Comparative analysis of antioxidant and anti-inflammatory activities of red, blue, and black tea for health benefits

Sanjana Patel[1], Rohit Dey[1], Khushi Verma[1], Radha Deshbhratar[1], Kamal Kishore Maru[1], Porshia Sharma[1], Rashmi Limaye[1] & Payal Puri[1]

[1] Institute for Innovative Learning and Research (IILR) Academy, Indore, India

Correspondence: Porshia Sharma, Institute for Innovative Learning and Research, Indore, India. E-mail: venice12051999@gmail.com

Received: January 11, 2023 Accepted: January 30, 2023 Published: April 01, 2023

DOI: 10.14295/bjs.v2i4.299 URL: https://doi.org/10.14295/bjs.v2i4.299

Abstract

The current COVID-19 predicament necessitates a greater emphasis on developing immunity. Herbal teas are abundant in antioxidants which are important for strengthening the immune system. Hot water decoction of *Hibiscus rosa sinensis* flowers (red tea), *Clitoria ternatea* flowers (blue tea) and commercially available black tea were evaluated by comparing for *in vitro* antioxidant and anti-inflammatory properties. Anthocyanin pigment in red, blue, and black tea demonstrated R*f* values of 0.52, 0.86 and 0.78 respectively. Blue and black teas exhibited dominance of polyphenols, flavonoids, tannins, glycosides, terpenoids, saponins as compared to red tea. The highest total phenolic (12.25 ± 0.245 mg GAE/gm extract^{-1}) and flavonoid (15.84 ± 0.268mg QE/gm extract^{-1}) content were observed with black and blue tea respectively. Blue tea, and black tea extracts exhibited FRPA values of 1.81 ± 0.413 mg and 1.93 ± 0.178 mg AAE per gram extract^{-1} respectively. Black tea exhibits the highest antioxidant capacity in reducing molybdate ions (1.94 ± 0.354 mg AAE per gram extract^{-1}) followed by blue tea (1.56 ± 0.199 mg AAE per gram extract^{-1}). Blue tea extract at a very low concentration showed highest percentage hemolytic inhibition (57.14 ± 0.567%). According to the study, blue tea is a rich source of antioxidants with significant anti-inflammatory properties. The research may offer a valuable supplementary strategyfor its therapeutic applications.

Keywords: anti-inflammatory, antioxidant, *Clitoria ternatea*, *Hibiscus rosa sinensis*.

Análise comparativa das atividades antioxidantes e anti-inflamatórias dos chás vermelho, azul e preto para benefícios à saúde

Resumo

A situação atual do COVID-19 exige uma maior ênfase no desenvolvimento da imunidade. Os chás de ervas são abundantes em antioxidantes, importantes para fortalecer o sistema imunológico. A decocção de água quente de flores de *Hibiscus rosa sinensis* (chá vermelho), flores de *Clitoria ternatea* (chá azul) e chá preto comercialmente disponível, foi avaliada comparando as propriedades antioxidantes e anti-inflamatórias *in vitro*. O pigmento antocianina no chá vermelho, azul e preto, demonstrou valores de R*f* de 0,52, 0,86 e 0,78, respectivamente. Os chás azul e preto, exibiram predominância de polifenóis, flavonoides, taninos, glicosídeos, terpenoides e saponinas em comparação com o chá vermelho. Os maiores teores de fenólicos totais (12,25 ± 0,245 mg EAG/gm extrato^{-1}) e flavonoides (15,84 ± 0,268 mg QE/gm extrato^{-1}) foram observados nos chás preto e azul, respectivamente. Os extratos de chá azul e chá preto exibiram valores de FRPA de 1,81 ± 0,413 mg e 1,93 ± 0,178 mg de AAE por grama de extrato^{-1}, respectivamente. O chá preto apresenta a maior capacidade antioxidante na redução de íons molibdato (1,94 ± 0,354 mg AAE por grama de extrato^{-1}), seguido pelo chá azul (1,56 ± 0,199 mg AAE por grama de extrato^{-1}). O extrato de chá azul em concentração muito baixa, apresentou a maior porcentagem de inibição hemolítica (57,14 ± 0,567%). De acordo com o estudo, o chá azul é uma rica fonte de antioxidantes com propriedades anti-inflamatórias significativas. A pesquisa pode oferecer uma valiosa estratégia complementar para suas aplicações terapêuticas.

Palavras-chave: anti-inflamatório, antioxidante, *Clitoria ternatea*, *Hibiscus rosa sinensis*.

1. Introduction

Tea, particularly black tea, is a popular beverage all around the world. *Camellia sinensis* leaves are used to make black tea. Catechins are major polyphenols (Quan et al., 2007) present in black tea which forms complex theaflavins which offers characteristic taste and color to black tea (Jain, 1999). Theaflavins inhibits xanthine oxidation and nitric oxidase action responsible for production of free radicals (Fatima et al., 2013). It contains L-theanine which raises GABA (gamma-aminobutyric acid) levels, promoting relaxation and releasing alpha waves in the brain, which improves relaxation, focus, and creativity (Nobre et al., 2008). It also contains caffeine (methyl xanthine), a psychostimulant that boosts alertness but is detrimental to one's health if consumed in excess.

Herbal tea comes into play as a healthy alternative. These are used as therapeutic vehicles in traditional medicine and are a popular global beverage choice by the fact that they are rich in phytochemicals like flavonoids, polyphenols, alkaloids, coumarins (Chandrasekara; Shahidi, 2018) etc. Red tea (made from *Hibiscus rosa sinensis* flowers) and blue tea (made from *Clitoria ternatea* flowers) have been shown to be extremely healthful owing to their antioxidant qualities. Antioxidants are free radical scavengers that reduce oxidative stress (Madani et al., 2010) which is linked to inflammatory disorders such as ageing, cancer, diabetes (Forman; Zhang, 2021) etc.

Both types of tea include antioxidants, phenols, and flavonoids in particular, which are responsible for their health benefits. The plant species *Hibiscus rosa sinensis*, widely referred to as China rose, belongs to the Phylum Spermatophyte. In the tropics, *Hibiscus rosa sinensis* (Malvaceae family) is frequently planted as a beautiful plant. *Hibiscus rosa sinensis* is known as Chinese *Hibiscus* in English. It is a 1.5-2.4 m tall, evergreen, woody, glabrous shrub (Faten et al., 2012). It contains flavonoids like rutin, quercetin, kaemferol, myricetin and anthocyanins like cyanidin-3-sabubioside and dephinidin-3-sabubioside (Kumari et al., 2021). It is high in antioxidants, anti-inflammatory, anti-aging, induces weight loss, skin and hair benefits, liver detoxification and has many other health advantages (Khristi; Patel, 2016).

Clitoria ternatea, commonly known as Butterfly pea plant, is a member of the Magnoliophyta division. Butterfly pea flowers are gigantic, stunning purple blooms that extend 1" to 3" in length (Chakraborthy et al., 2018). Anthocyanin, kaempferol (anti-cancer), *p*-coumaric acid (anti-inflammatory), and delphinidin-3, 5-glycoside are major phytochemicals in these flowerswhich can boost immunity (Jeyaraj et al. 2021). It comprises a plethora of antioxidants, is anti-inflammatory, improves cognitive and brain functions, decreases anxiety, fights sleeplessness, and improves mood (Kosai et al., 2015). Red tea and blue tea are healthier than black tea since they contain no caffeine and have no calories.

Although there have been numerous studies on flowers and their alcoholic extracts, none have been undertaken directly on aqueous flower infusion (tea) to our knowledge. Aqueous extracts are used because they are pure, concentrated and edible. The objective here is to perform comparative studies on phytochemical and antioxidant properties in black, red and blue tea by identifying, evaluating, quantifying phytochemicals, determining antioxidant potential, and assessing *in vitro* anti-inflammatory activity of the teas.

2. Materials and Methods

2.1 Preparation of tea extract

3.5 grams of flowers and black tea that were used to make hot water infusion (Figure 1) were washed initially then dried and weighed. After that, they were simmered in 30 mL of water for 5 minutes, or until the water's colour changed. They were sieve-filtered before being used in various tests.

Figure 1. Red, blue, and black tea extracts. Source: Authors, 2022.

2.2 Thin layer chromatography (TLC) for caffeine identification

TLC was used for detection and visualization of caffeine in tea extracts. Silica gel G was utilized as an absorbent in stationary phase. In a 20 mL *d/w*, 12 grams of silica gel G was introduced for slurry preparation and allowed to set for 15 min. After that, it was poured evenly over the glass slide and allowed to dry for 15-30 min. After that, the TLC plate was heat activated for 10 min in the oven at 110 °C. On the line of origin, 6-7 drops of extract were loaded. Ethyl acetate, methanol, and acetic acid in an 8:1:1 ratio was used to make the mobile phase (Rani et al., 2013). The plate was placed in a developing chamber that had been pre-saturated with mobile phase. Following development, the plate was placed in a beaker containing iodine crystals. The presence of caffeine in the extract was established by the appearance of brown spots under iodine vapour. R*f* values of caffeine was calculated using the following formula:

R*f* = Distance travelled by the solute/Distance travelled by the solvent

2.3 Paper chromatography for pigments

Anthocyanin identification and visualisation in the extracts were done using paper chromatography. As the stationary phase, Whatman filter paper number 1 was used. The mobile phase consisted of a 15:1 mixture of chloroform and methanol (Forgacs; Cserhati, 2002). The extracts were loaded on the origin line, and the paper was placed in a pre-saturated developing chamber containing the mobile phase. The paper was left to develop until the solvent had travelled 3/4 of its length. After the paper had dried, the anthocyanin pigment was visualised under visible light and the Rf value of the pigment was calculated using the following formula:

R*f* = Distance travelled by solute/Distance travelled by solvent

2.4 Qualitative phytochemical screening

The different qualitative chemical tests were performed to detect various phytochemicals present in tea extracts (Harbone, 1973; Raman, 2006). The tests were performed as follows (Table1):

2.4.1 Test for flavonoids

Alkaline reagent test: 2 mL of different extracts was mixed with 2 mL of 10% sodium hydroxide solution. An intense yellow colour was formed which turned colourless after addition of few drops of dilute acid indicated the presence of flavonoids.

2.4.2 Test for phenols

Ferric chloride test: 1-2 mL of different extract was treated with 1 mL of 5% ferric chloride solution. Appearance of blue black colour indicates the presence of phenolic compounds.

2.4.3 Test for terpenoids

Salkowski Test: 2 mL of each of the extract was treated with 1 mL of chloroform. Concentrated H_2SO_4 was carefully added along the side of the test tube to form a layer. A reddish-brown colouration at the interface indicates the presence of terpenoids.

2.4.4 Test for glycosides

Keller-Kellani test: 5 mL of different extracts was treated with 2 mL glacial acetic acid and 1 ml of 5% ferric chloride. After gentle heating transfer it to a test tube containing 2 mL of conc. H_2SO_4. Appearance of reddish-brown colour at junction of two liquid and bluish green colour of acetic acid layer indicates the presence of glycosides.

2.4.5 Test for tannins

Braymer's test: 1 mL of different extracts were treated with 2 mL of 5% ferric chloride solution. Appearance of blue-black colour indicates the presence of tannins.

2.4.6 Test for steroids

Salkowski test: 1 mL of different extracts was treated with 1 mL of chloroform and concentrated sulphuric acid was added along the side of the test tube and shaken well. Chloroform layer appears red and acid layer showed greenish yellow colour.

2.4.7 Test for saponins

Foam Test: 2 mL of extract was diluted with 5 mL distilled water in a test tube, and it was shaken vigorously. Formation of stable foam was taken as an indication for the presence of saponin.

2.4.8 Test for anthocyanins

Few drops of tea extract were treated with 2-3 drops of 10% NaOH (*m/v*) solution. Anthocyanins gives green coloration in alkaline medium and then concentrated HCl was added until the color turns red.

Confirmatory test for anthocyanin

H_2SO_4 test: 1 mL of concentrated H_2SO_4 was added to 2 mL of extract. Orange coloration at interface confirms presence of anthocyanin in the extract.

NaOH test: 2 drops of 1N NaOH (*m/v*) solution was added to 2 mL of extract. Appearance of blue color confirms presence of anthocyanin in the extract.

Table 1. Qualitative analysis of phytochemicals.

S. No	Metabolite	Test	Experiment	Observation
1.	Flavonoids	Alkaline reagent Test	2-3 mL extract + 2 mL 40% NaOH	Deep yellow colour appears
2.	Terpenoids	Salkowsky's Test	2 mL extract + 1 mL chloroform + few drops of conc, H_2SO_4	Reddish-brown colouration appears at the interface
3.	Glycosides	Kellarkialliani Test	5 mL extract + 2 mL glacial acetic acid + 1 mL 5% $FeCl_3$ + heat carefully then cool +transfer it to a TT containing 2 mL conc. H_2SO_4	Reddish- brown and greenish- blue ring appears at the junctions
4.	Saponins	Foam Test	2 mL extract + 5 mL D/W + shake TT	Stable foam
5.	Tannins	Braymer's Test	1 mL extract + 2 mL of 10% $FeCl_3$	Dark blue colour appears
6.	Phenols	Ferric chloride Test	1-2 mL extract + 1 mL of 5 % $FeCl_3$	Deep blue colour appears
7.	Steroids	Salkowsky's Test	1 mL extract + 1 mL chloroform +1 mL conc. H_2SO_4 along the sides of test tube	Chloroform layer appears red and acid layer shows greenish yellow colour.
8.	Anthocyanin	NaOH Test	2 mL extract+ few drops of 10% NaOH	Appearance of blue green colour.

Source: Authors, 2022.

2.5 Total phenolic content (TPC)

The total phenolic content of the tea extracts was measured using the *Folin-Ciocalteau* reagent (Singleton et al., 1999). Phenols reduce phosphomolybdate and tungstate to molybdenum blue, which yields the colour blue. As a standard analyte, gallic acid is used. Gallic acid (1mg/mL) with volume of 25, 50, 100, 150, and 200 µL were used to make a series of standard solutions. In test tubes, 20 µL of extracts were taken independently. Using *d/w*, the volume of all the test tubes was increased to 1 mL. 5 mL *Folin-Ciocalteau* reagent and 4 mL 1M Na_2CO_3

solution were added to them and thoroughly mixed. They were incubated at room temperature for 15 min before having their absorbance measured at 760 nm on a colorimeter. Triplicate readings were taken. The concentration of phenolics in extracts was estimated using a standard graph of gallic acid. The amount of total phenoilcs in each gram of extract was measured in mg of GAE (Gallic acid equivalents)/gm extract^{-1} using y = mx + c equation.

2.6 Total flavonoid content (TFC)

The Aluminium chloride assay was used to determine the total flavonoid content of tea extracts (Chang et al., 2002). The appearance of yellow color is caused by the formation of a complex between the Al^{3+} ion and the carbonyl and hydroxyl groups of flavonoids. Quercetin is employed as standard compound. To generate a series of standard solutions, quercetin (1mg/mL) was taken in varying concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 µL. Separately, 20 µL of tea extracts were collected. All the test tubes' volumes were increased to 1 mL. 0.1 mL of 10% Aluminum chloride, 0.1 mL potassium acetate, and 2.2 mL of *d/w* were added to them. They were then incubated at room temperature for 30 min. A colorimeter was used to measure their absorbance at 415 nm. The readings were done in triplicate. To determine the amounts of flavonoids in aqueous extracts of teas, a standard graph of quercetin was created. Each gram of extract has its total flavonoids quantified in mg of QE (quercetin equivalents)/gm extract^{-1} using y = mx + c equation.

2.7 Ferric reducing potential assay (FRAP)

Antioxidants reduces ferric ions to ferrous ions, which then react with ferricyanide ions to generate the ferro-ferricyanide complex, which gives the colour as prussian blue (Ahmed et al., 2014). The standard antioxidant used is ascorbic acid (1mg/mL). Varying concentrations of ascorbic acid (25, 50, 75, 100, and 125 µL) were used to make a series of standard solutions. Separately, 20 µL of extracts were taken. 1.5 mL of 0.2M sodium phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide were added to each test tube and incubated in a waterbath at 50 °C for 20 min. Following the incubation, 1 mL of 10% TCA and 0.1 mL of 0.1% $FeCl_3$ were added and incubated for another 10 min at room temperature. A colorimeter was used to measure absorbance at 700 nm. All assays were run in triplicates. The maximum antioxidant potential (ferric reducing capacity) in the extracts was calculated using a standard graph of ascorbic acid and evaluated in mg AAE (ascorbic acid equivalent) per gram of extract^{-1} using y = mx + c equation.

2.8 Total antioxidant capacity (Phosphomolybdate assay)

Antioxidants reduces the molybdenum ion from Mo (VI) to Mo (V), resulting in the formation of the phosphomolybdate complex, which is bluish-green in color. The assay was done according to (Battistelli et al., 2005) with minor modifications. Ascorbic acid (1mg/mL) is used as a reference analyte. A series of standard solutions with increasing doses (25, 50, 75, 100, and 125 µL) were prepared, and 20 µL of extracts were taken individually. To them 1.2 mL of *d/w* and 2.2 mL of phosphomolybdate reagent were added. The mouths of all test tubes were foiled to avoid evaporation and incubated in a waterbath at 95 °C for 15 min. Their absorbance was measured at 765 nm after they had cooled down. A set of 3 readings were taken. The highest antioxidant capacity of extracts was evaluated in terms of mg AAE (ascorbic acid equivalent) per grams of extract^{-1} using a standard graph.

2.9 In vitro anti-inflammatory activity assessment by (RBC) membrane stabilization

The study was performed according to (Sakat et al., 2010; Shinde et al., 1999) with slight modifications. RBCs have been used here in anti-inflammatory investigation because they imitate lysosomes. Tea extract's anti-inflammatory action is linked by its ability to inhibit haemolysis. The more haemolysis occurs, the more RBCs are broken down and Haemoglobin (Hb) is released into the solution. The greater the amount of Hb released, the higher the solution's absorbance. If the absorbance of the extract is less than the absorbance of the control, the extract is shown to have an anti-inflammatory effect.

2.10 Preparation of red blood cells (RBCs) suspension

Fresh whole human blood was collected from pathology and transferred to the heparinised centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% (*v/v*) suspension with normal saline.

2.11 Heat induced hemolysis

The standard anti-inflammatory drug taken here is Aspirin (15 mg/mL). To prepare the control, 20 µL phosphate buffer saline (PBS) and 3.2 mL of 10mM (PBS) were added to 100 µL of 10% RBC suspension. 100 µL of RBC suspension and 3.2 mL PBS were added to 2 µL extract in a test sample tube. In a standard tube, 20 µL of drug was added to 100 µL of RBC suspension and 3 mL of PBS. For 10 min, all the tubes were incubated at 37 °C. After that, they were heated for 20 min at 54 °C to rupture the RBC membrane and allowed extracts to fuse with it. The tubes were centrifuged for 5 min at 2500 rpm. The supernatant absorbance was then measured at 540 nm. The extract's hemolytic inhibition percentage was calculated using the following formula:

Absorbance of control – Absorbance of extract/Absorbance of control*100

By Sakat et al. (2010) and Shind et al. (1999).

2.12 Statistical analyses

All the experiments were carried out in triplicate and the results were given as the mean ± standard deviation (SD). The data were analyzed for statistical significance using Student's t-test and differences were considered significant at $p < 0.05$.

3. Results

3.1 Preparation of tea extract

The tea extract yielded following colors (Table 2).

Table 2. Colour of tea extracts.

Extract	Color
Red Tea	Reddish-pink
Blue Tea	Purple-blue
Black Tea	Brown

Source: Authors, 2022.

3.2 Thin layer chromatography

The extracts were subjected to TLC to detect caffeine. Pigments in red and blue tea could be seen as they were coloured. However, for caffeine, I^2 (Iodine) vapours were employed to visualize it, which resulted in brown spot exclusively in black tea (Figure 2), indicating that it contains caffeine. R*f* value of caffeine in black tea was found to be 0.77.

Figure 2. Caffeine in black tea under I^2 vapour. Source: Authors, 2022.

3.3 Paper chromatography for pigments

Anthocyanin pigment was detected and visualised through paper chromatography in red, blue, and black tea extracts (Figure 3). The R*f* values were determined, and the highest Rf value was obtained in blue tea extract (0.86). The R*f* values are presented in the Table 3 given below.

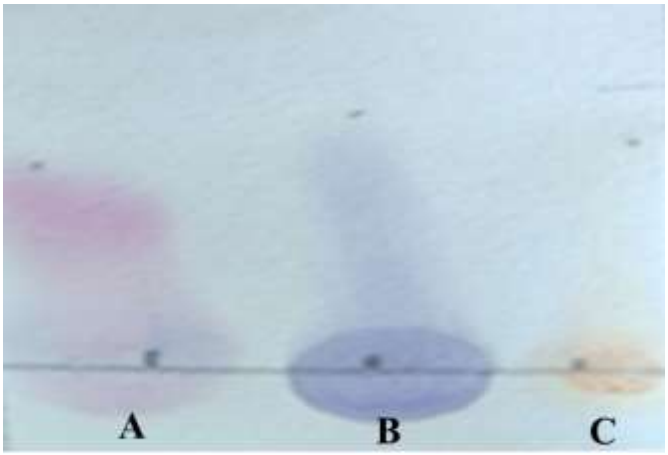

Figure 3. Paper chromatography profile for anthocyanin in (**A**) red, (**B**) blue and (**C**) black tea. Source: Authors, 2022.

Table 3. R*f* values of anthocyanins in tea extracts.

Extract	Distance travelled (cm)	Rf value
Red Tea	2	0.52
Blue Tea	3.3	0.86
Black Tea	3	0.78
Solvent	3.8	-

Note: (-) Not determined. Source: Authors, 2022.

3.4 Phytochemical screening

The presence of phytochemicals that contributes to the teas' antioxidant properties is determined through qualitative analysis. Flavonoids, phenols, tannins, saponins, terpenoids, steroids, glycosides, and anthocyanins were found abundantly in blue and black tea extract in tests (Figures 4, 5 and 6). Only flavonoids, phenols, tannins, saponins, and anthocyanins were detected in red tea extract (Table 4). As a conclusion, as compared to black and red tea extracts, blue tea extract contains an abundance of phytochemicals.

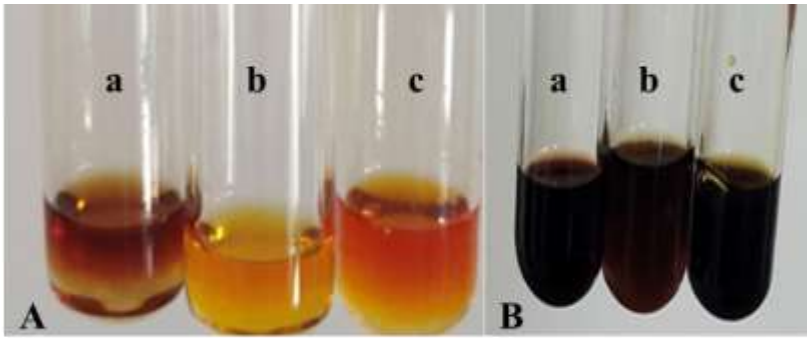

Figure 4. (**A**) Flavonoids and (**B**) polypheols respectively in (**a**) black, (**b**) red and (**c**) blue tea. Source: Authors, 2022.

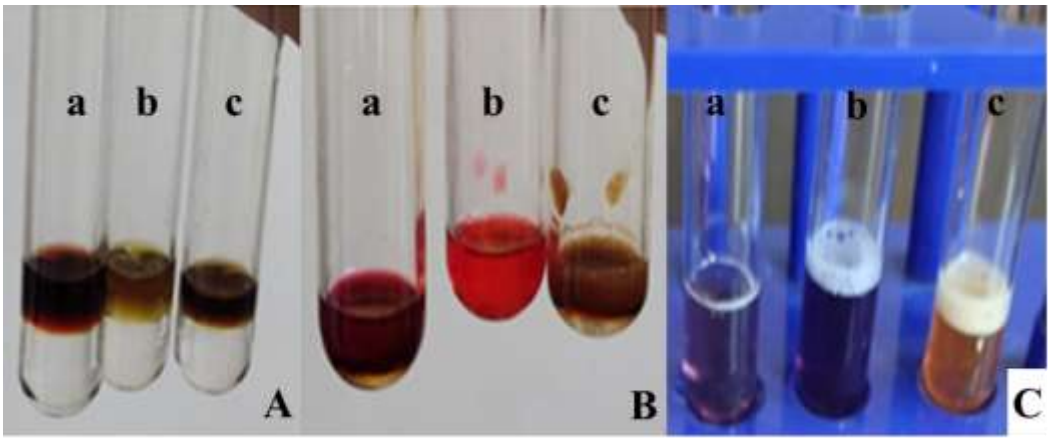

Figure 5. Coloured reactions for phytochemicals (**A** - Terpenoids, **B** - Glycosides, **C** - Saponins) (**a**) blue, (**b**) red and (**c**) black tea. Source: Authors, 2022.

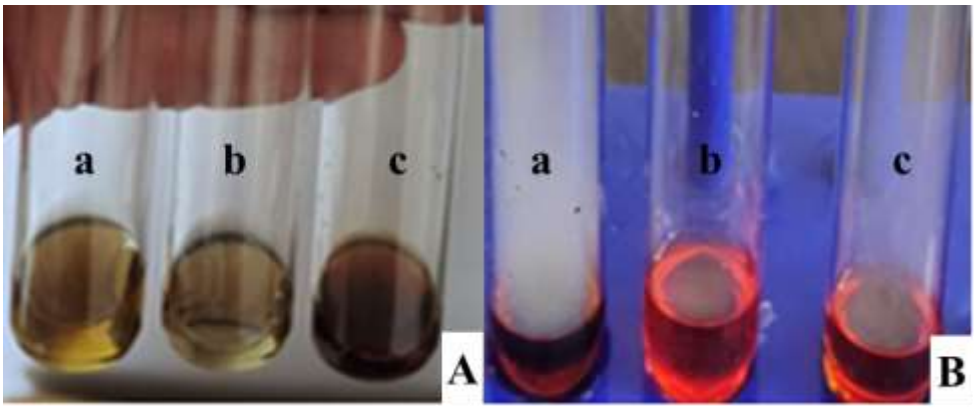

Figure 6. Anthocyanin indicated by green color with NaOH (**A**) and red colour with HCl (**B**) in (**a**), blue (**b**) red and (**c**) black tea. Source: Authors, 2022.

Table 4. Phytochemical analysis of tea extracts.

S. No.	Phytochemical	Red Tea	Blue Tea	Black Tea
1	Flavonoids	+	++	+
2	Phenols	+	++	++
3	Tannins	+	++	++
4	Saponins	+	++	+
5	Terpenoids and Steroids	-	++	++
6	Glycosides	-	++	++
7	Anthocyanins	++	++	+

Note: (-) absent. (+) present. (++) strongly present. Source: Authors, 2022.

3.5 Confirmatory anthocyanin test

Blue and red tea extracts showed highly significant coloration during preliminary tests for anthocyanin, whereas black tea extracts showed little or no coloration at all. Both blue and red tea exhibited good coloration in confirmatory tests (Figure 7), confirming that they have more anthocyanin. However, since anthocyanin is

converted down into other derivatives during processing thus in black tea, the coloration isn't quite as prominent.

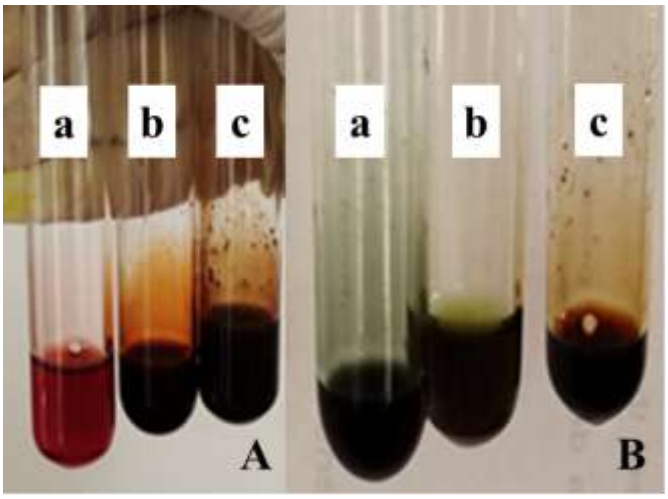

Figure 7. Anthocyanin presence as shown by orange (**A**) (**a**, **b** and **c**), and blue green (**B**) (**a**, **b**, and **c**) colour in tea extracts. Source: Authors, 2022.

3.6 Total phenolic content

The total phenolic content (TPC) of red tea, blue tea, and black tea was calculated in terms of mg GAE (Gallic Acid Equivalent) per gram of extract using gallic acid as standard from calibration graph (Figure 8). They were 2.41 mg, 10.83 mg, and 12.25 mg GAE per gram for red tea, blue tea, and black tea extracts^{-1} respectively (Table 5). Black tea (12.25 mg GAE/ gm extract^{-1}) seemed to have the highest phenolic content among the three extracts.

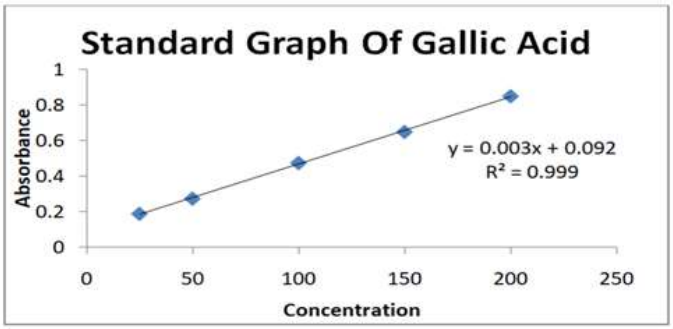

Figure 8. Standard curve of gallic acid. Source: Authors, 2022.

3.7 Total flavonoid content (TFC)

Total flavonoid contents were extrapolated from the straight line equation of quercetin standard curve (Figure 9). Red tea, blue tea, and black tea extracts exhibited total flavonoid concentrations of 5.30 mg, 15.84 mg, and 10.57 mg QE per gram extract, respectively (Table 5). The blue tea extract was shown to have the highest flavonoid concentration (15.84 mg QE per gram extract^{-1}).

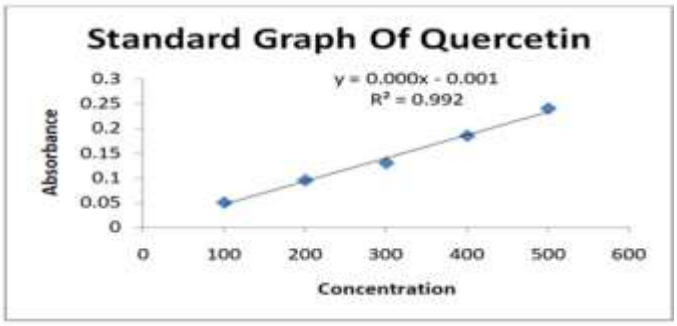

Figure 9. Standard curve of quercetin. Source: Authors, 2022.

3.8 Ferric reducing potential assay (FRPA)

This assay measures reducing potency of plant extract against the oxidative effects of reactive oxygen species. It is based on the reduction of ferric ion via the addition of hydrogen removed from phenolic antioxidant compound. The higher absorbance indicates higher reducing potency of the sample. Red tea, blue tea, and black tea extracts exhibited FRPA potential values of 0.56 mg, 1.81 mg, and 1.93 mg AAE per gram extract^{-1}, respectively. Ascorbic acid at the concentration of 1mg/mL was used as the reference antioxidant for comparison. Compared to red and blue tea extracts, black tea extract (1.93 mg AAE per gram extract^{-1}) seemed to have the highest ferric reducing potential. Experimental results have been recorded in the Table 5 given below.

3.9 Total antioxidant capacity (TAC)

This method evaluates both water soluble and fat soluble antioxidants. Molybdenum ions could be reduced by antioxidants. The extract's overall antioxidant capability is determined by the greatest potential at which it reduces molybdenum ions. The total antioxidant capacity is measured in mg AAE (ascorbic acid equivalent) per gram extract using ascorbic acid as standard (1mg/ml). Black tea exhibits the highest antioxidant capacity in reducing molybdate ions (1.94 mg AAE per gram extract^{-1}), followed by blue tea (1.56 mg AAE per gram extract^{-1}) and red tea (0.44 mg AAE per gram extract^{-1}) having the lowest. The TAC values of extracts are recorded in the (Table 5) given below.

Table 5. Quantitative phytochemical and antioxidant assay of tea extracts.

Tea extract	TPC (mgGAE/gm extract^{-1})	TFC (mg QE/gm extract^{-1})	FRPA (mgAAE/gm extract^{-1})	TAC (mgAAE/gm extract^{-1})
Red Tea	2.41±0.132	5.30±0.400	0.56±0.372	0.44±0.296
Blue Tea	10.83±0.329	15.84±0.268	1.81±0.413	1.56±0.199
Black Tea	12.25±0.245	10.57±0.314	1.93±0.178	1.94±0.354

Note: Values are expressed as mean± SD for three determinations. Source: Authors, 2022.

3.10 Membrane stabilization

Stabilization of RBCs membrane was studied to establish an additional mechanism for anti-inflammatory action of tea extracts. Due to resemblance of RBC membrane with lysosomal membrane, this effect may possibily inhibit the release of lysosomal content of neutrophils responsible for tissue damage at the site of inflammation. Red tea, blue tea, and black tea are exhibited hemolytic inhibition percentages of 47.61%, 57.14%, and 10.52%, respectively. With 57.14% hemolytic inhibition, blue tea proved to be the most efficient in anti-inflammatory action. Aspirin, standard anti-inflammatory drug showed hemolytic inhibition of % 39.45 ± 0.432% at 15 mg/mL. Hemolytic inhibition values are presented in the (Table 6) given below.

Table 6. Effect of tea extracts on membrane stabilization.

Tea extract	Percentage hemolyticinhibition
Red Tea	47.61 ± 0.361%
Blue Tea	57.14 ± 0.567%
Black Tea	10.52 ± 0.478%
Aspirin	39.45 ± 0.432%

Note: Values are expressed as mean ± SD for three determinations. Source: Authors, 2022.

4. Discussion

The outcomes of our investigation showed that tea extracts possess significant antioxidant and anti-haemolytic activities. Compared to red tea extract, blue and black tea extracts exhibited substantial proportions of phenols and flavonoids. Black and blue tea extracts revealed higher significant values for ferric reducing potential and total antioxidant capacity than red tea extract. Conversely, since caffeine is only found in black tea, blue tea is desirable as it is caffeine-free.

It was established that diluted blue tea extract exhibited greater anti-haemolytic potential over black and red tea extracts in anti-inflammatory analyses. Phytochemicals such as phenols and flavonoids have been reported in several studies to contribute to the antioxidant and anti-inflammatory properties of medicinal plants along with potential health benefits (Geronikaki; Gavalas, 2006; Jing et al., 2015).

The presence of phenols and flavonoids in significant concentrations was detected in red, blue, and black tea that also contributed to their ferric reducing potential, total antioxidant capacity, and anti-inflammatory properties. Researchers also reported three major flavanoids from petals of *C. ternatea* (Kazuma et al., 2003). The maximum value for TFC was recorded for blue tea extract as 15.84 mg QE per gram extract^{-1}. In previous study conducted (Vankar; Srivastava, 2008), promising amount of polyphenols and flavonoids were detected in flower extract of China rose.

Whereas, at 2 µL concentration, red and black tea extracts were demonstrated to have fairly significant anti-inflammatory activity in stabilization studies of RBCs membrane. In comparison to other tea extracts, blue tea extract displayed the highest haemolysis (57.14 percent) inhibition after 3.4 times dilution. This difference in anti-inflammatory activity of blue tea could be due to presence of specific type of polyphenols and flavonoids in it rather than their amounts. The results show a strong correlation between total phenolic, flavonoid content and anti-inflammatory assay (Talhouk et al., 2007). Thus altogether, having diverse phytochemical variety, anti-inflammatory and being powerhouse of antioxidant properties the blue tea showcases impressive nutritional profile, act as wonderful weight loss beverage, refreshing drink, potential detox liquid, immunity booster and much more. Blue tea also has potential to augment physical and psychological wellbeing and can be considered as a good medical treatment for respiratory and reproductive health, healthy eyesight, maintaining diabetic condition, healthy hair growth and in weight loss. In times to come the awareness of Blue tea benefits must be outspread in order to have a potential and natural alternative to treat or control various medical or health conditions.

4. Conclusions

Herbal beverages have experienced an upsurge in interest, advancement and uptake owing to their phytochemical content and potential implications to health and wellness. Due to this, our study provides the first information on the antioxidant capacity and analysis of red and blue tea and their comparison with black tea. As an outcome of the study's findings, blue tea is more relevant in terms of non-caffeinated beverage consumption due to its potent antioxidant and anti-inflammatory properties.

However, further research is required to isolate and formulate the active component found in blue tea into a drug along with clinical trials. Such research will be beneficial in establishing their implementations in the pharmaceutical sector to cure and prevent a multitude of emergent ailments.

5. Acknowledgments

We would like to thank all the anonymous individuals who helped with this study. The authors are grateful to

Indore Path Laboratory, Indore (M.P.), India for providing us the blood sample for the study.

6. Auhors' Contributions

Sanjana Patel: wrote the paper and performed analysis. *Khushi Verma*: performed analysis. *Radha Deshbhratar*: performed analysis. *Kamal Kishore Maru*: analysis tools. *Porshia Sharma*: scientific writing and manuscript editing. *Rashmi Limaye*: contributed and collected data. *Payal Puri*: concieved and designed the analysis.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Yes applicable. The experimental protocols were approved by the Institutional Ethical Committee of IILR (IEC No: 19/07/21) Academy, Indore.

9. References

Ahmed, F., Fatima, M., & Saeed, S. (2014). Phenolic and flavonoid contents antioxidative potential of epicarp and mesocarp of *Lageneria siceraria* fruit: a comparative study. *Asian Pacific Journal of Tropical Medicine* 7, S249-S255. https://doi.org/10.1016/S1995-7645(14)60241-8

Battistelli, M., De Sanctis, R., De Bellkis, R., Cucchiarini, L., Dacha, M., & Gobbi, P. (2005). Rhodiolarosea as antioxidant in red blood cells: Ultrastructural and haemolytic behaviour. *European Journal of Histochemistry*, 49(3), 243-254. https://doi.org/10.4081/951

Chakraborthy, G.S., Kumar, V., Gupta, S., Kumar, A., Gautam, N., & Kumari, L. (2018). Phytochemical and pharmacological aspects of Clitoria ternatea-A review. *Journal of Applied Pharmaceutical Sciences and Research*, 1, 3-9.

Chandrasekara, A., & Shahidi, F. (2018). Herbal beverages: Bioactive compounds and their role in disease risk reduction- A review. *Journal of Trdaitional and Complementary Medicine*, 8(4), 451-458. https://doi.org/10.1016/j.jtcme.2017.08.006

Chang, C., Yang, M., Wen, H., & Chern, J. (2002). Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178-182. http://lawdata.com.tw/File/PDF/J991/A04971003_178.pdf

Djenidi, H., Khennouf, S., & Bouaziz, A. (2020). Antioxidant activity of phenolic content of commonly consumed fruits and vegetables in Algeria. Progress in Nutrition 22(1), 224-235. 10.23751/pn.v22i1.7701

Fatima, M., Kesharwani, R. K., Misra, K., & Rizvi, S. I. (2013). Protective effect of theaflavin on erythrocytes subjected to invitro oxidative stress. *Biochemistry Research International*, 2013, 1-7. https://doi.org/10.1155/2013/649759

Faten, R., Ghaffar, A., Ibrahim, A., & Elaimy, E. I. (2012). Invitro, antioxidant and scavenging activities of *Hibiscus rosa sinensis* crude extract. *Journal of Applied Pharmaceutical Science*, 02(01), 51-58. https://japsonline.com/admin/php/uploads/379_pdf.pdf

Forgacs, E., & Cserhati, T. (2002). Thin-layer chromatography of natural pigments: New advances. *Journal of Liquid Chromatography & Related Technologies*, 25(10-11), 1521-1541. https://doi.org/10.1081/JLC-120005702

Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, 20(9), 689-709. https://doi.org/10.1038/s41573-021-00233-1

Geronikaki, A. A., & Gavalas, A. M. (2006). Antioxidants and anti-inflammatory diseases: synthetic and natural antioxidants with anti-inflammatory activity. *Combinatorial Chemistry & High Throughput Screening*, 9(6), 425-442. https://doi.org/10.2174/138620706777698481

Harborne, J. B. (1973). Phytochemical Methods; A Guide to Modern Techniques of Plant Analysis, 2nd edn., Chapman and Hall, London, New York, pp 49-188.

Jain, N. K. (1999). Biosynthesis of precursors for liquor and flavor characteristics in black tea. *In*: Global Advances in Tea Science, Aravali books international Ltd., New Delhi, 723-748 p.

Jeyaraj, E. J., Lim, Y. Y., & Choo, W.S. (2021). Extraction methods of butterfly pea (Clitore ternatea) flower and biological activities of its phytochemicals. *Journal of Food Science and Technology*, 58, 2054-2067. https://doi.org/10.1007/s13197-020-04745-3

Jing, L., Ma, H., Fan, P., Gao, R., & Jia, Z. (2015). Antioxidant potential, total phenolic and total flavonoid content of Rhododendron anthopogonoides and its protective effect on hypoxia induced injury in PC12 cells. *BMC Complementary and Alternative Medicine*, 15, 287. https://doi.org/10.1186/s12906-015-0820-3

Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals *Clitoria ternatea*. *Phytochemistry*, 62(2), 229-237. https://doi.org/10.1016/S0031-9422(02)00486-7

Khristi, V., & Patel, V.H. (2016). Therapeutic potential of Hibiscus rosa sinensis: A review. *International Journal of Nutrition and Dietetics*, 4(2), 105-123. http://dx.doi.org/10.17654/ND004020105

Kosai, P., Sirisidthi, K., Jiraungkoorskul, K., & Jiraungkoorskul, W. (2015). Review on ethnomedicinal uses of memory boosting herb, butterfly pea, *Clitoria ternatea*. *Journal of Natural Remedies*, 15(2), 71-76. https://doi.org/10.18311/jnr/2015/480

Kumari, P., Ujala, & Bhargava, B. (2021). Phytochemicals from edible flowers: Opening a new arena for healthy lifestyle. Journal of Functional Foods 78: 1-18.

Madani, A., Nehal, N., Haque, S. S., & Khan, A. (2010). Perspective of oxidative stress in a biological system and prevention by naturally occurring oxidant. Proceedings of the National Academy of Sciences, 80(4), 287-295. https://www.cabdirect.org/cabdirect/abstract/20113218611

Nobre, A. C., Rao, A., & Owen, G. N. (2008). L-theanine, a natural constituent in tea, and its effect on mental state. *Asia Pacific Journal of Clinical Nutrition*, 17, 167-168. https://doi.org/10.1016/j.jff.2021.104375

Quan, P. T., Hang, T. V., Ha, N. H., & Gang, B. L. (2007). Total polyphenols, total catechin content and DPPH free radical scavenger activity of several types of Vietnam commercial green tea. *Journal of Science & Technology Development*, 10(10), 6-10. https://doi.org/https://doi.org/10.32508/stdj.v10i10.2827

Raman, N. (2006). Phytochemical methods. New Indian Publishing Agencies, New Delhi, pp. 19.

Rani, N., Sagar, B.P.S., & Shukla, S. K. (2013). To investigate and asses why some food/ dietary supplements are health hazardous. *Journal of Drug Delivery & Therapeutics*, 3(1), 65-69. https://doi.org/10.22270/jddt.v3i1.392

Sadique, J., Al-Rqobah, N. A., Bulghaith, M. F., & El-Gindy, A. R. (1989). The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. *Fitoterapia*, 60, 525-532.

Sakat, S., Juvekar, A. R., & Gambhire, M. N. (2010). *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 2(10), 146-155. https://innovareacademics.in/journal/ijpps/Vol2Issue1/322.pdf

Shinde, U. A., Kulkarni, K. R., Phadke, A. S., Nair, A. M., Mungantiwar, A. A., Dikshit, V. J., & Saraf, M. N. (1999). Mast cell stabilizing and lipoxygenase inhibitory activity of Cedrus deodara (Roxb.) Loud. Wood Oil. *Indian Journal of Experimental Biology*, 37, 258-261. http://nopr.niscpr.res.in/handle/123456789/19006

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and oxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology*, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Talhouk, R. S., Karam, C., Fostok, S., El-Jouni, W., & Barbour, E. K. (2007). Anti-Inflammatory Bioactivities in Plant Extracts. *Journal of Medicinal Food*, 10(1). https://doi.org/10.1089/jmf.2005.055

Vankar, P. S., & Srivastava, J. (2008). Comparative study of total phenol, flavonoid contents and antioxidanT activity in *Canna indica* and *Hibiscus rosa sinensis*: Prospective natural food dyes. *International Journal of Food Engineering*, 4, 1-15. https://doi.org/10.2202/1556-3758.1232

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).