents were found to be 4.52% and 1.55% in Fine Dust tea respectively. Similarly, maximum average value of Na and Ca contents determined to be 0.17% and 1.37% in Danadar tea whereas minimum average value of Na and Ca contents were determined to be 0.95% and 0.07% `in Fine Dust tea respectively. In case of P and Fe content, the highest average value was detected to be 0.28% in Premium tea bag and 0.055% in Danadar Tea even as the lowest average value was detected to be 0.17% and 0.029% in Fine Dust tea respectively. The Present study concludes that Danadar tea was revealed to be superior over the other brands and all the studied brands may therefore, be ranked as: Danadar tea > Premium tea Bag > BOP tea > Super Clone Tea > Golden Leaf tea > Hotel Special tea > Fine Dust tea.

Cai *et al.* (2012) investigated the effect of solid-state fermentation with filamentous fungi (Aspergillus oryzae var. effuses, Aspergillus oryzae, and Aspergillus niger) on total phenolics content (TPC), flavonoids, and antioxidant activities of four subfractions of oat, namely, n-hexane, ethyl acetate (EA), n-butanol, and water, and compare them to their corresponding subfractions of unfermented oat. The TPC and total flavonoids increased dramatically, especially in EA subfractions ($P<0.05$). The levels of antioxidant activity of subfractions were also significantly enhanced ($P <0.05$). The highest antioxidant activities were also found in the EA subfractions. The polyphenols in EA were analysed by high-performance liquid chromatography at 280 nm. Most polyphenols were increased remarkably, especially ferulic and caffeic acids. There was a clear correlation between the TPC and antioxidant activity. In conclusion, fungi fermentation is a potential bioprocess for increasing the TPC, flavonoids, and antioxidant activities of oat-based food.

Atomssa and Gholap (2015) showed that EGCG had highest molar decadic absorption coefficient in methanol than in the other solvents. ECG had the highest absorption cross - section, transitional dipole moment, and oscillator strength of all the catechins in water. On the other hand, EGC had the least optical transition properties of all the catechins in water Limits of detection (LOD) were comprised in the range 3.1x10 gmL^{-}

^{1} to 1.6x10 gmL^{-1} and reproducibility's with RSD lower than 2 %. After characterization of the electron transition, a method was developed for UV - Visible determination of total catechins. Using the developed method, the content of total catechins in Ethiopian and Sri Lanka green tea leaves at room temperature was determined. The result of the experiment indicated that, Ethiopian green tea leaves have the greater total catechins (17 ± 0.01 %) than Sri Lanka green tea leaves (7.17 ± 0.12 %).

Shaheen *et al.* (2018) investigated total phenol content (TPC), Anti-inflammatory, Anti-allergic and Antioxidant activity (AA) in differently processed Camellia Sinensis (tea) of Bangladesh. TPC varied in the range of 1056.74 (branded tea) – 2348.60 (green tea) mg GAE/g respectively. Radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) was found in the range of 1269.91- 2432.76 μ mol TE/ g while total Oxygen Radical Absorbance Capacity (ORAC) value in the range of 1262.81-2834.51 μ mol TE/g. Both green and organic tea showed highest ORAC value when brewed for 5 min in boiling water extract while tea BT-2 and branded (Mirzapur) tea exhibited highest ORAC value for 10 min brewing. Anti-inflammatory activity potential was found in the order of green tea> tea BT-2> organic tea> branded tea where both green and organic tea exhibited dose-response relationship. Anti-allergic activity ranged from (60.9-83%) with highest and lowest in green and branded tea respectively.

Sari *et al.* (2007) reviewed Total Polyphenol, Antioxidant and Antibacterial Activities of Black Mate Tea. They showed that Total polyphenol content of the extracts ranged from 97.01 to 119.28 mg gallic acid equivalent (GAE)/g dry weight (dw) tea depending on the solvent used and extraction time applied. In general, methanol was the least efficient solvent for polyphenol extraction from black mate tea and the efficiency of the others was found to be similar. All extracts showed antioxidant activity by 2,2-diphenyl-1-picryhydrazyl (DPPH) radical and reducing power. Different trend was observed for each method with respect to solvents used. The extracts possessed antibacterial activity depending on the solvent used and bacterium tested and the results showed that black mate tea extracts had strong antimicrobial activity against selected bacteria, except for E. coli 0157: H7. While S. aureus was found to be the most sensitive to all extracts, E. coli was the most resistant among bacteria tested.

David *et al.* (2015) described the voltametric behaviour and the quantitative determination of caffeic acid (CA) on a disposable pencil graphite electrode (PGE). The anodic peak current of CA recorded by differential pulse voltammetry (DPV) varied linearly with CA concentration in the range 1×10^{-7} to 3×10^{-3} M. The detection and quantification limits were 8.83×10^{-8} M and 2.94×10^{-7} M caffeic acid, respectively. The mean recoveries of CA from Turkish green, white and black teas were 98.30%, 99.57% and 91.46%. For these three teas types the corresponding total polyphenolic contents (TPC) evaluated by DPV on PGE were 35.81, 34.59 and 31.21 mg caffeic acid equivalent/g tea, respectively. These TPC values were in good accordance with those obtained by the Folin–Ciocalteu method. The developed DPV on PGE method constitutes a simple and inexpensive tool for the rapid assessment of TPC of tea samples.

Xiong *et al.* (2015) revealed that the predictive model by MSI using partial least squares (PLS) analysis for tea leaves was considered to be the best in non-destructive and rapid determination of TPC. Besides, the ability of MSI to classify tea leaves based on storage period (year of 2004, 2007, 2011, 2012 and 2013) was tested and the classification accuracies of 95.0% and 97.5% were achieved using LS-SVM and BPNN models, respectively. These overall results suggested that MSI together with suitable analysis model is a promising technology for rapid and non-destructive determination of TPC and classification of storage periods in tea leaves.

Chen *et al.* (2008) determined total polyphenols content in green tea with near infrared (NIR) spectroscopy coupled with an appropriate multivariate calibration method. Partial least squares (PLS), interval PLS (iPLS) and synergy interval PLS (siPLS) algorithms were performed comparatively to calibrate regression model. The number of PLS components and the number of intervals were optimized according to root mean square error of cross-validation (RMSECV) in calibration set. The performance of the final model was evaluated according to root mean square error of prediction (RMSEP) and correlation coefficient (R) in prediction set. Experimental results showed that the performance of (PLS) model is the best in contrast to PLS and iPLS. The optimal model was achieved with R = 0.9583 and RMSEP = 0.7327 in prediction set. This study demonstrated that NIR spectroscopy with siPLS algorithm could be used successfully to analysis of total polyphenols content in green tea, and revealed superiority of siPLS algorithm in contrast with other multivariate calibration methods.

Anesini *et al*. (2008) calculated the total polyphenol content according to the International Organization for Standardization method (ISO) 14502-1 for the determination of substances characteristic of green and black tea. The antioxidant capacity was determined by the ferric thiocyanate method (FTC) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay. Green tea showed a higher polyphenol content than black tea. The total polyphenol concentration in green tea was found to vary from 21.02 (1.54 to 14.32 (0.45% of gallic acid equivalents (GAE), whereas in black tea, the polyphenol content ranged from 17.62 (0.42 to 8.42 (0,55% of GAE ($P <0.05$). A similar profile was observed for the antioxidant capacity determined by both methods. The antioxidant activities were well correlated with the total polyphenol content (r^2) 0.9935 for the ferric thiocyanate method and (r^2) 0.9141 for the 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay). This is the first systematic screening for the quantification of polyphenols and antioxidant activity in tea commercialized in Argentine markets. The results obtained herein allow one to conclude that Argentine tea is of very good quality when compared to teas from other sources.

Turkmen *et al.* (2007) measured total polyphenol contents and antioxidant and antibacterial activities of black tea. The polyphenol content was found to be in the range of 0.44-114.01 mg gallic acid equivalents (GAE)/g dry weight tea, depending on the solvent used and the length of the extraction process. In general, aqueous acetone displayed the highest polyphenol contents and antioxidant activity, while absolute acetone was the least efficient solvent. Antioxidant activities of tea extracts tested using the reducing power and 2,2-diphenyl-1-picryhydrazyl (DPPH) radical methods ranged from 0.09 to 1.18 and from 2.60 to 95.42 %, respectively, depending on the extraction conditions and the antioxidant activities correlated well with the polyphenol concentrations.

Chen *et al.* (2013) examined the total antioxidant capacity and total polyphenol content of 9 tea juices following in vitro digestion model. They showed that there was a significant variation in total antioxidant capacity [16,392.30 ± 111.64–22,340.23 ± 46.73l mol VC/L (DPPH), 3,194.24 ± 14.76–13,795.07 ± 37.46l mol Trolox/L (ABTS), 2,540.61 ± 59.79–7,951.57 ± 31.91l mol Trolox/L ferric reducing antioxidant power (FRAP)] and total polyphenol content (265.84 ± 9.52–876.62 ± 6.59l g GAE/mL). Following the in vitro digestion, most of the juices of antioxidant capacities and 7 juices of the total polyphenol content were decreased. Before the in vitro digestion, a highly positive correlation was found between the total polyphenol content and FRAP value. After the gastric phase of digestion, there was a very strong positive linear correlation between the total polyphenol content and total antioxidant capacity. After the duodenal phase of digestion, the correlation between the total polyphenol content and total antioxidant capacity was very weak, and also, a very weak linear correlation was found between the antioxidant assays.

Rahman *et al.* (2019) carried out a study to compare the black and green tea available in Bangladesh in respect to proximate composition and antioxidant activity. Eight brands of black tea and two brands of green tea were bought from local supermarket. The variance between every proximate content (moisture, protein, fat, ash and carbohydrate) of black and green tea was found statistically insignificant ($p > 0.05$). The total antioxidant activity was determined by phosphor - molybdenum assay method and ferrous chelating activity was monitored by measuring the generation of the ferrous ion ferrozine complex. Green tea performed significantly higher total antioxidant activity (792.87 AAE) than black tea (330.72 AAE). The variance of ferrous chelating activity between black and green tea was found statistically significant ($P < 0.05$) at concentration of 10 ppm, 100 ppm, 200 ppm, 400 ppm, and 600 ppm. Black tea showed maximum inhibition, 50.33 % at 600 ppm and the lowest 1.93 % at 10 ppm. The highest percent inhibition for green tea was found 32.14 at 600 ppm and the lowest 1.25 at 10 ppm. IC50 values were found 5.50 ppm and 4.50 ppm for black and green teas respectively.

Alam *et al.* (2015) studied five different popular marketed brands of tea produced in Bangladesh viz; Tetley-Premium, Lipton-Taaza, Finlay-Premium, HRC-Clevedon, Ispahani-Blender's choice before expiry dates to evaluate their status of qualitative parameters viz; Caffeine, Total Polyphenol Content (TPC), Theaflavin (TF), Thearubigin (TR), Highly Polymerized Substances (HPS), Total Liquor Colour (TLC), Briskness Index (BI) and Colour Index (CI). In the study, the values of caffeine and TPC were found to be varied from 2.65% (Finlay-Premium) to 3.28% (Tetley-Premium) and 24.58% (Lipton-Taaza) to 12.76% (Tetley-Premium) respectively. Similarly, the estimated values of TF, TR and TLC were detected to be maximum in Lipton-Taaza tea but maximum HPS was in Finlay-Premium tea whereas minimum TF and TLC was in Tetley-Premium tea and minimum TR and HPS was in HRC-Clevedon tea. In contrast, BI and Cl were found to be the highest in Ispahani-Blender's choice and HRC-Clevedon tea respectively and the lowest in Tetley-Premium tea. The present study concludes that the Lipton-Taaza tea is superior over the other brands in respect to qualitative status and all the studied brands may, therefore, be ranked as: Lipton-Taaza > Finlay-Premium > Ispahani-Blender's choice > HRC-Clevedon > Tetley-premium.

CHAPTER III

MATERIALS AND METHODS

3.1. Sample collection and sample description

Fourteen different brands of teas were purchased from departmental stores (Figure 4) of Dhaka city, capital of Bangladesh. Teas were used as sold by the vendor without further identification. A voucher specimen was retained in the Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University for further reference. Of those fourteen samples seven samples are black tea, six samples are green tea and one sample is white tea. Tea samples are shown in Figure 5.

Figure 4. Experimental sites of the studies. (Google)

Figure: 5(A). Experimental samples: (a) Matcha green tea; (b) Kazi and kazi green tea; (c) Taaza (Brooke Bond); (d) Bengal classic tea; (e) Jafflong tea; (f) Kazi and kazi black tea;

Figure: 5(B). Experimental samples; (g) Ispahani premium green tea; (h) Fresh premium tea bags; (i) Jafflong green tea; (j) Ispahani Mirzapur tea; (k) Lipton green tea; (l) Fresh premium tea

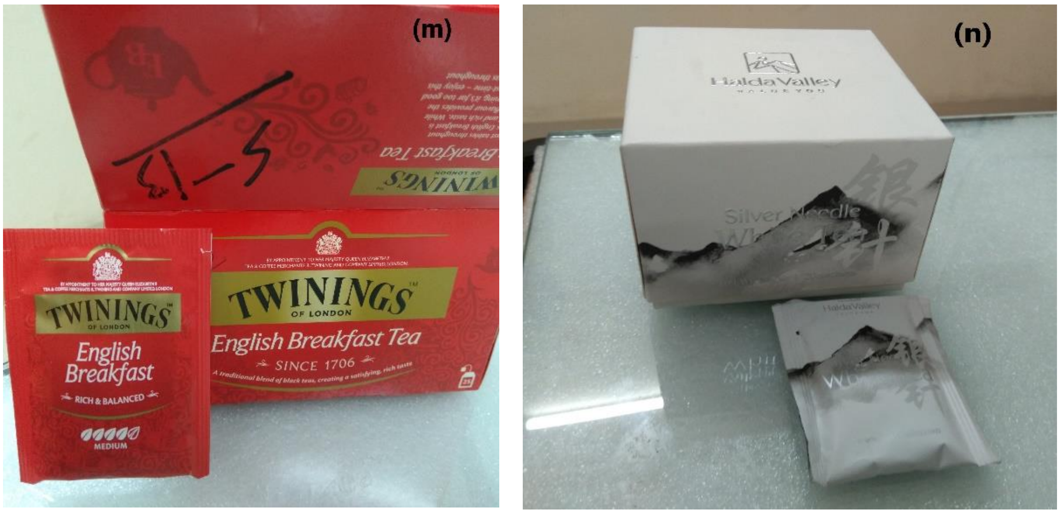

Figure: 5(C). Experimental samples; (m) Twinings of London; (n) Halda valley white tea

3.2. Equipment

1. Beaker

2. Centrifuge (800 Centrifuge, TIN- 148-105-0491, China)

3. Centrifuge tube (15 mL & 50 mL, Corning Incorporated, Mexico)

4. Cuvette (2mm)

5. Eppendorf tubes (2 mL & 5 mL, Beijing Lab Technology, China)

6. Micropipette (10µL – 100µL, 100µL - 1000µL, 1000µL - 5000µL)

7. Parafilm (Bemis, USA)

8. Reagent bottle (100 mL, 500 mL)

9. Rotter machine (FAB-LAB, SAU)

10. Shaking incubator (Model no: BJPX-103B, Biobase, China)

11. Spectrophotometer (Dynamica; HALO DB-20S, UV-VIS double beam, UK)

12. Volumetric flask (50 mL, 100 mL)

13. Vortex (Model no: VM-2000, Digisystem, Taiwan)

14. Weighing balance (New FGH Series, A&D company, Korea)

3.3. Chemicals

1. Ferulic acid (M. W= 194.18 g/ mol, FUJIFILM WAKO, JAPAN)

2. Folin & Ciocalteu's Phenol reagent (FCR) (M. W=1.2 g/ mol; Lab Fine, India)

3. HPLC grade methanol (M. W= 32.4 g/ mol, Merck, Germany)

4. Hydrochloric Acid (HCl) (37%, M. W= 1.19 kg/ L, Merck, Germany)

5. Sodium Carbonate (Na_2CO_3) (M.W = 1.19 kg/ L, Merck, Germany)

3.4. Reagent preparation

a) Sodium carbonate Na_2CO_3 (20%) solution: In a reagent bottle, 20 g of Na_2CO_3 was taken and 100 mL of distilled water was added in it to. Total content was mixed properly to dissolved sodium carbonate properly.
b) Folin-Ciocalteu reagent (FC) reagent (1:2): A volume of 2 mL FCR was take in a 15mL centrifuge tube and 4 mL of distilled water was added in it and mixed properly. This reagent was freshly prepared before each experiment.
c) Preparation of standards

Preparation of ferulic acid standard: An amount of 100 mg of ferulic acid was dissolved in 15 mL of methanol (HPLC grade) in a 100 mL volumetric flask. Final volume was made up to 100 mL with distilled water. This volume was considered as stock amount. Then, 4 mL of stock solution was transferred in a 50 mL volumetric flask and final volume was made up to 50 mL with distilled water.

3.5. Extraction of polyphenols

A) Extraction with methanol

To prepare the Methanolic extract of samples, 1 g of each sample was taken in 15 mL centrifuge tubes. 4 mL of HPLC grade methanol was added to each tube. Then sample was mixed properly using a vortex mixture. Later the tubes with mixture were set to a mixture machine to mix properly for 1 hour at 2500 rpm. After 1 hour, the mixture machine was stopped and tubes were kept aside for 5 minutes. Then the tubes were centrifuged for 5 minutes at 2500 rpm. After centrifugation, the supernatant was transferred to Centrifuge tubes (15 ml). The Centrifuge tubes were tightly capped and wrapped with parafilm. The methanolic extracts of samples were labelled properly and preserved at 4°C.Extraction of samples with methanol is presented in Figure 6.

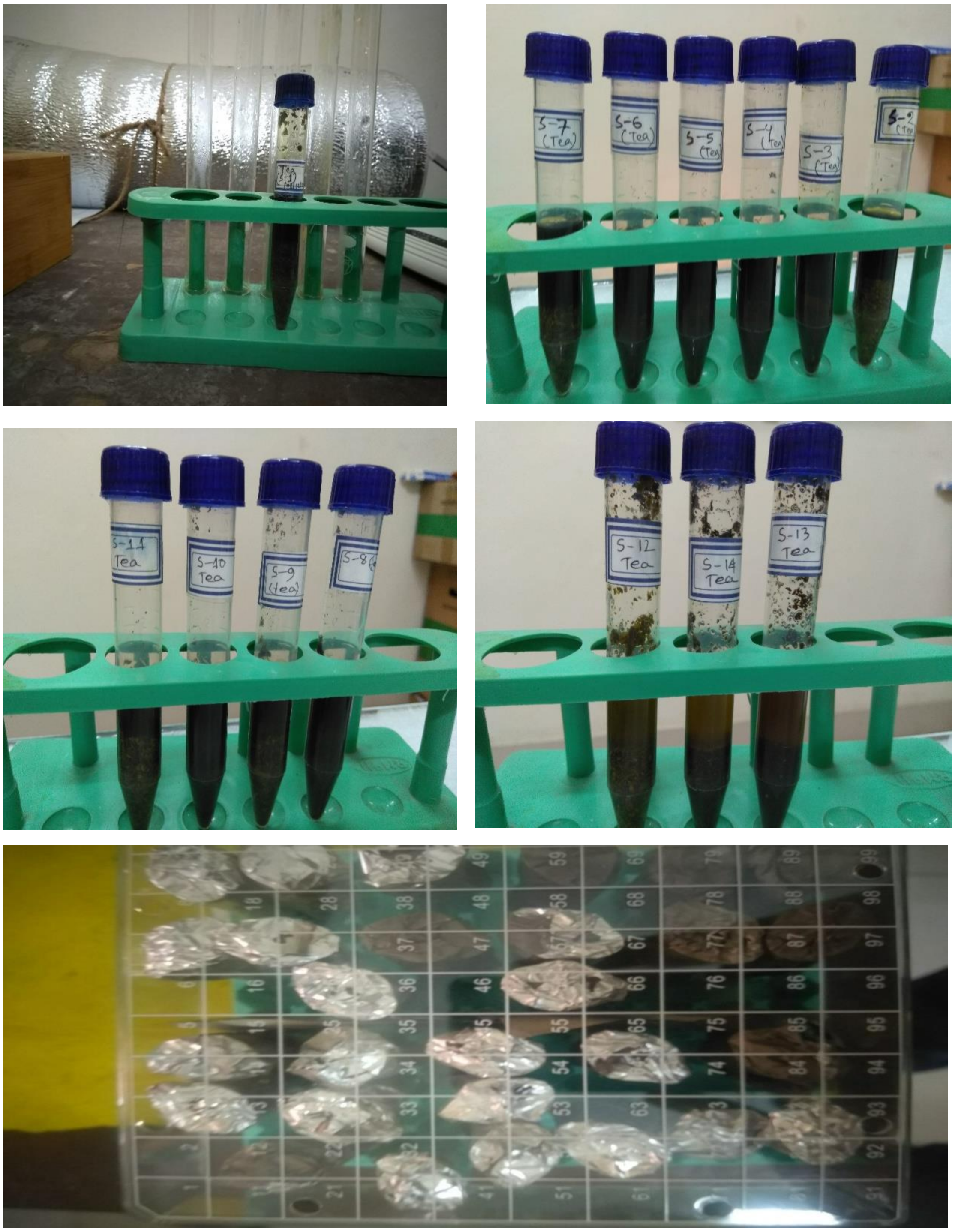

Figure:6. Extraction of polyphenol from tea samples with methanol

B) Extraction with hot water

To prepare the hot water extract of samples, one gram of each sample was taken in 15 mL centrifuge tubes, then 10 mL of hot water was added to each tube. Samples and solvents were mixed well by vortex mixture. Afterwards mixing of samples with solvent and collection of supernatants from centrifuge tubes were carried out following the steps mentioned in the above. The samples were labelled properly and preserved at 4 °C. Extraction of samples with hot water is presented in Figure 7.

Figure:7. Extraction of polyphenol from tea samples with hot water

3.6. Determination of methanol soluble polyphenol

Methanol soluble polyphenol in tea was determined by Folin-Ciocalteu reagent earlier described by Singleton, Orthofer, & Lamuela-Raventós, (1999). Briefly, in a 15 mL centrifuge tube, methanolic extract (in different volume) was taken. Final volume of extract was adjusted to 250 μL with distilled water. Then, 250 μL Folin-Ciocalteu reagent was added with it. Later 500 μL of 20% Na_2CO_3 was added in it and shaken well. Final volume was made up to 5 mL with distilled water. Total content was mixed well by vortex for few seconds. The mixture was kept in dark for 30 min undisturbed. After 30 min, the mixture was centrifuged at 2500 rpm for 5 min. In the similar way, a blank was prepared for the study. Then, the absorbance of the solution was taken at 760 nm by using spectrophotometer. Finally, soluble polyphenol in tea sample was calculated from the standard curve of ferulic acid. Polyphenol concentration was determined by using ferulic acid equivalent scale (1 OD equivalent to 0.0227 μg of ferulic acid). Soluble polyphenol of the tea sample was expressed as mg of ferulic acid equivalent/ 1 g sample dry weight.

3.7. Determination of hot water-soluble polyphenol

Hot water-soluble polyphenol in tea samples was determined from hot water extract of tea following the procedure described in above. Hot water-soluble polyphenol was expressed as mg of ferulic acid equivalent per 1 g sample dry weight.

3. 8. Preparation of standard curve (linearity curve) of ferulic acid

The standard curve of ferulic acid was prepared from the straight-line equation, $y= mx + c$. Linear regression was confirmed from various concentration (3.2,4,4.8,…..8,8.8) of ferulic acid plotted on X axis and OD obtained from this concentration on Y axis. The standard curve is shown in Figure 8.

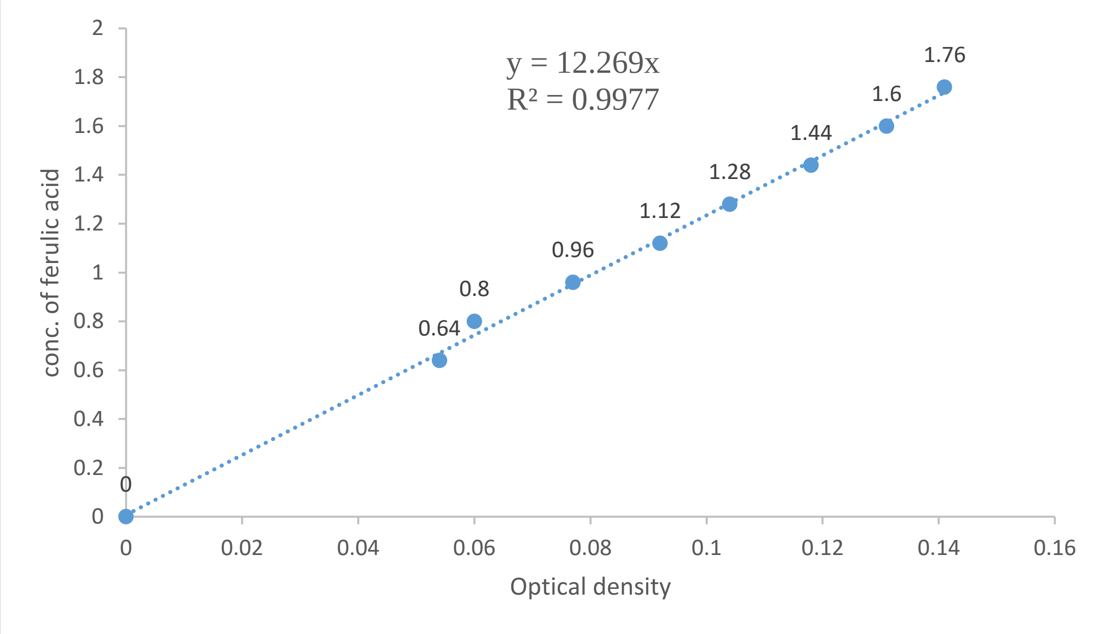

Figure:8. Standard curve of ferulic acids

3.9. Statistical analysis

Values are presented as mean ∓ standard deviation (SD) of 3 replication of each experiment and calculated as dry weight basis. Means of components in samples were compared by one way ANOVA and Tukey's test at the confidence level of 95% using Statistix 10 statistical software. (Version 10.0. 2105 Miller Landing Rd,Tallahassee FL 32312,USA)

CHAPTER IV

RESULT AND DISCUSSION

4. 1. Phenolic contents in tea

4.1.1. Methanol soluble polyphenols

The contents of methanol soluble or free phenolics was estimated among the fourteen tea brands of tea. The polyphenol contents ranged from 9.86 ± 3.95mg FAE/ 1 g of tea to 138.25 ± 15.95 mg/1 g of tea dry weight basis. (Figure 09). Among the group of samples Matcha green tea and Ispahani Premium green tea had the highest polyphenol content which were 138.25 ± 15.95 mg FAE /1 g of tea and 95.28 ± 14.22 mg FAE/1 g of tea respectively. On the contrary, Taaza (Brooke Bond) black tea and Fresh premium black tea had the lowest amount 9.86 ± 3.95mg FAE/1 g and 12.29 ± 2.21 mg FAE /1 g of free phenolics respectively. Bangal classic tea, Jafflong black tea, Ispahani Mirzapur tea, Twinings of London black tea had significally similar free phonolics.

Marilena and Shicong (2017) reported the polyphenols in green tea and black tea ranged from 2.70 ± 0.02mg /g to 90.94 ± 1.12 mg /g of tea. Among green tea were reported highest levels of free polyphenols. The lower level of polyphenol found in present study was relatively similar to the values reported by Marilena and Shicong (2017). However, the higher level of polyphenol reported by the authors was much lower than the present findings. similar to their study we also observed that green tea brands content higher polyphenols compare to black tea brands.

Zhang *et al.*, (2011) compared the chemical constituents of aged pu-erh tea, ripened pu-erh tea, and other teas and demonstrated the similar result of the present study. In his experiment TPC of the 32 ripened Pu-erh teas ranged from 3 to 27 mg/g with a mean value of 17 ± 6 mg/. Their reported value was similar to the lower range of polyphenol of black tea.

As for the 6 green teas, TPCs ranged from 60 to 138 mg/g with a mean of 84 ± 29 mg/g, again, in good accordance with those from the literature (Perez-Jimenez *et al*., 2010).

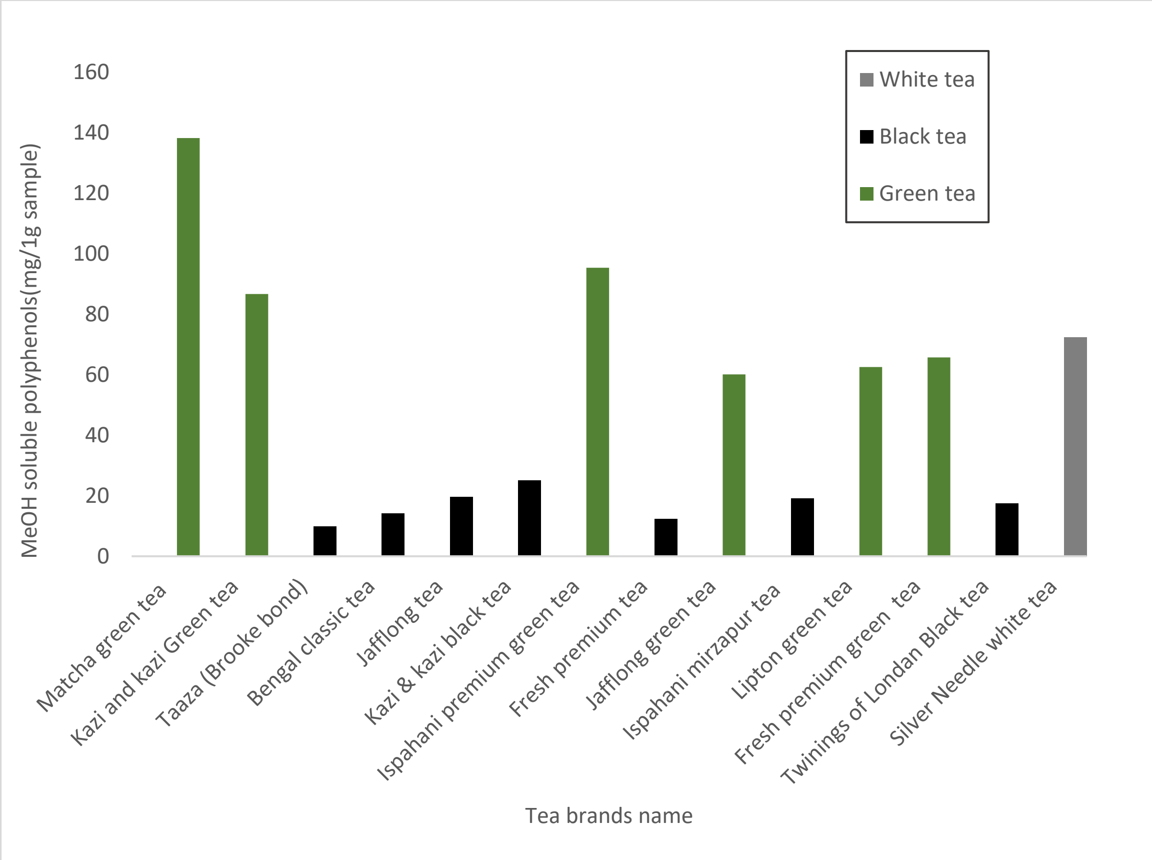

Figure 9. Amounts of methanol soluble phenolics in collected tea brands

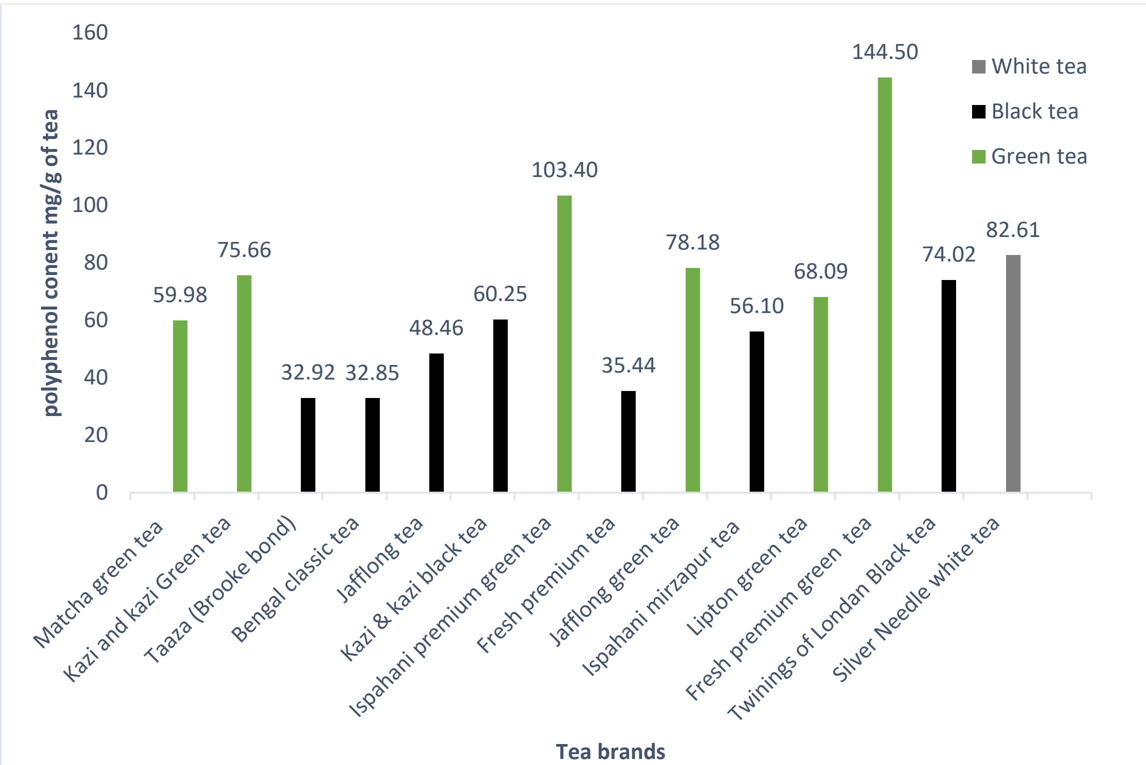

Figure 10. Amounts of hot water soluble phenolics in collected tea brands

4.1.2. Hot water-soluble polyphenols of tea

The contents of hot water-soluble polyphenols ranged from 32.85± 1.6 to 144.50± 18.21mg FAE/g of tea (Figure 10). Among the groups, fresh premium green tea and Ispahani premium green tea had the highest water-soluble polyphenol content which were 144.50± 18.21mg FAE/g of tea and 103.40± 8.89 mg FAE/g of tea respectively. Matcha green tea (59.98±2.01 mg FAE/g of tea), Kazi & kazi green tea 75.65±15.5 mg FAE/g of tea), Ispahani mirzapur tea (56.09 ± 2.9 mg FAE/g of tea), Lipton green tea (68.09±8.02) had similar water-soluble polyphenol content. Among the samples Bengal classis tea (32.85±1.6 mg FAE /g of tea) and Taaza (Brooke bond) tea (32.92 ±4.08 mg FAE /g of tea) performed the lowest water-soluble polyphenol respectively (Figure 10).

Alam *et al.* (2015) reported the water-soluble polyphenol contents ranged from 117.89-211.39 mg/g in tea. The range of polyphenol content in Bangladesh tea is 221-310 mg/g (Choudhury, 1990; Chowdhury and Alam, 2001). The present study observed much lower water-soluble polyphenol than the reported value.

Table 2. Total phenolics (mg FAE/ g dry weight basis) in two different extractions solution of different tea brands

Tea brands	Amount of hot water-soluble polyphenol (mg FAE/g)	Amount of methanol soluble polyphenol (mg FAE/g)
Matcha green tea	59.98±2.02 cde	138.23±15.97a
Kazi and kazi green tea	75.66±15.54c	86.70±5.67bc
Taaza (Brooke bond)	32.92±4.08f	9.86±3.96e
Bengal classic tea	32.85±1.65f	14.17±1.27e
Jafflong tea	48.46±7.07def	19.60±3.83e
Kazi & kazi black tea	60.25±6.65cde	25.02±2.60e
Ispahani premium green tea	103.40±8.90b	95.28±14.22b
Fresh premium tea	35.44±3.10ef	12.29±2.21e
Jafflong green tea	78.18±8.61bc	60.09±4.01d
Ispahani mirzapur tea	56.10±2.98cdef	19.07±2.13e
Lipton green tea	68.09±8.02cd	62.51±3.60d
Fresh premium green tea	144.50±18.22a	65.70±14.41cd
Twinings of Londan Black tea	74.02±3.54cd	17.36±2.66e
Silver Needle white tea	82.61±13.95bc	72.27±3.09bcd

All values are presented as mean ± SD (standard deviation) at dry weight basis; different alphabets in each column show the significant difference ($p < 0.05$).

Table 3: Comparison of present study data with some other research works.

Types	Result	References
Black tea	9.86±3.96 to 74.02±3.54 mg/ g of tea	This study
Green tea	62.51±3.60 to 144.50±18.22 mg/ g of tea	This study
White tea	72.27±3.09 to 82.61±13.95 mg/ g of tea	This study
	National	
Red dust (Black tea)	8.20 ± 0.49 mg GAE/ g of extract	*Nibir et al. (2017)*
Green tea	26.33 ± 1.73 mg GAE/ g of extract	*Nibir et al. (2017)*
Black tea	242.46±12.42 mg GAE /g dry extract	*Rahman et al. (2021)*
Green tea	763.42± 10.51 mg GAE /g dry extract	*Rahman et al. (2021)*
	International	
White tea	112 ± 0.1 mg GAE/ g of tea leaves	*Quesille-Villalobos et al (2013)*
Green tea	111 ± 1 mg GAE/g of tea leaves	*Quesille-Villalobos et al (2013)*
Black tea	37 ±1 mg GAE/g of tea leaves	*Quesille-Villalobos et al. (2013)*
Jasmine honey tea (Unified)Green tea	876.62 ± 6.59 µg GAE/mL of tea juice	*Chen et al. (2013)*
Ice tea (Unified)Black tea	265.84 ± 9.52 µg GAE/mL of tea juice	*Chen et al. (2013)*
Chinese green tea, *Camellia sinensis*	1216±32.0 mg GAE/ cup	*Ali et al. (2005)*
Green tea	211 mg GAE/ g	*Anesini et al. (2008)*
Black tea	84 mg GAE/g	*Anesini et al. (2008)*

According to Table 2, a high variability was observed in the total phenolic contents among all analysed tea brands. Total phenolics ranged from 9.86 ± 3.95 to

144.50±18.22 mg FAE /g of tea dry weight basis. Overall, white and green tea infusions showed higher total phenolic contents than the black tea groups. Difference in the total phenolic contents in tea samples can be influenced by several factors, such as the diversity of used raw material (sinensis or assamic ecotypes), the geographic origin, type of processing (fermented or nonfermented), and the extraction conditions for their analysis. Considering the effect of manufacture conditions, it is well known that during the fermentation process, the quantity of simple catechins, commonly found at high levels in nonfermented teas, decreases due to oxidation reactions catalysed by natural polyphenol oxidases. These reactions lead to the increase of larger phenolic molecules, such as theaflavins and thearubigins in black teas (Ankolekar *et al.,* 2011). In this way, Deetae *et al.* (2012) found that total catechin contents were significantly higher in green teas than in oolong (semifermented) and black teas (fermented). Also, other studies by Moraes *et al.* (2008) revealed that green teas have more total phenolic compounds than black teas. Anesini, *et al.* (2008) reported that total polyphenol concentration was higher in green teas (nonfermented) than in black teas (fermented) from Argentina. Levels varied from 211 to 143 mg GAE/g in green teas, and from 176 to 84 mg GAE/g in black teas. (Shown in Table 2) These ranges are higher than values obtained for green and black teas in current study and may be related to the fact that above authors used methanol for phenolics extraction. The analysis of water-soluble phenolic compounds in tea infusions would give more physiologically relevant results for overall potential phenolic-linked health benefits.

According to Table 2 hot water extraction show highest amounts of polyphenol. Sari *et al.,* (2007) reviewed that methanol extraction show highest polyphenol content. The difference between this two-extraction process because duration of extraction process. There is another cause for difference land condition of tea production. Bangladeshi tea cultivation land comparatively low altitude.

CHAPTER V

SUMMARY AND CONCLUSION

Present study was conducted to measure nutritional properties of tea available in Bangladesh. The experiment was carried out in Sher -e-Bangla Agricultural University. A wide range of local and foreign tea brands were evaluated for the nutritional properties such as polyphenols. Total fourteen brands were selected. of those seven tea brands were black tea, six tea brands were green tea and one white tea. Matcha green tea, Kazi and kazi green tea, Taaza (Brooke bond), Bengal classic tea, Jafflong tea, Kazi & kazi black tea, Ispahani premium green tea, Fresh premium tea, Jafflong green tea, Ispahani mirzapur tea, Lipton green tea, Fresh premium green tea, Twinings of Londan Black tea, Silver Needle white tea were the study materials.

Free phenolics of tea was estimated from the absolute methanolic extract of tea samples. Among the group of samples, Matcha green tea and Ispahani Premium green tea had the highest polyphenol content which were 138.25 ± 15.95 mg FAE /1 g of tea and 95.28 ± 14.22 mg FAE/1 g of tea respectively while Taaza (Brooke Bond) black tea and Fresh premium black tea had the lowest amount 9.86 ± 3.95mg FAE/1 g and 12.29 ± 2.21 mg FAE /1 g of free phenolics, respectively.

Water soluble polyphenol of tea was measured from the hot water extract of tea samples Fresh premium green tea and Ispahani premium green tea had the highest water-soluble polyphenol content which were 144.50± 18.21mg FAE/g of tea and 103.40± 8.89 mg FAE/g of tea respectively. Bengal classis tea (32.85±1.6 mg FAE /g of tea) and Taaza (Brooke bond) tea (32.92 ±4.08 mg FAE /g of tea) performed the lowest water-soluble polyphenol, respectively.

Contribution of total polyphenol in tea composition ranged from 9.86 ± 3.95 to 144.50± 18.21mg FAE/g of tea. Green teas showed the highest polyphenol content as compared to black tea. There was a relationship between fermentation process with polyphenol content. so fully fermented tea like black teas showed lower polyphenol content. These tea brands should have further more studies for other nutritional contents and their potential health benefits.

CHAPTER VI

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