

**In vitro compositional and phytochemical analysis of green and black tea**<sup>b</sup> Iram Aslam\*, <sup>a</sup> Asif Ahmad, <sup>c</sup> Muhammad Usman Raja, <sup>a</sup> Saleha Ahmed<sup>a</sup> Institute of Food and Nutritional Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan,<sup>b</sup> School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan,<sup>c</sup> Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.**Authors' Contribution**

Aslam, I. &amp; A. Ahmad designed the study, analysis, results, and discussions, U. Raja and S. Ahmed analysed antimicrobial activity.

**\*Corresponding Author's Email Address:** iramaslam92@gmail.com**ABSTRACT****Review Process:** Peer review

The traditional beverage that people consume more after water is tea. The ethanolic extract of green and black tea have antioxidant and antimicrobial activity and these activities may differ in their processing methods. In this study, a physiochemical analysis of green and black tea was carried out. The physiochemical analysis showed that green tea has a higher moisture content, crude fat, and crude fiber except for crude protein and ash content which was found higher in black tea. The maceration process was used for extraction purposes. This extract was used to analyze the total phenolic and flavonoid content, antioxidant activity, and antimicrobial activity. A significant difference was found in the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of green and black tea. The flavonoids and phenolic content in T<sub>1</sub> (ethanol 100%) acquired average and significant in the extract of green and black tea. The maximum value of TPC was observed in green tea extract 152.17<sup>a</sup> ± 0.28 mg GAE/g in treatment T<sub>1</sub>, and the value of TFC in green tea was found 40.26±11.387mg QE/g and the minimum value was found in black tea at 36.85±13.68 mg QE/g of extract. The antioxidant activity of green tea extract ranged from 90.64% to 69.07%, and black tea extract ranged from 89.05% to 50.93% in each extract. The parallel determination of the inhibitory effect of gram-negative and positive bacterial strains (*Streptococcus pyogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*) was found sensitive and resistant towards extract of tea. The positive and negative effect was found in antimicrobial activity by using antibiotics against these bacterial strains.

**Keywords:** Extraction technique, antioxidant potential, antimicrobial activity.

**INTRODUCTION:** Tea (*Camellia sinensis*) is a common beverage that is consumed more around the world. Black tea is consumed worldwide and green tea is consumed mostly in North Africa and Asia. Black, green, and white tea is commonly consumed more than is attained from the buds or leaves of the *Camellia sinensis* plant. But there is a difference between them regarding processing methods. The history of tea is a wide range, it was originated in South Eastern China but now it has a wide range of varieties and is cultured across countries tropical and sub-tropical regions around the world. There are more than 82 species have been developed. Pakistan has less cultivation of tea as compared to other countries because in Pakistan, tea is cultivated mainly in the district Mansehra. In that region, an institute was developed, National Tea Research Institute (NTRI) which was established in 1986 in Pakistan. The highest concentration of antioxidants is typically found in tea (green and black tea). The compounds present in green and black tea are flavonoids that naturally occur in tea and have the colour significance, and taste that help in body functioning. The most important phytochemicals or polyphenols in green tea are catechins which include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGC) (Cabrera *et al.*, 2006). Green tea is non-fermented, in that catechins are a more important compound which is responsible for higher antioxidant quality. In green tea, some pigments are present like chlorophylls and carotenoids, and after the heating process, they are reduced turned into pheophorbide and pheophytin. Pheophytin is present in plants and responsible for the electron transfer pathway of the photosystem in plants (Hsu *et al.*, 2013). Black tea is also responsible for this quality as it also contains theaflavins, thearubigins, theanine, flavonols, protein, carbohydrates, and many more. Furthermore, compounds like polysaccharides, minerals, volatile compounds, alkaloids, and trace elements are present in it. Besides antioxidant activity, black tea and green tea also possess antimicrobial activity against various microorganisms. The polyphenols in tea, anthocyanidins, catechins, and tannins that are hydrolyzable show antimicrobial activity. In green tea, epigallocatechin gallate and epicatechin gallate inhibit the growth of many bacterial species and it has an anticarcinogenic effect which is why their antimicrobial activity is important to determine (Shankar *et al.*, 2007). The comparative study of green and black tea can provide information for consumers, researchers, and the tea industry, helping individuals make informed choices and better understand the unique characteristics associated with both types of tea.

**OBJECTIVES:** To evaluate the compositional and phytochemical analysis of green and black tea, (2) to measure the antimicrobial potential of green and black tea.

**MATERIALS AND METHODS:** **Collection and preparation of sample:** The samples of black and green tea were procured from the

National Tea Research Institute, Mansehra, Pakistan. The chemical analysis was done in the Food Analysis lab of the Department of Food Technology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi (PMAS-AAUR), Pakistan. Both samples were dried, ground into fine powder, and stored in polyethylene zip bags until further analysis. All the chemicals and reagents required for this study were purchased from Sigma Aldrich.

**Compositional analysis of black and green tea:** Moisture content, crude fat, crude fiber, crude protein, and ash content were determined by the standard methods of AOAC (2002) with slight modifications.

**Extraction of green tea and black tea:** Ethanolic extraction of black and green tea was prepared. The extract of both teas was made by combining 20g of powder (green and black tea) with different concentrations of control (T<sub>0</sub>), 100% (T<sub>1</sub>), 75% (T<sub>2</sub>), and 50% (T<sub>3</sub>) (w/v) ethanol in separate Erlenmeyer flasks. The mixture was then shaken for 48 hours at room temperature (25°C) at a speed of 150 rpm. The extract and solvent were filtered through Whatman filter paper No. 1, decreased in volume (1/4<sup>th</sup>) by rotary evaporator, and then kept in airtight bottles at 40°C in the refrigerator for further analysis.

**pH determination of black and green tea:** The pH of tea extract was determined using a pH meter (Inolab, WTW series) following the standard method of AOAC (2002). For this, 5g extract of both teas was taken and mixed with 10mL distilled water. The pH meter was standardized with buffer solution (pH 4 and 7). After standardizing the pH meter, the samples were carried out to find the pH of the samples.

**Phytochemical analysis**

**Total phenolic content (TPC):** The Folin-Ciocalteu (FC) method, as described by Turkmen *et al.* (2006), was used to calculate the TPC of tea samples. For the calibration curve, the standards for gallic acid were made. Each 0.5g extract was made into a solution by dissolving it in 5mL of ethanol. Each extract was diluted to 0.5 mL with 2.5 mL of FC-Reagent (10%) and 2.5 mL of Na<sub>2</sub>CO<sub>3</sub> (7.5%) for neutralization. The sample spent 30 minutes in the incubator. At 765 nm, the absorbance was measured using a spectrophotometer (UV1100), and the result was converted to mg GAE/g of extract.

**Total flavonoid content (TFC):** Tea sample TFC was examined using the techniques outlined by (Mohammed, 2014) and Turkmen *et al.* (2006). In a 10 mL round bottom flask, 4 mL of distilled water and 1 mL were added on purpose. 0.3mL of NaNO<sub>3</sub> (5%) was then added to the solution. 0.3mL of 10% AlCl<sub>3</sub> was added after 5 min and allowed to sit for around 6 minutes. Then 2mL of 1M NaOH was added to each sample after a 6-minute stay. The capacity was increased by adding distilled water to make it 10mL. After thoroughly combining all the solutions, use a spectrophotometer (UV1100) to measure the absorbance at 415 nm. In mg of Quercetin equivalents/g of extract, TFC was determined.

**Antioxidant activity (DPPH Assay):** The free radical scavenging activity of both tea extracts was determined as described by Omar *et al.* (2016). 100 mL of methanol was added to 24 mg of DPPH to create the stock solution. Using 0.02g of each extract, various concentrations of each were created (400g/mL to 50g/mL). Each extract was then given 3mL of the DPPH solution. 30 minutes were spent incubating the reaction mixture in a dark area after it had been well mixed. A 517nm spectrophotometer (UV1100) was used to measure the absorption. The percentage of DPPH was calculated as:

$$\text{Inhibition (\%)} = \frac{AB - AA}{AB} \times 100$$

AB = Absorbance of a blank sample

AA = Absorbance of sample

**Antimicrobial activity:** The microbial content of tea extract was analyzed by using the disc diffusion method (Nibir *et al.*, 2017). The bacterial strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa* were used to determine the antimicrobial activity of both tea extract. The test microorganisms were spread on a separate Muller-Hinton media that had been prepared for the growth of bacteria. Then wells were prepared on the media plates with the help of an aluminum bore (6mm diameter). After that 500µL of each extract (diluted) was taken in the wells and solvents (ethanol and distilled water) were used as a negative control. Augmentin, Amikacin, and Moxifloxacin were used as antibiotics to determine the minimum inhibitory concentration for antimicrobial susceptibility. The plates were stored at 37°C for 48hr.

**Statistical analysis:** All the data were analyzed with the statistical software Minitab 17 to check the level of significance 5%, while the intragroup comparison was made using Tukey's HSD test.

**RESULTS AND DISCUSSIONS: Compositional analysis of black and green tea:** The results of the compositional analysis of black and green tea were statistically analysed and presented in figure 1.

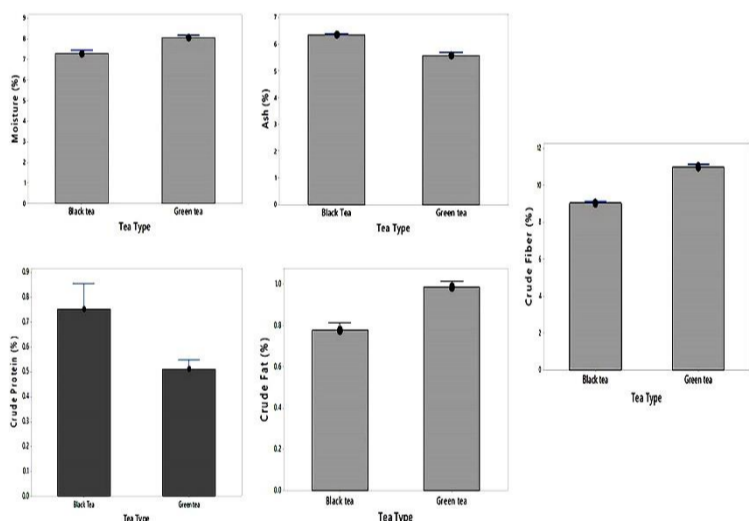


Figure 1: Compositional analysis of black and green tea.

The results of this study showed that green tea has higher compositional compounds except for crude protein and ash content which was found higher in black tea. The statistical results were found to be significant different among both tea samples. The moisture content in the green tea sample was found higher as compared to black tea. The higher moisture content in green tea may be due to the difference in processing methods of both teas. The green tea has the exclusion of fermentation process as compared to black tea. In the processing of black tea, and enzymatic changes are involved. The previous research also indicated that the non-fermented character of green

tea may relate to its quality, but it depends on the type or condition of tea that has been used (Yao *et al.*, 2006). This study validates the previous study of Olowolagba, (2011) that reveals both types of tea are influenced by the fermentation process. Time and temperature are also important parameters to retain the quality of tea in such a way that drying leaves through the evaporation process may lead to loss of water. The crude protein content in green tea ranged from 0.48% to 0.56% and in black tea, it ranged from 0.67% to 0.89%. The present study showed that black tea has more protein content than green tea. It might be the presence of higher nitrogen content in the soil where it was grown. Protein in tea can protect biological cells against various mutagenic effects (Shen *et al.*, 2008). It is a minor constituent of tea and has a substantial impact on the quality of tea.

Due to the difference in the processing method of both types of teas, in black tea theanine is the main compound that has the amino acids more than 50% of the total amino acids of black tea. But in green tea, it is different in such a way that amino acids depend on the clone and type of tea (Yao, 2006). The protein content in black tea is owed to oxidation of leaves throughout processing. Enzymatic oxidation occurs in black tea in which catechins are transformed into theaflavin and thearubigins. It is directly measured by analysis of amino acids and indirectly measured by determining the nitrogen content (Cabrera *et al.*, 2003). The crude fat in green tea was found higher (0.98%) as compared to black tea. A non-significant difference was found in the comparison of both tea samples. (Figure 1). Crude fat is a minor component of tea that has less impact on the quality of tea. The leaves of green tea are prepared by steaming them and after drying leaves at a specific temperature to evade polymerization and oxidation of phenolic compounds present in tea (Sajilata *et al.*, 2008).

The higher crude fiber content in green tea may be due to the processing methods which done with impurities like crushing, curling, and tearing. The low fiber content in tea samples may be attributed to the young leaves of tea (Carter and Drewnowski, 2012). This study validates the Śmiechowska & Dmowski, (2006) that reveals crude fiber content regarding quality does not increase from 16.5% in black tea. The green tea is important in this point that have more fiber content. The crude fibers help to prevent constipation and work against the movement of the bowel. Further, researchers are also concerned that this parameter allows the determination of tea age in which leaves are an important part of plant quality in terms of commercial use (Kottawa-Arachchi *et al.*, 2011). This study suggested that, the low percentage of fiber content in black tea could be attributed to the enzymatic processing during fermentation, and the fiber content in black tea is not linked with the quality characteristics. It also showed that the tannins in tea may depend on the crude fiber (Kottawa-Arachchi *et al.*, 2011). The ash content was found 6.36% in black tea as compared to green tea. The Ash content of the food determines its mineral content which is required in minute quantities but vital for the normal physiological functioning of the body. This previous study of Olowolagba, (2011), revealed that the higher ash content as leaves develop, the water-soluble potash and phosphoric acid content decrease, resulting in a variation in the composition of tea ash with leaf age. The ash content provides the total presence of minerals in part of plants (leaves). The presence of mineral content in black tea relate to the quality of tea. Mohammed and Sulaiman (2009) also revealed that black tea contains several minerals including Mg, K, Na, Ca, Mn, and Cu that are important for the plant itself and for humans from a nutritional point of view. The amount of these minerals in black and green tea varies according to environmental and processing conditions. The raw leaves of the black tea when undergoing the fermentation process may have the possibility of adulteration that will ultimately reduce the quality of the tea. The previous work by Rahman *et al.* (2019) predicted a positive relation between the quality and ash content of black tea. The quality of black tea is associated with the availability of higher ash content in black t

**pH analysis of black and green tea:** The average pH in green and black tea samples was found between 4.16 and 3.02 respectively. The results of pH in both samples showed that green tea has a higher value of pH presented in Figure 2.

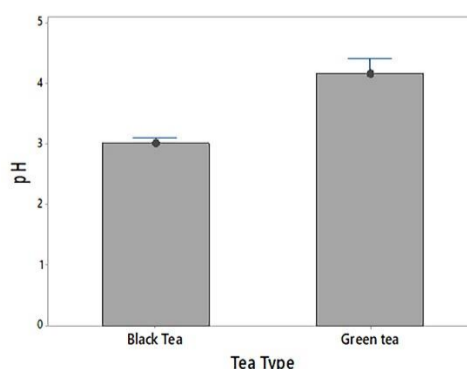


Figure 2: The pH analysis of green and black tea.

The pH in green tea is higher because of the presence of catechins which are abundant in green tea. In green tea, catechins are more stable at low pH that is ranged from 4 to 6 (Saadeh *et al.*, 2009). Another reason for pH stability in green tea was due to the effect of

extract at different solvents and its extract at low pH inhibited dissociated phenolic groups of reactants as these phenolic groups have stronger properties of electron-donating. Other than this, catechins in green tea are degraded to form semiquinones (Saadeh *et al.*, 2009). The current study corresponds with (Lunkes and Hashizume, 2014) and claims that green tea has a higher pH content than black tea because of the presence of phytochemicals and natural antioxidant compounds. These antioxidant compounds are more active during storage. The pH of green tea and black tea may also vary according to extraction technique, types of solvents used, and amount of concentration of extract. Similar results were described by Horanni and Engelhardt (2013) who observed extracts having low pH value possess strong buffering capacity. It was deemed necessary to control the pH on stability and extractability of green tea constituents.

**Phytochemical analysis:**

**Total phenolic content and total flavonoid content (TPC and TFC):** The results of TPC and TFC of each extract of tea are shown in table 1. The results indicate that T<sub>1</sub> had a higher concentration of TPC in green tea in comparison to black tea. The statistical value varied with the trend (T<sub>1</sub> > T<sub>2</sub> > T<sub>0</sub> > T<sub>3</sub>) of TPC in both teas. It showed that treatment that contains ethanol as an organic solvent has more phenolic content than distilled water because of polar. The results of TFC in green tea extract in T<sub>1</sub> were found less as compared to black tea. There was a non-significant difference found in T<sub>2</sub> of both green and black tea samples.

Tea Types	Treatments	TPC (mg GAE/g)	TFC (mg QE/g)
Green Tea	T <sub>0</sub>	53.02 <sup>g</sup> ± 0.05	24.03 <sup>f</sup> ± 0.54
	T <sub>1</sub>	152.17 <sup>a</sup> ± 0.28	49.75 <sup>b</sup> ± 0.45
	T <sub>2</sub>	70.05 <sup>c</sup> ± 0.42	46.13 <sup>c</sup> ± 0.13
	T <sub>3</sub>	37.62 <sup>e</sup> ± 0.40	41.21 <sup>d</sup> ± 0.20
Black Tea	T <sub>0</sub>	57.54 <sup>f</sup> ± 0.56	23.97 <sup>f</sup> ± 0.41
	T <sub>1</sub>	88.71 <sup>b</sup> ± 0.73	51.35 <sup>a</sup> ± 0.58
	T <sub>2</sub>	64.9 <sup>d</sup> ± 0.64	45.61 <sup>c</sup> ± 0.76
	T <sub>3</sub>	58.98 <sup>h</sup> ± 0.48	26.40 <sup>e</sup> ± 0.85

Table 1. Total phenolic and flavonoid content in green and black tea extract.

The phenolic (TPC) and flavonoid (TFC) content was found significantly higher in green tea when compared with black tea. The maximum value of TPC was observed in green tea extract *i.e.*, 152.17<sup>a</sup> ± 0.28 mg GAE/g in treatment T<sub>1</sub>. The findings showed that the treatments containing ethanol have more phenolic content than distilled water because of the polar nature of the solvent. The results revealed that T<sub>1</sub> had a higher concentration of phenolic compounds in comparison to black tea at the same concentration. The higher phenolic content in green was due to the presence of higher catechins in it in comparison to black tea. The solvent (ethanol) had a significant effect on the phytochemical extraction as polar solvents have a greater ability to yield higher concentrations of polyphenolic components (Lorenzo and Munekata, 2016). The findings of a previous study conducted by Orak *et al.* (2013) are in accordance with the current research. They explored that green tea is a valuable medicinal plant due to its

phenolic compounds as compared to black tea. In green tea catechins are predominant phenolic compounds in samples and higher phenolic content in green tea depends on extraction efficiency, infusion time, and temperature. The results of this study relate to another scientific study conducted by Bastos *et al.* (2007), who found that the ethanolic extract of green tea had higher TPC as compared to the aqueous extract. The variation can be attributed to phenolic content because of several factors such as the age of leaves, climate, species, and horticultural practices. Water, minerals, fertilizers, and soil are the main factors that can constitute an important influence on the polyphenols of tea. Phenolics constitute 30% of dry weight in green tea leaves. In addition, the formulation of tea is important because it gives a direct indication of the health-enhancing properties of green tea (Cabrera *et al.*, 2003). Fakheri *et al.* (2015) also analysed total phenolic content using a Folin-Ciocalteu assay in the same range as found in the current study. Polyphenols in a sample of green tea may also vary because of the non-fermentation process in comparison to black tea, which is present in fermented form. The fermentation process varies in both types of teas and it may lead to the variation in phenolic compounds because during the fermentation process, enzymatic activity occurs in black tea as compared to green tea (Almajano *et al.*, 2008). The TFC was measured as mg Quercetin equivalents (QE)/g of extract. The maximum value of TFC in green tea was found

at 40.26 ± 11.387 mg QE/g and the minimum value was found in black tea at 36.85 ± 13.68 mg QE/g of extract. The results of total flavonoid content in green tea extract were found lower as compared to black tea at the same concentration (T<sub>1</sub>). The flavonoids in green tea constitute a special class of phenolic compounds that exhibit higher antioxidant activity than phenolic acids. The high level of flavonoid content in green tea ethanol extract may be due to its richness in caffeoyl derivatives. These derivatives include chlorogenic acid, caffeic acid and dicaffeoylquinic acid (Higdon and Frei, 2003). This study validates the previous study of Orak *et al.* (2013) that revealed that green tea is a good source of phenolic and flavonoid content because green tea catechins are predominant in samples and contribute significant effects on color and taste. The ethanolic extract of green tea has a higher content of flavonoids than aqueous extract because they have biological activity to work in human body against various diseases. All teas from the *Camellia sinensis* plant, such as black, green, oolong and white teas naturally contain between 100 to 300 mg of flavonoids per serving (Bhagwat *et al.*, 2003).

**Antioxidant activity (DPPH Assay):** The results of antioxidant activity of black and green tea extract are shown in table 2. The statistical results were found to be significantly different in each extract. Antioxidant activity of green tea extract ranged from 90.64% to 69.07% followed by each extract and black tea extract ranged from 89.05% to 50.93% in each extract. The highest antioxidant activity was reported in T<sub>0</sub> at 400 µg/mL concentration and it decreases gradually at concentration in 100 µg/mL (T<sub>3</sub>). The same trend was found in both types of tea extract. It indicates that both treatments have a significant influence on free radical scavenging activity in each extract.

Tea Type	Concentrations (µg/mL)	Treatments			
		T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Green Tea	400	90.64 <sup>a</sup> ± 0.36	90.05 <sup>a</sup> ± 0.15	81.90 <sup>d</sup> ± 0.30	78.98 <sup>e</sup> ± 0.69
	200	71.21 <sup>f</sup> ± 0.365	85.21 <sup>b</sup> ± 1.2	87.17 <sup>a</sup> ± 1.07	73.74 <sup>e</sup> ± 0.29
	100	66.07 <sup>c</sup> ± 0.62	69.07 <sup>a</sup> ± 0.11	61.96 <sup>d</sup> ± 0.41	55.87 <sup>f</sup> ± 0.50
Black Tea	400	85.27 <sup>c</sup> ± 0.25	89.05 <sup>b</sup> ± 0.32	70.86 <sup>f</sup> ± 0.4	64.54 <sup>g</sup> ± 0.48
	200	75.22 <sup>d</sup> ± 0.59	78.95 <sup>c</sup> ± 0.25	68.19 <sup>g</sup> ± 0.39	55.14 <sup>h</sup> ± 0.64
	100	65.36 <sup>c</sup> ± 0.71	67.03 <sup>b</sup> ± 0.40	57.25 <sup>e</sup> ± 0.63	50.93 <sup>g</sup> ± 0.72

Table 2. Antioxidant activity (DPPH Assay) of black and green tea extracts.

The higher value was observed in ethanolic extract of green tea (T<sub>1</sub>) in 400 µg/mL concentration and it decreased gradually in 100 µg/mL concentration (T<sub>3</sub>). The results of DPPH radical scavenging activity indicated that ethanolic and distilled water extract have a significant influence on the radical scavenging activity of both tea samples. It was observed that higher values were found in green tea in T<sub>1</sub> and T<sub>4</sub> at 400 µg/mL concentration in comparison to black tea as it decreases T<sub>1</sub> to T<sub>3</sub> with low concentration. The same trend was found in 200 µg/mL concentration and 100 µg/mL concentration in both samples of tea. This study cleared that T<sub>1</sub> and T<sub>4</sub> have the highest antioxidant activity in both samples as compared to T<sub>2</sub> and T<sub>3</sub>. As the concentrations of solvents increase, antioxidant activity increases because it depends on the tea type, infusion time, and temperature to inhibit free radicals. The differences among tea extracts seem more obvious at low concentrations and these extracts possessed higher antioxidant activity due to the presence of antioxidant compounds such as phenolic acids, glycosylated flavonols, and their derivatives (Orak *et al.*, 2013). According to a literature review, the three side hydroxyl groups on the beta rings of catechins, which are found in green tea, give them their antioxidant qualities. In green tea compared to black tea, EGC, GCG, EGCG, and GC have a greater capacity to scavenge free radicals than two adjacent hydroxyl groups. Black tea is mostly credited with having antioxidant qualities since it contains theaflavins, phenolic acids, and thearubigins (Rahman *et al.*, 2019). Many other previous kinds of research also revealed that the DPPH radical scavenging activities are concentration-dependent, increasing with concentration and decreasing with the same sequence. The reducing power of chemical complexes in tea is related to the transfer of electrons so it has the potential to scavenge free radicals (Hsu *et al.*, 2013; Orak *et al.*, 2013).

**Antimicrobial activity:** The results of the antimicrobial activity of

each extract of tea are shown in table 3. The maximum zone of inhibition (25mm) was measured in green tea (T<sub>1</sub>) against *S. pyogenes* and 11.29mm was measured in T<sub>3</sub> against the same strain.

Bacteria used	Treatments (Zone of inhibitions) in mm				
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	
Green Tea	<i>Staphylococcus aureus</i>	Resistant	18.84±0.9	14.50±1.7	10±1.0
	<i>Streptococcus pyogenes</i>	20±2.3	25.95±0.6	16±1.6	11.29±1.4
	<i>Escherichia coli</i>	Resistant	Resistant	Resistant	Resistant
	<i>Pseudomonas aeruginosa</i>	Resistant	Resistant	Resistant	Resistant
Black Tea	<i>Staphylococcus aureus</i>	Resistant	9±0.9	7±1.02	6.24±0.99
	<i>Streptococcus pyogenes</i>	5.3±0.87	7.41±1.02	5.32±0.98	2.41±0.5
	<i>Escherichia coli</i>	Resistant	Resistant	Resistant	Resistant
	<i>Pseudomonas aeruginosa</i>	Resistant	Resistant	Resistant	Resistant

Table 3. Antimicrobial activity of black and green tea extract.

The maximum zone of inhibition (9mm) was measured in black tea (T<sub>1</sub>) against *S. aureus* and 2.41mm was measured in T<sub>3</sub> against *S. pyogenes*. *E. coli* and *P. aeruginosa*, both bacteria species were found resistant in each treatment of tea. Antibiotics (positive control) give a zone of inhibition except Augmentin which was resistant to *Escherichia coli* (table 4).

Bacteria used	Antibiotics (Zone of inhibition in mm)		
	Augmentin	Moxifloxacin	Amikacin
<i>Staphylococcus aureus</i>	>17	16	>17
<i>Streptococcus pyogenes</i>	>17	>21	>21
<i>Escherichia coli</i>	Resistant	>16	>17
<i>Pseudomonas aeruginosa</i>	>16	>17	>17

Table 4. Effect of different bacteria against common antibiotics drug.

The antimicrobial activity of green and black tea extract against different pathogenic bacteria was observed in this study. It validates that the extract of green tea had a higher zone of inhibition against pathogenic bacteria as compared to black tea. T-test showed the difference between antimicrobial activity that varied with the trend T<sub>1</sub> > T<sub>2</sub> > T<sub>3</sub> in green tea extract against gram-positive bacteria (*S. aureus* and *S. pyogenes*). Gram-negative bacteria were found resistant green tea and the same trend was observed in black tea extract. This result of antimicrobial activity correlated with previous findings that showed green tea has more antimicrobial activity than black tea because zone of inhibition in different concentrations of extract varies due to solvent and bacterial strains (Nibir *et al.*, 2017). Additionally, these researchers demonstrated that the polarity and concentration of the solvent had an impact on the antibacterial activity level. Similar to this, a prior study indicated that *E. coli* and *P. aeruginosa* were resistant to both forms of tea, whereas *Staphylococcus aureus* and *S. pyogenes* were shown to be sensitive to both types of tea. The concentration of extract and solvent had a significant impact on the zone of inhibition. Additionally, they discovered that ethanolic and distilled water extracts of green and black tea had the largest zone of inhibition against gram-positive bacteria (Fazal and Rauf, 2015). Solvents were utilized as a negative control in the current investigation, while antibiotics were used as a positive control because they did not affect the microorganisms. Except for Augmentin, which is resistant to, all bacteria showed a zone of inhibition by antibiotics. The antimicrobial activity of green tea and black with conventional antibiotics were the same. Infections caused by these bacteria are often treated with all of these medications. As a result, the extract of black and green tea could be utilised as a natural medicine to manage infections caused by these harmful microorganisms.

**CONCLUSIONS:** The total phenolic and flavonoid content, antioxidant, and antibacterial activities of green and black tea were considerably impacted by different factors such as solvents, its concentration, and polarity. It is advised that more polar organic solvents would be better for extract extraction because they would have no negative effects. The overall results showed that, green tea has higher physiochemical constituents, phenolic contents, antioxidant and antimicrobial potential than black tea.

**CONFLICT OF INTEREST:** Authors have no conflict of interest.

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