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COMPARATIVE PERFORMANCE OF POLYPHENOL AND OTHER BIOACTIVE COMPOUNDS IN FOUR GREEN TEA CANDIDATE VARIETIES

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Abstract

The study of the phytochemical composition and antioxidant activity of four green tea varieties from the NTHRI Shinkiari, Pakistan were selected (NTHRI-I, NTHRI-II, Rustam Khan, and Abdul Waheed) for analysis. Tea leaves were first plucked then processed through various steps. Standard methods were carried out to analyze proximate composition of moisture and ash content. The moisture contents of tea samples Abdul Waheed, Rustam Khan, NTHRI-II and NTHRI-I were 0.33%, 6.8%, 10.00 % and 15.3% respectively. Tea samples were evaluated and analyzed for chemical parameters i.e. polyphenols, caffeine and anti-oxidant analysis, the cumulative antioxidant activity was validated by assessing the free radical scavenging ability of all samples. The findings showed that the IC_{50} values ranged from 1.43 to 5.55 μg/mL. It was also discovered that the level of caffeine varied between 2.06% and 2.58%, depending on the four selected varieties of green tea. The average polyphenol content in different green tea varieties which is locally produce at NTHRI Shinkiari, Mansehra i.e. Rustam Khan, Abdul Waheed, NTHRI-II and NTHRI-II is 1.25896mg/ml, 1.6765mg/ml, 1.62333mg/ml and 1.2623mg/ml respectively in the levels of these important antioxidant compounds. For qualitative analysis of green tea samples extracts of different solvents were used i.e. distilled water and methanol. The results indicated that all the four varieties contain phytochemicals (tannins, saponin, alkaloids, flavonoids, phenols, terpenoid and glycosides). This study contributed to the understanding of the potential health benefits and physicochemical profile of green tea produced in Pakistan, which may have been useful for the Pakistani tea industry in fostering the production and use of Pakistani-origin green tea for health benefits.

INTRODUCTION

Tea, a beverage derived from the leaves and buds of the *Camellia sinensis* plant, is the second most popular beverage in the world, drunk far more than soft drinks with carbonation, beer, wine, and coffee combined. Originally from China, tea has been popular throughout the world in the two thousand years since its invention (Cabrera *et al.*, 2006). Pakistan has a long history of tea drinking, which has

now become a vital component of the country's social fabric. In 1958, the Pakistan Tea Board conducted the first tea tests in West Pakistan (now Pakistan), in the village of Baffa (district Mansehra, KPK). These initiatives were not maintained since tea output in then-East Pakistan (now Bangladesh) fulfilled the nation's demands (Hamid, F. S. 2007).

Tea is thought to contain a variety of medical benefits, including antihypertensive, antioxidant, and anticarcinogenic. These various biological functions are assumed to be related to a group of polyphenol chemicals found in tea leaves, known as green tea catechins (GTCs). GTCs are a combination of epicatechin isomers, namely (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG). Green tea is a nonfermented product in which GTCs are usually preserved (Zhu *et al.*, 1997). Green tea contains chemical components that are deeply associated with human health, according to evidence provided by several studies. The constituents that are isolated and extracted from green tea, such as tea polyphenols, caffeine, theanine, and tea polysaccharides, have pharmacological properties (Zhao *et al.*, 2022).

The unique taste and appearance of teas are caused by a complicated combination of components, including methylxanthines, polyphenols, and over 600 volatile ingredients, plus sugars and amino acids. Generally, dried tea possesses around 25–35% polyphenolic components, 3-5% caffeine, and 2-5% amino acids. Tea's bitterness is triggered by catechins (Piyasena *et al.*, 2023). In the green tea market, bud-processed tea is considered the best grade and most costly, followed by bud-processed teas with one, two, or three leaves. The price of green tea is determined by the maturity of the tea leaves and perceived quality (Xu, C., Liang, *et al.*, 2021). In the past, tea leaves were ignored for their spiritual benefits instead of their capacity to repair physical issues and remove poisons from the body. In addition to being an internationally recognized and popular beverage, it also contains natural ingredients such as proteins, polyphenols, chlorophyll, alkaloids, and carbohydrates (Samiullah *et al.*, 2021).

Recent studies have indicated that catechins have a substantial impact on weight loss. Green tea has a major role in conditions including arthritis, high bone density, cardiovascular diseases, and more since it contains antioxidants and other bioactive ingredients (Kar, S., & Saloni, S. 2016). The caffeine concentration varies based on the type of tea used in the tea beverage and the quantity of tea in the components; therefore, different varieties of tea have varied amounts of caffeine, resulting in variable results in tests (Shao, J., & Zhang, Y. 2019).

Tea polyphenols are one of the main ingredients in the development of the color and flavor of tea, as well as vital constituents for tea with health properties (Zhao *et al.*, 2022). Tea leaves contain many components, including polysaccharides, volatile oils, vitamins, minerals, purines, alkaloids (caffeine), and polyphenols (catechins and flavonoids). While all varieties of tea have antibacterial and antioxidant properties, the efficiency reduces as the tea becomes darker. This is due to reduced levels of anti-oxidant polyphenols in the leaves (Rani *et al* 2014).

1.1 Objectives:

The present study was developed with following aims and objectives

1. Collection and processing of green tea.
2. The phytochemistry of green tea was assessed both qualitatively and quantitatively.

METHODS AND MATERIAL

All the research work relating to this investigation was carried out at PARC NTHRI, Shinkiari

Sample: Green tea

varieties: Rustam Khan, Abdul Waheed, NTHRI-I, NTHRI-II

2.1 Collection and processing:

Two to three leaves with bud of four varieties of green tea namely NTHRI-I, NTHRI-II, Abdul Waheed and Rustam Khan were collected randomly and processed at NTHRI Research Institute Shinkiari Mansehra. Samples were dried and powdered for further investigation

Fig-1 (plucking)

Fig-2 (Before dry)

Fig-3 (After dry)

Fig-4 (Grinding)

2.2 Proximate Analysis:

2.2.1 Moisture content test:

To determine the amount of moisture. Three grams of each variety were taken. Placed the green tea samples in the preheated oven for 90 minutes at 105°C cooled it by placing it in the desiccator for a while. Weighted it again and calculated the moisture content using the provided the guidelines.

$$\text{Moisture content (\%)} = \frac{M_0 - M_1}{M_0} \times 100$$

2.2.2 Ash Content Determination:

Estimation of ash content was done accordance with the process demonstrated by AACC (2000) method 08-01. Taken weighted crucibles with 2g of each tea sample and ignited through an oxidizing flam for few minutes then placed in a furnace at 550c for 6 h. The crucibles then shifted to desiccators for coolness, reweight and determined.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.3 Phytochemistry:

All tea samples were powdered and placed in desiccators to prevent gaining moisture. Powdered samples were used to prepare aqueous and ethanolic extract by soaking them in 50 ml solvent for 24 hours then filtered through a filter paper (125 mm). These both extracts were used for the analysis of phytochemistry by standard chemical tests. Presence of phytochemicals was confirmed by the intensity of color change.

2.3.1 Tannin test:

1ml of each extract was taken out to perform the tannin test. Four test tubes were filled with 1.5ml of distilled water each, and the test tubes were then strongly shaken on the shaker device. After that, each of the test tubes received two drops of dil. ferric

chloride (1%). The presence of tannin was shown by changing solution color from yellow to dark green. Harbone (1973) described the use of the ferric chloride test to determine the amount of tannin.

2.3.2 Saponin test:
1ml of extract were pipetted out in order to identify whether the saponins were present. soon after, 3ml of distilled water was introduced and the mixture was well stirred on a shaker. Formation of the froth confirmed the presence of saponins. The presence of saponins was identified using the technique described by Harbone (1973).

2.3.3 Alkaloid test:
Test tubes were filled with 1ml of each extract. Each test tube was then filled with 2.5ml of 2% HCl. Following, this was progressively heated in the water bath. This was then filtered using Whatman filter paper, and 1 milliliter of each filtrate was mixed with 0.5 milliliter of Wagner's reagent. Iodine and potassium iodide in a water-based solution is Wagner's reagent. The presence of alkaloids was revealed by change in color of sample from yellow to reddish-brown or light red. (Okwu, 2005) developed the method for identifying alkaloids.

2.3.4 Flavonoid test:
2ml of extracts were poured into the test tubes. A few drops of concentrated ammonia (NH_3) were added. The development of dark yellow at the end indicated the presence of flavonoids (Adegoke, *et al.*, 2009).

2.3.5 Phenol test:
1ml of the extract were taken in a test tube. Subsequently added 10% FeCl3 was in it. The yellow color of sample turned into dark blue at the end which confirmed the presence of phenol. This test is also known as FeCl3 Test.

2.3.6 Test for steroid:
In a test tube, pipette out 2ml of extract. Carefully add six drops of concentrated H2SO4 from the test tube's side wall. The existence of steroids is indicated change in color of extract.

2.3.7 Test for terpenoid:
1mL from every extract was taken out and treated with 2ml of chloroform. Following that, 3ml of concentrated H2SO4 was added. Reddish brown color indicates the presence of terpenoid (Adegoke, *et al.*, 2009).

2.3.8 Test for carotenoids:
1ml of each extract were placed in a test tube. Each test tube received 5ml of chloroform and were properly stirred. The mixture was filtered and treated with 85% sulfuric acid. The change in color at the end indicates the presence of carotenoids.

2.3.9 Glycoside test:
1ml of extract was introduced into a test tube.2.5ml of concentrated H2SO4.This was then heated for over 15 minutes in a water bath. After that, the test tubes were cooled and 20% KOH was used to neutralize the mixtures. In the end, a few drops of FeCl3 were added, resulting in the formation of green to black color, indicating the presence of glycoside.

2.3.10 Protein test:
Take 2 ml of plant extract into a test tube and add 1 drop of 1% CuSO4 solution and 1 ml of ethanol to it. At this point NaOH or KOH will be added. The presence of pink color in the ethanol layer indicates the presence of a volatile oil.

2.4 Antioxidant:
Take a 1-gram powder sample and add 25 mL of 99% methanol to it. Seal the sample using aluminum foil. Place the sample in a water bath while shaking it at 100 rpm at room temperature for 2.5 hours. For the preparation of 1M DPPH solution, dissolve 4 mg of DPPH in 100 mL of 99% methanol. Cover the DPPH solution and keep it in a cool condition. After 2.5 hours, remove the sample from the water bath and centrifuge it for 15 minutes at 6000–8000 rpm. Filter the supernatant. Prepare a solution series using the extracted solution and methanol in volumes of 1 mL, 2 mL, 3 mL, 4 mL, and 5 mL, or alternatively, 50µL, 100µL, 150µL, 200µL, 250µL, and 300µL. Take each concentration and add 3 mL of DPPH solution to it. Dilute each measured sample solution with 99% methanol to

make a total of 10 ml. Keep it in the dark for 30 minutes, then take the reading at 517 nm.

2.4.1 Formula;

$$\%RSA=\frac{(ABS\ of\ control)-(ABS\ of\ sample)}{(ABS\ of\ control)}\times 100$$

where (ABS of control) is the absorbance of the blank (solvent + DPPH) and (ABS of sample) is the absorbance of the solvent extracts and %RSA is radical scavenging activity.

2.5 Polyphenol analysis:

Sample Preparation:

5 mg of the extract weight then extract is diluted with 5 ml of methanol final concentration is 1000 ppm.

Preparation of Follin ciocalteu Reagent:

About 5 ml of Follin C reagent is pipetted in the solution no 1.45 ml of distilled water is added.

Preparation of 7.5% of sodium carbonate:

7.5 g is prepared in 100 ml of distilled water to form 7.5% solution.

7.6 Protocol:

To a glass reaction 0.5 ml of sample (1000 ppm) is reacted with 2.5 ml of FC reagent. The reaction is stood for 2 mints. To the solution 2 ml of sodium carbonate is mixed and incubated for 30 minutes. The sample is reacted 765nm by spectrophotometer. The blank is prepared without extract.

FORMULA:

$$Y = mx+c$$

$$C= x(v/m)$$

2.6 Caffeine test:

Take 5 g of each sample in 50 mL of distilled water. Next, boil and filter each sample. After filtering, combine 10 mL of HCL and 2 mL of lead acetate in 20 mL of extract. After adding the lead acetate and HCL, dilute up to 250 mL with distilled water and shake thoroughly and evenly. Lay aside for clarification. Again, filter the dilute extract/sample, and then take 50 ml from the filtered sample. After that, add 0.2 mL of 9N sulfuric acid. Finally, examine the spectrophotometer measurement at 380 nm wavelength. Then look for the caffeine content from the standard curve as per optical density E. The caffeine content (mg/ml) checked out from the standard curve as below.

$$\text{Caffeine (\%)} = \frac{(C/1000\times L\times 250/20\times 100/50)}{M\times M1}\times 100$$

RESULTS AND DISCUSSION

Tea is a widely consumed beverage in daily human diet with proved beneficial effects (Sharangi AB, 2009), currently being the major source of dietary flavonoids in U.S. adults (Song WO and Chun OK, 2008). In addition, non-fermented tea such as green and white tea considered good sources of natural antioxidants. Whereas green tea composition and bioactivity has been in-depth investigated (Chen Q *et al*, 2008).

3.1 Moisture content test:

The moisture content of green tea is very important as it affects shelf life and quality. The ISO 1573 tea standard specifies that green tea has a moisture content in the range of 3% to 8%. The moisture content of green tea samples collected from NTHRI Mansehra ranged from 0.33% to 15.3%. The large variation between these samples is due to the time of their collection, weather and environment, and processing delays. The moisture contents of tea samples Abdul Waheed, Rustam Khan, NTHRI-II and NTHRI-I were 0.33%, 6.8%, 10.00% and 15.3% respectively.

TABLE -1 Moisture Content of Tea Samples

Tea verities	NTHRI-I	NTHRI-II	Rustam Khan	Abdul Waheed
Moisture content	15.3%	10%	6.8%	0.33%

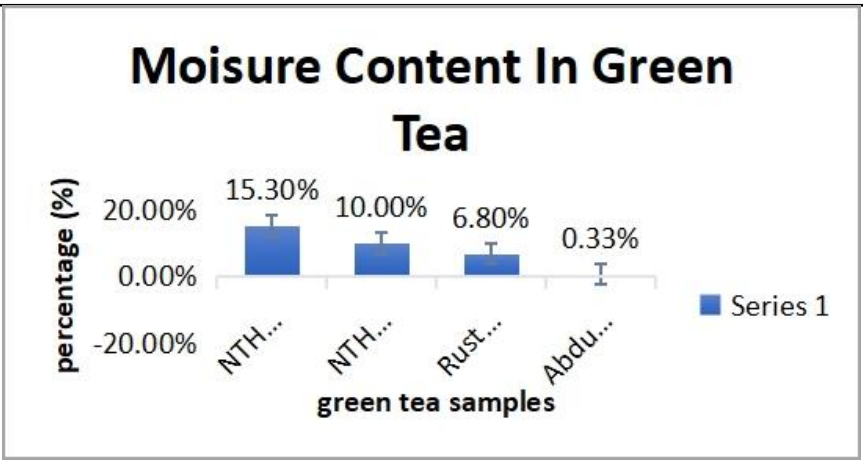

Fig-5 Moisture Content of Tea Samples

3.2 Ash Content determination:

Ash content in green tea has a vital role in the determination of its quality. Ash content is inversely related to moisture content. Ash content below 5.54% ensures the good quality of green tea and also preserves the tea freshness (Adnan *et al.*, 2013).

Table-2 Ash content (%)

S. No	Tea varieties	Ash content (%)
1	NTHRI-I	5.75
2	NTHRI-II	5.2
3	Abdul Waheed	5.7
4	Rustam Khan	5.6

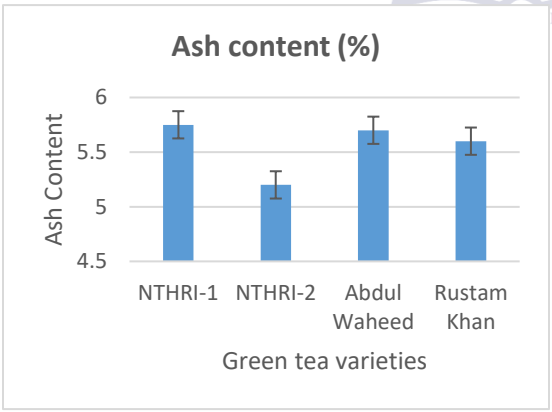

Fig-6 Ash content of tea sample

3.3 Phytochemical analysis:

Phytochemicals are the bioactive compounds which are present in the medicinal plants (Rathee JS *et al.*, 2007). Green tea is more popular as the beverage and has ascribed many health benefits such as reduction in cholesterol and protection against cardiovascular disease (Ushimaru pi *et al.*, 2007). Evaluations of tea samples were conducted to establish preference rating of tea for flavor, taste and color.

Table-3 Phytochemical analysis of distilled water extract and Methanol extract

Tests	NTHRI-I		NTHRI-II		Rustam Khan		Abdul Waheed	
	D.water	Methanol	D.water	Methanol	D.water	Methanol	D.water	Methanol
Tannin	+	+	+	+	+	+	+	+
Saponin	+	-	+	-	-	+	+	+
Alkaloid	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+
Steroids	+	-	-	-	-	-	-	-
Terpenoid	+	+	+	+	+	+	+	+
Carotenoid	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+	+
Glycoside	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+

3.3.1 Tannin test:

The green tea has some tannin content in it and it helps in fighting high blood pressure and protecting the kidney. The effects of green tea tannins as an antioxidant and antihypertensive can all be attributed to the fact that they have a high ability to convert some chemicals. (Yokozawa, *et al.,* 1994). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which were examined in NTHRI, tannins are present in both water and methanol extract.

3.3.2 Saponin test:

Saponin possesses antioxidants that reduce oxidative stress and the probabilities of chronic diseases like cancer and heart disease (Malinow, *et al.,* 1977). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, saponin are present in both water and methanol extract.

3.3.3 Alkaloid test:

Alkaloids found in green tea, such as caffeine, theobromine, and theophylline, act as mind stimulants and enzyme stimulants. These compounds play an important role in providing both short-term and long-term health effects (Smith, A. 2002). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, alkaloid is present in both distilled water and methanol extract.

3.3.4 Flavonoid test:

There are flavonoids in green tea, of which catechins are powerful antioxidants. They help to remove free radicals from the body, decrease inflammation, and may prevent heart diseases and cancer. The protective effects for healthy blood vessels and metabolic health are also present from their consumption (Khan, N., & Mukhtar, H. 2007). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, flavonoid is present in both distilled water and methanol extract.

3.3.5 Phenol test:
Green tea contains the phenolic compounds catechins, which provide antioxidant protection by limiting oxidative damage to cells. It is assumed that the items related to lower risk of cancer, cardiovascular diseases and neurological diseases. In addition, these chemicals increase food shelf life by minimizing quality degeneration through oxidation effects (Lorenzo *et al.,* 2016). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, phenol is present in both distilled water and methanol extract.

3.3.6 Test for steroid:
Steroids are related to testosterone and such diseases as anemia, for muscle development and prevention of fractures The use of steroids is important in conditions that may include anemia and promotion of muscle health. But if not used rightly, they can lead to severe problems such as liver failure or even cancer (Gupta, *et al.,* 2009). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, both methanol and distilled water extracts lack steroid, except distilled water extract of NTHRI-I.

3.3.7 Test for Terpenoid:
It is considered that terpenoids have an important role in the fragrance of tea by giving a contribution towards the formation of certain volatile components. These compounds are all responsible for floral and fruity flavor and aroma, and increased the total flavor of tea considerably (Han *et al.,* 2016). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, terpenoid is present in both methanol and distilled water extract of green tea samples.

3.3.8 Test for carotenoids:
Carotenoids are essential for tea aroma formation as precursors of some highly crucial volatile aldehydes, such as norisoprenoids, that are predominant in green tea and are perceived easily by the human nose (Han *et al.,* 2016). In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, the carotenoids in absence.

3.3.9 Glycoside test:
Glycosides in the green tea are unique in that they not only play a part in providing some of the health benefits but are also involved in the formation of flavor and taste. In all varieties of

Green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, glycoside is present.

3.3.10 Protein test:
The proteins compounds may interact with green tea components i.e. polyphenols, which reduce the antimicrobial and antioxidant properties. This study shows that though proteins may cover up some benefits of green tea varieties, but it still possessed unique antioxidant and antimicrobial potentialities. In all varieties of green tea, namely NTHRI-I, NTHRI-II, Abdul Waheed, and Rustam Khan, which are examined in NTHRI, protein is present in the extracts.

3.4 Antioxidant Analysis
Antioxidants, those in green tea, have tremendous value in shielding against ROS in the body. Green tea contains phytochemicals that act as antioxidants and directly combat free radicles, thereby decreasing oxidative stress. Oxidative stress is associated with various chronic diseases, including cancer, cardiovascular disease, dementia, and others. By using green tea, which has a high concentration of antioxidants, the threat of illness can be reduced (Wiseman, *et al.,* 1997).
All four types of green tea contain antioxidants, but in varying amounts. Comparing NTHRI-II to, Rustam Khan, NTHRI-I and Abdul Waheed, the former has more antioxidant compounds. NTHRI-II absorbance ranges from 0.390 to 1.044 nm. Additionally, Abdul Waheed has 0.209 to 0.541 nm, NTHRI-I has 0.524 to 1.019 and Rustam Khan has 0.367 to 1.019 range.

Table-4 Absorbance of Abdul Waheed

Serial no	Control	Conc. (µg/ml)	ABS Abdul Waheed	%RSA	IC50
1	1.054	5000	0.921	12.6185	5.1099
2	1.054	4000	0.891	15.4648	5.1099
3	1.054	3000	0.784	25.6166	5.1099
4	1.054	2000	0.679	35.5787	5.1099
5	1.054	1000	0.491	53.4155	5.1099

Total average of abdul waheed %RSA (Inhibition of DPPH) values divided by 5 = 28.538 %

Table-5 Absorbance of Rustam Khan

Serial no	Control	Conc. (µg/ml)	ABS Rustam Khan	%RSA	IC50
1	1.054	5000	1.019	3.3206	4.537
2	1.054	4000	0.922	12.5237	4.537
3	1.054	3000	0.872	17.2675	4.537
4	1.054	2000	0.659	37.4762	4.537
5	1.054	1000	0.367	65.18026	4.537

%RSA 27.154% average

TABLE-6 Absorbance of NTHRI-I

Serial no	Control	Conc. (µg/ml)	ABS NTHRI-1	%RSA	IC50
1	1.054	5000	1.019	3.3206	5.136
2	1.054	4000	0.988	6.2618	5. 136
3	1.054	3000	0.856	18.785	5. 136
4	1.054	2000	0.658	37.571	5. 136
5	1.054	1000	0.524	50.284	5. 136

%RSA 23.244 %

TABLE-7 Absorbance of NTHRI-II

Serial no	Control	Conc. (µg/ml)	ABS NTHRI-I	%RSA	IC50
1	1.054	5000	1.019	0.94877	4.588
2	1.054	4000	0.988	6.35674	4.588
3	1.054	3000	0.856	18.9753	4.588
4	1.054	2000	0.658	37.4763	4.588
5	1.054	1000	0.524	62.9981	4.588

%RSA 25.351%

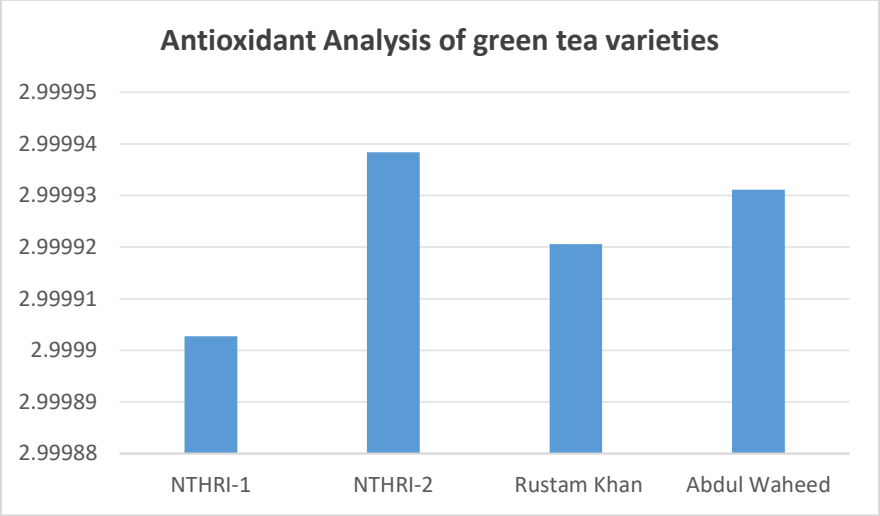

Fig-25 Antioxidant Analysis of green tea varieties

3.5 Caffeine analysis:

Caffeine is considered one of the most important components of green tea. A caffeine content value in Green Tea is specified by some world standard range is from 1.50% to 4.00% (Shatta, A. A. 1999). In green tea, catechins help reduce the level of an enzyme that breaks up norepinephrine, COMT (catechol-O-methyltransferase). The caffeine content percentage of green tea varieties i.e. NTHRI-I, NTHRI-II, Rustam Khan and Abdul Waheed is 2.06, 2.40, 2.20 and 2.58 respectively.

TABLE-8 Caffeine Content of tea samples

S.NO	Tea Varieties	Absorbance	Caffeine (%)
1	NTHRI-I	0.947	2.06
2	NTHRI-II	1.105	2.4
3	Rustam Khan	1.034	2.2
4	Abdul Waheed	1.126	2.58

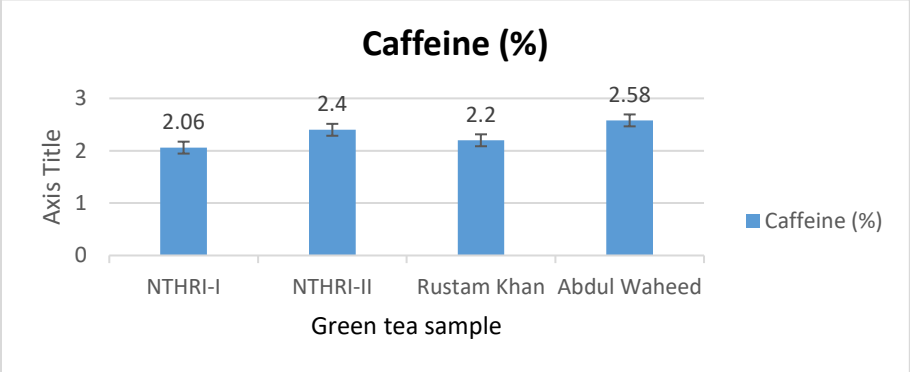

Fig-26 Caffeine Content of tea samples

3.6 Polyphenol analysis:

Green tea polyphenols, especially catechins and EGCG, contribute significantly to its health-promoting properties. These alternatives have shown that they have anti-inflammatory, antioxidant, anticancerous, and

hypoglycemic values, augment the breakdown of fats, have a positive impact on heart health, and prevent chronic diseases such as diabetes, Parkinson's, and Alzheimer's diseases (Afzal *et al.*, 2015). The average polyphenol content in different green tea varieties which is locally produce at NTHRI Shinkiari, Mansehra i.e Rustam Khan, Abdul Waheed, NTHRI-II and NTHRI-II is 1.25896mg/ml, 1.6765mg/ml, 1.62333mg/ml and 1.2623mg/ml respectively.

TABLE-9 Total Polyphenol content in Green Tea Var.Rustam Khan

S.NO	Concentration (µg/ml)	Absorbance of GAE	Absorbance of Rustam khan	Polyphenol conc. in Rustam Khan(mg/ml)	Total Polyphenol conc. in Rustam Khan(mg/ml)
1	500	1.98	1.785	0.4406	8.812
2	250	1.07	1.472	0.3623	7.246
3	125	0.520	1.065	0.2606	5.212
4	62.5	0.226	0.871	0.2121	4.242
5	31.25	0.156	0.549	0.1316	2.633
6	15.625	0.084	0.436	0.1033	2.067
Average polyphenol content in Rustam Khan				**5.035 mg GAE/g**	

TABLE-10 Total Polyphenol content in Green Tea Var.Abdul Waheed

S.NO	Concentration (µg/ml)	Absorbance of GAE	Absorbance of Abdul Waheed	Polyphenol conc. in Abdul Waheed(mg/ml)	Total Polyphenol conc. in Abdul Waheed(mg/ml)
1	500	1.98	2.161	0.534625	10.692
2	250	1.07	1.869	0.461625	9.232
3	125	0.52	1.539	0.379125	7.582
4	62.5	0.226	1.142	0.279875	5.597
5	31.25	0.156	0.97	0.236875	4.737
6	15.625	0.084	0.501	0.119625	2.392
Average polyphenol content in Abdul Waheed				**6.705 mg GAE/g**	

TABLE-11 Total Polyphenol content in Green Tea Var.NTHRI-I

S.NO	Concentration (µg/ml)	Absorbance of GAE	Absorbance of NTHRI-I	Polyphenol conc. in NTHRI-I (mg/ml)	Total Polyphenol conc. in NTHRI-I (mg/ml)
1	500	1.98	1.98	0.489375	9.787
2	250	1.07	1.594	0.392875	7.857
3	125	0.52	1.329	0.326625	6.532
4	62.5	0.226	1.21	0.296875	5.937
5	31.25	0.156	1.005	0.245625	4.912
6	15.625	0.084	0.809	0.196625	3.932

Average polyphenol content in Ver.NTHRI-I	6.493 mg GAE/g

TABLE-12 Total Polyphenol content in Green Tea Var. NTHRI-II

S.NO	Concentration (µg/ml)	Absorbance of GAE	Absorbance of NTHRI-II	Polyphenol content (mg/ml)	Total Polyphenol conc. in NTHRI-II (mg/ml)
1	500	1.98	2.121	0.524625	10.492
2	250	1.07	1.566	0.385875	7.717
3	125	0.52	1.249	0.306625	6.132
4	62.5	0.226	1.107	0.271125	5.422
5	31.25	0.156	0.095	0.018125	0.362
6	15.625	0.084	0.056	0.008375	0.167
Average polyphenol content in Var.NTHRI-II				5.049 mg GAE /g	

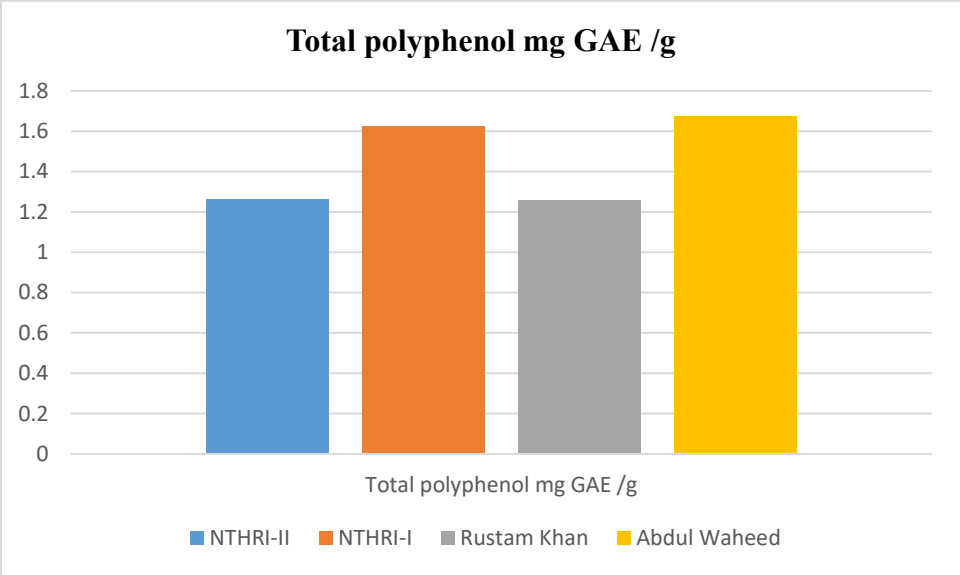

Fig-27 Total polyphenol mg GAE /g

Conclusion:

The aim of this research was to assess the phytochemical content and antioxidant potential of four green tea types produced in the Shinkiari, Mansehra, Pakistan. The results point out that these locally produced green teas are, in fact, as good as other green tea brands. It is concerned with moisture content and caffeine content, polyphenol content, and antioxidant activity levels. It means that it has similar benefits for human health, for instance, reducing oxidation, better heart health, and perhaps cancer protection.

Collectively, the study adds knowledge to the effects of green tea as well as the physicochemical properties of green tea manufactured in Pakistan. This information can be useful for the Pakistan tea industry to popularize the green tea from Pakistan due to some beneficial impacts on human health that can be provided by it. Further research might look at exploring the content of actual antioxidant compounds in these tea types and their health applications.

Recommendation:

To determine the best green tea variety for human use, we looked at several factors i.e. polyphenol composition, moisture, ash, caffeine, and antioxidant properties. The best option indeed is the option Abdul Waheed, because it has the highest level of polyphenol and is very low in moisture, which is good for the health and longevity of the product. NTHRI-I has high antioxidant and polyphenol values, but it contains more moisture, enough to compromise on the freshness. The third position is held by Rustam Khan, who has average scores for all of the dimensions with no truly outstanding value. Thus, the NTHRI-II is the least preferred as compared with other brands because the NTHRI-II apparently contains low polyphenols and other benefits.

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