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# A Review on Antioxidant and Antimicrobial Potentials of Beverages – Green Tea, Hibiscus Tea, Rooibos Tea and Black Tea

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**ABSTRACT:** Antioxidant and antimicrobial potentials of tea infusions of commercially available green tea, hibiscus tea, rooibos tea and black tea were assessed. The aqueous extracts were screened for total phenolic content, total flavonoid content. Antioxidant activity evaluation with DPPH radical scavenging activity, FRAP, BCB and ATBS methods. The ranking based on antioxidant activity was: Green Tea > Black Tea > Rooibos Tea > Hibiscus Tea. Antimicrobial activity was evaluated by Time kill assays, Anthocyanin and Phenolic content test, MIC Assays, FICI Assay and by Disc Diffusion Method. Gram positive bacteria (*S. aureus*, *E. faecalis*, *B. cereus*), Gram negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) and Yeast (*C. albicans*) were used. Green Tea had maximum antimicrobial activity whereas Rooibos tea was relatively lower. *E. coli* was the most inhibited organism whereas *S. aureus* was resistant.

**KEYWORDS:** Antioxidant, Antimicrobial, Flavonoid, Phenolic content, Tea.

## I. INTRODUCTION

Tea is the most widely consumed beverage in the world, second to water. Off the total amount of tea that is produced and consumed in the world 78% is Black, 20% is Green and 2% Oolong. Black tea is produced from fully fermented leaves and have a characteristic taste and smell. In green tea, the leaves retain their green color and most of their initial polyphenol content.<sup>[1]</sup>

Rooibos tea is a red herbal tea that is obtained from the leaves of *Aspalathus linearis* a plant native to Republic South Africa. It is known to contain lots of antioxidants and is a completely caffeine free alternative to black or green tea. Some antioxidants present has an anti-diabetic effect.<sup>[2]</sup>

Hibiscus tea is an herbal tea made from the dried parts of the hibiscus plant. It is caffeine free and is believed to help in the treatment of heart diseases and reduce blood pressure. The health benefits can be attributed to the higher content of anthocyanins.<sup>[3]</sup>

Humans are exposed to various environmental stressors like UV radiation, microbes, allergens, increased ozone levels, polycyclic aromatic hydrocarbons which amplify the generation of reactive oxygen species.<sup>[4]</sup> Excessive free radical production in the body results in a condition referred to as oxidative stress which produces degenerative effects on human health, leading to oxidative deterioration of lipids, proteins, and DNA, activation of procarcinogens, inhibition of cellular and antioxidant defense systems, and changes in gene expression contributing to serious pathological changes within the body that leads to premature ageing and numerous neuro degenerative diseases, cancer, diabetes, osteoporosis and cardiovascular diseases.<sup>[5-7]</sup>

The Human body has in place several strategies to counteract the effects of free radicals and oxidative stress, this can be enzymatic (e.g., SOD, CAT, and GPx) and nonenzymatic (e.g., lipoic acid, glutathione, L-arginine, and coenzyme Q10) antioxidant molecules, these constitute the endogenous antioxidants.<sup>[8]</sup> Some common exogenous antioxidants are Vitamin C, Vitamin E, Flavonoids, Polyphenols. Synthetic antioxidant like Butylated Hydroxy Anisole (BHA), Butylated Hydroxytoluene (BHT), Tert-Butyl Hydroquinone (TBHQ) and propyl, octyl and dodecyl gallates are usually added to food to not only improve their shelf life<sup>[4]</sup> but also to be marketed as 'antioxidant enriched food'.



There is an increasing interest in replacing synthetic antioxidants since number of them have restricted use in foods because of their toxicological effects on various species and suspected carcinogenic potential.<sup>[9]</sup>

Epidemiological studies suggest that consumption of plant polyphenols on a daily basis can protect against developments of disorders caused by free radical accumulation.<sup>[7]</sup> Tea is a major source of dietary polyphenols and an even better source of the flavanols, the catechins and catechin/gallate esters.<sup>[10]</sup>

Previous studies have shown a high correlation between antioxidant capacity and total phenolic content indicated that phenolic compounds could be one of the main components responsible for antioxidant activities of these beverages.<sup>[11]</sup> Polyphenolic compounds function as good electron and hydrogen atom donors and therefore are able to terminate radical chain reaction by converting free radicals and ROS to more stable products.<sup>[5]</sup>

An anti-microbial agent is a chemical or substance, either synthetic or natural,<sup>[12]</sup> that retards microbial growth or even kills micro-organisms.<sup>[13,12]</sup> More specifically, there are antibacterial, anti-viral, anti-fungal and anti-protozoan agents depending on the type of microorganism affected by the anti-microbial agent<sup>[13]</sup> as antimicrobial agents can vary in their selective toxicity. Certain of these chemicals act in a non-selective manner and have similar effects on all types of cells.

The action of anti-microbial agents can either be highly selective or even more toxic to a specific microbial species. The action spectrum of each anti-microbial agent is, therefore, different.<sup>[12]</sup>

Some anti-microbial agents damage microbial cells by altering the normal selective permeability of the cytoplasmic membrane and the cell wall, causing leakage of vital intracellular substances. Protein denaturation also occurs when the permeability of the membranes is influenced, resulting in the inactivation of the proteins and enzymes involved in the normal functioning of the cell.<sup>[13]</sup>

Antibiotics are the major drugs used for the treatment of infectious diseases, in recent years, antibiotic resistance has been increasing, and is becoming a serious problem.<sup>[14]</sup>

Some microbes may develop resistance to a single antimicrobial agent while others, like "Multidrug Resistant (MDR)" Strains, are resistant to several agents. Infections caused by strains often fail to react to standard treatment and create a greater risk of death due to the spread of the resistance to other microorganisms.<sup>[15,16]</sup> In some cases, MDR microorganisms, which are called "pan-resistant organisms", have become resistant to all the available antibiotics and cannot be treated with any single antibiotic alone.<sup>[17]</sup> The failure of the existing antibiotics to control infections makes it crucial to find alternative agents with new mechanisms of action.

A novel therapeutic approach involves the use of natural antimicrobial compounds such as plant derived products such as spices, essential oils, extracts, or the consumption of herbal teas alone or in combination with antibiotics.

Many studies have been done on the anti-microbial activity of green and black teas.<sup>[18-25]</sup> Catechins are reported to play a major role in the anti-microbial effect of the black and green teas, especially epicatechin gallate and epigallocatechin gallate which showed the strongest anti-microbial activity against a wide range of microbes.<sup>[19,26,21,27,28]</sup> Some of the bacteria tested included *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Clostridium perfringens* and *Pseudomonas fluorescens*, but it was found that these teas are not effective against yeasts and moulds.<sup>[18,26,23,29]</sup>

The aim of this study was to assess and compare the different teas such as green tea, black tea, rooibos tea and hibiscus tea on their antioxidant and antimicrobial potentials at conditions usually used in tea preparations at home.

## II. MATERIALS

### 1) Sample Preparation for Antioxidant activity

200mg of tea is added to 5mL of distilled water and steeped for 10 minutes at 96°C, the mixture is then centrifuged at 200g for 10 minutes and is filtered to get rid of leaves and any other kind of debris. The filtrate is then used in further tests.

The tea extracts are made according to the manufacturer's instructions, analyzed for Ascorbic Acid immediately after which its stored at -20°C for future use.<sup>[30]</sup>

Each plant leaf (1.5g) was mixed with 100mL of boiling water for 5 minutes, with constant shaking and the samples were then filtered through Whatman No. 1 filter paper.

Infusions for the TEAC and TP assays were treated in three different ways:

- i. Direct extraction to obtain the infusion.
- ii. Freeze-drying the direct infusion and keeping the solid at 20°C until further analysis.
- iii. Freezing (20°C) the direct infusion and analyzing after 2–5 weeks. <sup>[31]</sup>

Tea leaves are powdered and 1g is added to 50mL of methanol and continuously stirred for one hour at room temperature. The extracts were then filtered and stored at -20°C for future use. <sup>[1]</sup>

In general, we can conclude that aqueous extracts of the tea as seen in the case of McAlpine, et al, 2016 and M. Pilar Almajano, et al, 2008 made according to the manufacturer's instructions were better suited for further studies as it was closer to the form they were meant to be consumed in as compared to the alcoholic extraction by Chan, et al, 2007.

## 2) Preparation of Extract for Antimicrobial Activity

Dried calyces of the selected plants were finely ground (1mm). 3.6mL of sterile water was added to 1g of ground tissue and incubated for 2 hours at 4°C in the dark with occasional mixing to make aqueous extracts. Mixture was then centrifuged at 5000xg for 30 minutes. Aqueous extracts were then dialyzed for 36 hours against deionized water with three water changes at 4°C in the dark. Resulting extracts were centrifuged at 5000xg for 30 minutes and frozen at -80°C. Frozen extracts were lyophilized with a Freeze Dry system and stored in a sealed container at room temperature. <sup>[32]</sup>

Aqueous tea infusions of the teas were prepared by adding 100mL of boiling water to 10g of dried leaves. After 30 minutes of infusion, the teas were filtered through 0.40mm and 0.22mm filters. These 10% tea infusions were aliquoted and stored at -20°C. <sup>[33]</sup>

Tea leaves were harvested from shrubs and the dry leaves were ground into a fine powder using a high-speed grinder. The aqueous extract was prepared by submerging the ground powder in sterile distilled water and left in a platform shaker/incubator, for 24 hours at 25°C. The liquid was then filtered, and the filtrate stored at -80°C before lyophilization. The lyophilized aqueous extract was then left under UV light overnight to ensure the elimination of any microbial contamination.

The plant material was submerged in a mixture of methanol and dichloromethane (1:1), for 24 hours at 37°C, in a platform shaker/incubator to prepare the organic extract. The liquid was filtered and left for complete evaporation of the solvent under a fume hood, leaving behind the solid organic extract.

The percentage yields were calculated at 1.37% and 1.98% for the aqueous and organic extract respectively, after which the extracts were stored in sealed sterile bottles, at room temperature and protected from light, until further analysis. <sup>[34]</sup>

## 3) Preparation of Cultures

To represent the three main classes of micro-organisms,

- Gram-positive bacteria – *S. aureus*, *Enterococcus faecalis*, *Bacillus cereus*, MSSA, MRSA, *Micrococcus luteus*, *Lactobacillus acidophilus*
- Gram-negative bacteria – *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, *A. baumannii*
- Yeast – *C. albicans* and *Cryptococcus neoformans* were selected.

Cultures were grown in Tryptic Soy Broth (TSB) and stored in glycerol at -20°C. Working cultures were obtained by inoculating 50mL of TSB with 200µL of stock cultures and then incubating them for 24 hours at 35 – 37°C. *S. aureus* strains were subcultured once due to slower growth. After incubation, cultures were diluted to ca. 5.0 to 6.0 log CFU/mL and tested for antimicrobial activity. <sup>[31–35]</sup>

## 4) Antibiotics and Antifungals

Antimicrobials selected were Ampicillin, Ceftazidime, Cefuroxime, Amikacin, Erythromycin, Ciprofloxacin, Linezolid, Ampicillin Sulbactam, Doxycycline, Fluconazole, Itraconazole, Nystatin. A concentration of 1280mg/L for the antifungals and 5120mg/L for the antibiotics of the dry powders were taken respectively to prepare stock solutions. They were filtered for sterilization and stored frozen at -80°C for up to six months. <sup>[33]</sup> Erythromycin, Gentamicin, Nystatin, Penicillin G, Tetracycline, Ciprofloxacin, and Amphotericin B, which were all selected. The antimicrobials were prepared in sterile distilled water, while the antifungal, Amphotericin B, was dissolved in 1% DMSO in sterile water. <sup>[34]</sup>



### 5) Media

The Media utilized were:

- Mueller-Hinton broth
- Roswell Park Memorial Institute 1640 medium (RPMI) with L-glutamine and Morpholine Propane Sulfonic Acid.
- For colony counts, Tryptic Soy Agar were used. [33]

The method for the preparation of extract used by Booth, et al, 2014 is the most feasible method as it uses a grinder, incubator shaker, lyophilizer and a Laminar Air Flow which are basic lab utilities. It is the only method which uses UV light for the elimination of microbial contamination.

The most common microbes used were: *S. aureus*, *Enterococcus faecalis*, *Bacillus cereus* (Gram positive bacteria), *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram negative bacteria), *C. albicans* (Yeast). These microbes were chosen because they are commonly found as well as they cause food borne diseases in most cases i.e., they contaminate food. So, the idea was to use tea extracts to inhibit their growth.

The most used Media for the growth of microbes is Tryptic Soy Agar/Broth.

Commonly used antimicrobials were Erythromycin, Ciprofloxacin, and Nystatin.

## III. METHODS

### 1) TOTAL FLAVONOID CONTENT – ALUMINUM CHLORIDE ASSAY

250µL of the infusion is added to 1.25mL of distilled water. 75µL of 5% Sodium nitrate is then added and the mixture is allowed to stand for 6 minutes. After 150µL of 10% Aluminum chloride is added, the volume is made up to 2.5mL by addition of 0.5mL 1M Sodium hydroxide and 575µL of distilled water. A blank is made by substituting the infusion for 250µL of distilled water. Absorbance is then read at 510nm. The Results are given as grams of Quercetin equivalent/gram of dry tea leaves by plotting a calibration curve. [7]

### 2) TOTAL PHENOL CONTENT – FOLIN-CIOCALTEU'S METHOD

Tea infusions are diluted 1:100 with distilled water. 250µL aliquots are added to 1.25mL of 10% Folin-Ciocalteu's reagent. The mixture is left undisturbed for 5 minutes, followed by addition of 1mL of 7.5% Sodium carbonate. The reaction mixture is kept at 27°C for 1 hour. The standard was Gallic acid. The absorbance is read at 765nm. The TPC is expressed as milligrams of Gallic acid equivalents/gram of tea. [30]

### 3) ANTIOXIDANT ACTIVITY

#### a. DIPHENYLPICRYLHYDRAZYL ASSAY

0.5mL of the infusions of different dilutions is pipetted into test tubes. This is followed by the addition of 3mL of Ethanol, 0.3mL of 0.5mM of ethanolic DPPH. A blank is prepared by substituting the DPPH with 0.3mL of Ethanol. The reaction mixture is kept at 27°C for 45 minutes. The absorbance is read at 517nm.

The Percentage of Inhibition is then calculated:

$$\text{Percent inhibition} = \frac{AC - AA}{AC} \times 100$$

Where, AC is the absorbance value for negative control, AA is the absorbance value of the test samples after 45 minutes of incubation.

A Percent inhibition v/s Concentration graph is plotted to obtain the concentration at which 50% inhibition occurs. The higher the value, lesser is the antioxidant activity. [36]

The concentration of DPPH inhibited is to be obtained by performing the following calculation:

$$\text{DPPH Inhibited (mM)} = \text{Percent Inhibition} \times \text{Concentration of DPPH (mM)} [30]$$

The results are expressed as Ascorbic Acid equivalents per gram of dry tea (AAE/g), extrapolated from a calibration curve of L-ascorbic acid (0-12µg/mL). [7]

#### b. β-CAROTENE BLEACHING (BCB) METHOD

1.1µL of β-carotene (1mg/mL, 8.2µM) is added to 4.4µL of Linoleic Acid (0.1g/mL, 628µM) and 22µL of Tween 40 (0.2g/mL). All the solutions are made in Chloroform. It is mixed thoroughly, and the solvent is then removed using vacuum and its immediately resuspended in 2.4mL of PBS (0.2M, pH 7). Add 50µL of the infusion and incubate in a water bath at 50°C for 120 minutes. Control contains 50µL of sample instead of infusion. The optical density is then



read at 470nm using a UV spectrophotometer. Reference substances (BHT and  $\alpha$ -tocopherol) concentration was 90mg/mL.

The percentage of inhibition is then calculated by:

$$\text{Percent Inhibition} = \frac{(AA_{120} - AC_{120})}{(AC_0 - AC_{120})} \times 100$$

Where,  $AA_{120}$  is Absorbance of sample after incubation for 120 minutes,  $AC_{120}$  is the Absorbance of control after incubation for 120 minutes,  $AC_0$  is the Absorbance of negative control at the moment of solution preparation. <sup>[36]</sup>

#### c. ABTS RADICAL ASSAY

An aqueous solution of 7mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2.45mM Potassium Persulphate is separately and mixed in the ratio 1:1 in a foil wrapped container and incubated in the dark for 16 hours prior to use. Methanol is added to dilute the solution so as to obtain an optical density of 0.7 at 734nm. PBS is used as blank. The Percentage of Inhibition is then calculated by:

$$\text{Percent Inhibition} = \frac{AC - AA}{AC} \times 100$$

Where AC is the Absorbance of radical and methanol and AA is the Absorbance of the mixture containing the infusion after 30 minutes of incubation.

With the percentage, a Percent inhibition v/s Concentration graph is plotted it is compared with the Trolox calibration curve to obtain results as Trolox/gram of tea. <sup>[31]</sup>

#### d. FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

1mL of different dilutions of extract were added to 2.5mL Phosphate Buffer (0.2M, pH 6.6) and 2.5mL of Potassium Ferricyanide (1% w/v). The mixture is incubated for 20 minutes at 50°C. 2.5mL of 10% w/v of Trichloroacetic Acid solution was added. 2.5mL water is added to 2.5mL of the mixture. 500 $\mu$ L of 0.1% w/v Ferric Chloride solution is added and allowed to stand for 30 minutes. The absorbance is then read at 700nm. <sup>[1]</sup>

Aluminum Chloride Assay is often used to determine flavonoid content as Aluminum chloride is specific to flavanols and flavones Luteolin in the extract, while in other methods there might be some interference by phenolic acids in the extract.

Folin Ciocalteu's method stands out in their capacity to interact with any type of phenols and so has become one of the widely used methods for analyzing the phenol content in samples.

Among the various models of antioxidant activity, two commonly used ones are DPPH and BCB as they are sensitive, quick and the relative simplicity of the reagents and equipment. An added advantage is that DPPH Radical Scavenging Activity is not affected by substrate polarity and BCB is advantageous because of adding effective In Situ Lipidic Oxidation to the system.

In DPPH there is a better interaction with antioxidants soluble in organic solvents. BCB method is not used often because of the complexity in the preparation of reagents and low reproducibility. Comparing the methods used to assess antioxidant properties the following conclusions can be made. FRAP reagents are inexpensive and the results are reproducible. But only water-soluble antioxidants are detected by FRAP assay. The advantage of ABTS is the simplicity, reproducibility, and its flexibility in interacting with both hydrophilic and lipophilic antioxidants. The highly pigmented, hydrophilic antioxidants are usually better reflected in ABTS and ABTS has faster reaction kinetics and a heightened response to antioxidants hence making it more sensitive.

### 4) ANTIMICROBIAL ACTIVITY

#### a. TIME KILL ASSAYS

Lyophilized extracts were resuspended in 10mL of sterile water and autoclaved at 121°C for 30 minutes. Resuspended extracts were combined with 2.5mL of bacterial cultures which were harvested at late logarithmic phase and diluted to ca. 5.0 to 6.0 log CFU/mL. Bacterial cultures and extracts were incubated in 12.5mL of TSB at 35 – 37°C at room temperature. At regular intervals, a bacterial suspension sample was collected, serially diluted in 0.1% peptone, plated in Tryptic Soy Agar, and incubated for 24 hours at 35 – 37°C. Controls were prepared with sterile water. <sup>[32]</sup>

### b. ANTHOCYANIN AND PHENOLIC CONTENT

Anthocyanin content in tea extracts was tested by the pH differential method. Utilizing a colorimeter, absorbance was read at 520nm and 700nm at pH 1.0 and 4.5 with a dilution factor of 3 expressed in milligrams of cyanidin-3-glucoside equivalents per liter.

Tea extracts were further bleached with a bisulfite solution to evaluate quantity of polymerized color, the absorbance was evaluated at 420nm, 520nm, and 700nm utilizing a colorimeter. Percent polymeric color was calculated by:

$$\text{Percentage Polymeric Color} = \frac{\text{Polymeric color}}{\text{Color density}} \times 100$$

Polymeric color and color density were calculated as follows:

*Polymeric color and Color density* =

$$[(A_{420nm} - A_{700nm}) + (A_{520nm} - A_{700nm})] \times \text{Dilution Factor}$$

Color density depends on water diluted extract, whereas Polymeric color depends on the extract diluted with the bisulfite solution. Phenolic content was assessed by Folin – Ciocalteu's phenol reagent. Extracts were filtered through Whatman No. 4 filters after cooling.

The phenolic content was quantified with Gallic Acid (standard) and articulated in milligrams of Gallic acid equivalents (GAE) per gram of dry calyx extract after the absorbance was read at 725nm. <sup>[32]</sup>

### c. MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAYS

The micro-titer plate method was used to determine the antimicrobial activity of plant samples. 100µL of sterilized distilled water and samples were introduced into the wells. Plant samples were introduced at starting concentrations of 32mg/mL in Acetone (organic extracts) or sterile water (aqueous extracts). Conventional antimicrobials were exposed at a concentration of 0.01mg/mL and 0.1mg/mL for antibiotics and antifungals, respectively. The positive control was either Ciprofloxacin (0.01mg/mL) or Amphotericin B (0.1mg/mL) for bacteria and yeasts, respectively. Three negative controls were used – media control, negative culture control of TSB, solvent control, which consisted of Acetone (32mg/mL) for organic extract testing.

A serial doubling dilution was undertaken after all samples had been plated and the plates then inoculated with the relevant pathogen, ensuring an inoculum of approximately  $1 \times 10^6$  colony forming units (CFU)/mL.

Plates were sealed with a sterile adhesive sealer, and then incubated at 25°C for 48 hours and 37°C for 24 hours for yeasts and bacteria, respectively. After incubation, 40µL of p-iodonitrotetrazolium violet (INT; 0.4mg/mL) was added to each well, microbial growth was indicated by the appearance of purple pink. <sup>[34]</sup> Tea extracts were double diluted in sterile peptone water as a non-inhibitory media in sterile test tubes. Uninoculated peptone water was used as NC (negative control), whereas inoculated peptone water was used as PC (positive control). The standard strain against which the MIC needs to be measured was inoculated in peptone water and incubated for 3 – 4 hours to be in log phase. <sup>[35]</sup>

### d. DETERMINATION OF FRACTIONAL INHIBITORY CONCENTRATION INDEX (FICI)

The Microbroth Checkerboard technique was used to determine the Fractional Inhibitory Concentration Index (FICI). Mixture of teas and antimicrobials in different final concentrations, ranging from 2×MIC to 1/8×MIC was inoculated with fresh cultures into each microtiter well. After incubation at 37°C for 18 – 20 hours, the following formulas were used to calculate the FIC index:

$$FIC_A = \frac{MIC_A \text{ in combination}}{MIC_A \text{ alone}} \quad FIC_B = \frac{MIC_B \text{ in combination}}{MIC_B \text{ alone}}$$

$$FIC \text{ INDEX} = FIC_A + FIC_B$$

A FICI of  $\leq 0.5$  was considered synergistic, FICI of  $> 0.5-4$  was considered to be additive, and FICI of  $> 4.0$  was considered to be antagonistic. <sup>[33]</sup>

Combinations of aqueous and organic extracts with the conventional antimicrobial were further assessed using the sum of the fractional inhibitory concentration ( $\Sigma$ FIC), which would allow the classification of the type of interaction occurring. <sup>[34]</sup>

### e. DISC DIFFUSION METHOD

Strains were tested against doubling dilution of the extract prepared by percolation, along with the positive and negative controls. Similar double dilutions were also prepared of the infusion extract. The Mueller-Hinton Agar (MHA) plates were lawn cultured with the standard strains after matching the broth prepared with the 0.5-McFarland (MF)

turbidity standard. A total of 6 wells were cut of 2mm and filled with the 20 $\mu$ L of serially double-diluted green leaf extract prepared in deionized water.

The disk of primary drug was placed as positive control (PC). A well with a sterile deionized water served as negative control (NC). The plates were incubated at 37°C for 18 – 24 hours and were looked for the zone of inhibition (ZOI).<sup>[35, 33]</sup>

Infusion was concentrated to a fifth of the volume on a rotary evaporator. The prepared samples were filtered through 0.45mm membranes and kept in small sterile bottles and stored at -20°C prior to analysis. All microorganisms were grown at 30°C for 18 hours in nutrient broth, except Lactobacillus which was grown in MRS for 48 hours.

Suspensions of microorganisms, adjusted to 4 – 5 log CFU/mL, were placed in flasks containing 5mL of sterile nutrient or MRS agar at 45 – 46°C. The mix was poured into Petri plates with 10mL of basal medium. When the agar was solidified, sterile discs (6mm) were inserted into the plates.

Finally, 50 $\mu$ L of solution of each extract were transferred to the discs. The plates were incubated at 30°C for 48 hours and read at 8, 24 and 48 hours. At the end of the incubation period, inhibition zones formed in the medium were measured.<sup>[31]</sup>

Lyophilized tea extracts were used to perform the Time Kill Assays. Time Kill Kinetic Assay is used to study the antimicrobial activity against a microbial strain. The lyophilized tea extracts were divided into two sets – one set was autoclaved, and one set was filtered, and both these sets were tested. There will be a difference in both the results as the filtered extracts did not undergo any kind of treatment and might not have higher antimicrobial activities. The result of the autoclaved extract cannot be predicted as it may have higher antimicrobial activities as it has been subjected to heat which may enhance properties, or the heat might have reduced the properties leading to lower antimicrobial activities.

Polyphenols such as Anthocyanin have antioxidant as well as antimicrobial properties. They can inhibit microbial growth by denaturing proteins and disrupting cell membranes of the microbes thereby leading to apoptosis. pH differential method is used to assess the anthocyanin content. It is based on the assumption that pure anthocyanins have little or no absorbance in pH 4.5 buffer, and that polymeric or degraded anthocyanins will have an absorbance at this pH. Phenolic content was determined by Folin – Ciocalteu's test.

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antibiotic that inhibits the growth of a given strain of microbe. A lower MIC value implies that less drug is necessary for inhibiting growth of the organism; therefore, drugs with lower MIC scores are more efficient antimicrobial agents.

Synergy measurement by Checkerboard Analysis is used to determine the impact on potency of the combination of antibiotics in comparison to their individual activities. This comparison is represented as the Fractional Inhibitory Concentration (FIC) index value. The FIC index value represents the combination of antibiotics that produces the greatest change from the individual antibiotic's MIC.

- Synergy: When the combination of compounds results in an FIC value of <0.5, then the combination of the compounds increases the inhibitory activity (decrease in MIC).
- Additive: When the combination of compounds results in an FIC value of 0.5 – 4, the combination has no increase in inhibitory activity or a slight increase in inhibitory activity from the additive effect of both compounds combined.
- Antagonism: When the combination of compounds results in an FIC value of >4, the combination of compounds increases the MIC, or lowers the activity of the compounds.<sup>[37]</sup>

The Disk-Diffusion Agar method tests the effectiveness of antibiotics on a specific microorganism. The extracts were either percolated or filtered. The microbes are lawn cultured onto the media plates and the antibiotic discs are inserted. The plates are incubated, and the zone of inhibition is observed. Difference in results is not expected as the methods are very similar.

## IV. RESULTS

### 1. TOTAL FLAVONOID CONTENT

After 5 minutes of steeping, the highest flavonoid content estimated by Chloride Assay was seen in green tea (1640 $\mu$ g QE/g), black tea (1480 $\mu$ g QE/g) was next, followed by rooibos (183 $\mu$ g QE/g) and hibiscus tea (87.17 $\mu$ g QE/g). The final values are expressed as grams of Quercetin equivalent/gram of dry tea leaves.

However, the teas that were used cannot be subjected to a direct comparison as parameters like geographic locations, harvesting time, and environmental conditions of the test samples have an impact on flavonoid content.<sup>[7]</sup>





**2. TOTAL POLYPHENOL CONTENT**

Theoretically the health benefits that are related with the consumption of tea are because of the presence of polyphenols, so having a greater quantity would be beneficial and would support the supposition of steeping tea longer to extract more polyphenols.

The phenol content assayed with Folin – Ciocalteu’s method showed a rise with increase in steep time in one-minute intervals. The phenol content after 10 minutes of steeping was greater than five minutes of steeping. The high polyphenol content was seen in Green tea (1.17mg GAE/g) which was succeeded by black tea (0.84mg GAE/g), rooibos tea (0.746mg GAE/g) and hibiscus tea (0.65mg GAE/g). The whole phenol content is expressed as mg Gallic acid/gram of tea. [7]

**TABLE 1: Flavonoid and Phenol Contents in Teas**

| TEA SAMPLE   | TOTAL FLAVONOID CONTENT | TOTAL PHENOL CONTENT |
|--------------|-------------------------|----------------------|
| Green tea    | 1640µg QE/g             | 1.17mg GAE/g         |
| Black tea    | 1480µg QE/g             | 0.84mg GAE/g         |
| Rooibos tea  | 183µg QE/g              | 0.746mg GAE/g        |
| Hibiscus tea | 87.17µg QE/g            | 0.65mg GAE/g         |

**3. ANTIOXIDANT ACTIVITY**

**a. DPPH ASSAY**

Green tea showed (12390µg AAE/g) the highest DPPH Radical Scavenging Activity followed by rooibos tea (9890µg AAE/g), black tea (1900µg AAE/g) and then hibiscus tea (639µg AAE/g). [7] A number of the flavones in rooibos tea undergo cyclization which contribute to the higher scavenging of DPPH radicals even though it has a lower flavonoid content in comparison to black tea. [38] The results are expressed as Vitamin C equivalents. [7]

**b. BCB METHOD**

BCB method is usually used to evaluate the presence of antioxidant compounds in emulsions, following the β-Carotene/Linoleic Acid Oxidation. Antioxidant activity of herbal infusions were determined by BCB using BHT and α-tocopherol as controls and percentage inhibitions were calculated. Statistically, BHT presented the highest antioxidant activity (81.8%), followed by the infusions of black tea (80%), green tea (72%), rooibos tea (69%) and hibiscus tea (61.7%). [36]

**c. ABTS RADICAL ASSAY**

The Radical-Scavenging Activity of the infusions was also analyzed by the 2, 20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay and the results are expresses as TEAC values. TEAC value is the Trolox equivalent capacity (mmol of Trolox per liter of infusion) determined by the ABTS method. The highest TEAC value was found in green tea (6344 ± 72.8) and the rest of the order was as follows black tea (3771 ± 21.2), rooibos tea (746 ± 12.9), hibiscus tea (383 ± 69.4). [31]

**d. FRAP ASSAY**

After five minutes of infusion green tea (5250µg TE/g) showed the highest ferric reducing power followed by black tea (2077µg TE/g), rooibos tea (630µg TE/g) and hibiscus tea (124µg TE/g). It was noticed that identical samples gave different results when subjected to different brewing conditions. Tea bags were infused at 90°C for time periods from 0.25 to 15 minutes, and green tea had an antioxidant activity almost 3 times of black tea. The results are expressed as Trolox/gram of tea. [7]

TABLE 2: Antioxidant Properties of Teas

| TEA SAMPLE   | DPPH ASSAY           | BCB METHOD | ABTSRADICAL ASSAY | FRAP ASSAY        |
|--------------|----------------------|------------|-------------------|-------------------|
| Green tea    | 12,390 $\mu$ g AAE/g | 72%        | 6344 $\pm$ 72.8   | 5250 $\mu$ g TE/g |
| Black tea    | 1900 $\mu$ g AAE/g   | 80%        | 3771 $\pm$ 21.2   | 2077 $\mu$ g TE/g |
| Rooibos tea  | 9890 $\mu$ g AAE/g   | 69%        | 746 $\pm$ 12.9    | 630 $\mu$ g TE/ g |
| Hibiscus tea | 639 $\mu$ g AAE/g    | 61.7%      | 383 $\pm$ 69.4    | 124 $\mu$ g TE/g  |

#### 4. ANTIMICROBIAL ACTIVITY

##### a. TIME KILL ASSAYS

Hibiscus extracts demonstrated antimicrobial activity against *E. coli* and *S. aureus* strains. *E. coli* strains had an increased lag phase within 3 hours in filtered extracts at 20mg/mL. There was growth inhibition observed in filtered extracts at 40mg/mL and these strains were inactivated to below the level of detection after 24 hours in 60mg/mL filtered extracts. At 20mg/mL, autoclaved extracts were more effective than filtered extracts against *E. coli* strains.

At 20mg/mL, population of strain Cider was reduced. At 40mg/mL, autoclaved extracts, inhibited Cider similar to filtered extract. At 60mg/mL, autoclaved extracts were similarly effective at inactivating both strains of *E. coli* after 24 hours and strain Cider after 6 hours.

Lower concentrations of filtered and autoclaved extracts inactivated *S. aureus*. At 2.5mg/mL and 20mg/mL, filtered extracts caused an increase in the lag phase of *S. aureus*, but the 20mg/mL concentration showed bacteriostatic activity after 24 hours.

At 20mg/mL, 40mg/mL for filtered extracts and at 2.5mg/mL for autoclaved extracts, *S. aureus* strain populations reduced after 24 hours. Inactivation of *S. aureus* to undetectable levels was observed after 9 hours when grown at 35 – 37°C at 40mg/mL in autoclaved extracts. [32]

##### b. ANTHOCYANIN AND PHENOLIC CONTENT

Lyophilized extract from 100g of whole dried calyx yielded 1.78 to 2.14g. Phenolic content ranged from 48.7 to 53.8mg GAE/g. Monomeric anthocyanin content ranged from 2.63 to 14.27mg of cyanidin-3-glucoside equivalents per liter. In case of the crude extracts and phenolic amounts, the filtered treatment had a lower value whereas the lower value for anthocyanins was the autoclaved treatment. 52% was the polymerized color of the extracts in the filtered treatment and 70% for the autoclaved treatment. [32]

##### c. MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAYS

In this method, Conventional antimicrobials were tested individually as well as in combination with microbial strains, to evaluate the antimicrobial activity of *A. linearis* aqueous and organic extracts. The antimicrobial activities of the conventional microbes are already known.

The MIC values obtained were compatible with the break point expectations (derived from published antimicrobial analysis of samples against various pathogens); with the exception of tetracycline against *E. faecalis* and *P. aeruginosa*, where these two strains exhibited an enhanced vulnerability towards tetracycline.

There was no noteworthy antimicrobial activity observed in organic as well as aqueous extracts when *A. linearis* was accessed individually. Both the aqueous and the organic extracts displayed the best antimicrobial activity against *E. coli* (MIC value of 1.50mg/mL). [34]

The aqueous extract of the green tea was evaluated against the standard strains of *E. coli*, *S. aureus*, and *P. aeruginosa* to determine its Minimum Inhibitory Concentration.

The infusion extract failed to show any activity against any of the standard strain tested, so no further MIC evaluation was done for the same.

The activity was seen only with percolated extract in *P. aeruginosa* (PA), so this strain was tested further for MIC. Strain PA showed MIC of 12.5 $\mu$ g/mL with no growth and visible turbidity in higher concentrations.

The concentration ranges from 12.5 to 3.12 $\mu$ g/mL was then subcultured on brain–heart infusion agar, and no growth was observed in 12.5 $\mu$ g/mL, whereas growth of PA was seen when subcultured from 6.25 to 3.12 $\mu$ g/mL. [35]



**Figure 1 – MIC of Green Tea Extract against *P. aeruginosa* showing MIC value of 12.5 $\mu$ g/mL which shows no turbidity whereas 6.25 $\mu$ g/mL showing the turbidity with Positive control at left end and Negative (un-inoculated) control at right end.**

#### d. DETERMINATION OF FRACTIONAL INHIBITORY CONCENTRATION INDEX (FICI)

A total of 56 combinations were evaluated and  $\Sigma$ FICI calculations recorded for the Gram-positive, Gram-negative bacteria and yeasts. 19.64% of these combinations were synergistic, 7.14% were antagonistic, 19.64% were additive and 53.57% were indifferent in nature. The most synergistic interactions were observed against the Gram-positive bacteria, when *A. linearis* along with Penicillin G, was most synergistic against *S. aureus*. Antagonistic interactions were minimal, with only 4 antagonistic  $\Sigma$ FICI values identified when *A. linearis* was combined with Ciprofloxacin, Gentamicin, and Amphotericin B. <sup>[34]</sup>

Microbroth Checkerboard Assay was used to determine the Fractional Inhibitory Concentration Index. FICI of  $\leq 0.5$  was considered as borderline. Synergistic interactions were observed between Ampicillin and almost all tea combinations against MSSA followed by some Ampicillin-sulbactam combinations against *E. coli*, or *A. baumannii* clinical strain, or Nystatin and black tea and green tea combinations against *C. albicans*.

Moreover, with a FICI of  $> 4$ , antagonistic effects were observed particularly between rosehip, pomegranate, or rosehip bag teas, and Ciprofloxacin against all tested strains followed by Cefuroxime against *E. coli*, *K. pneumoniae* and *P. aeruginosa*, Erythromycin, Ampicillin-Sulbactam, Amikacin, or Doxycycline against each one or two strains. There were no antagonist interactions between the teas and the antifungals tested. <sup>[33]</sup>

#### e. DISC DIFFUSION METHOD

Of the 31 teas, only 15 teas showed inhibition zones against one or more microorganisms in the disk diffusion assays. The clinical isolates were more sensitive to teas than the standard strains. Rosehip, rosehip bag, and pomegranate blossom were the most effective teas against bacteria. Thyme, wormwood, mint, black, and green teas were highly effective against *S. aureus*. Additionally, Echinacea bag and Cinnamon teas were active against the clinical strains of *S. aureus* and *C. albicans*. <sup>[33]</sup>

The inhibition zones formed depend on the strain, the kind of extract and the concentration of extract. Strain *B. cereus* is most sensitive, showing the largest inhibition diameter in the presence of the tea extracts. Extracts from all the teas had an inhibitory effect against this strain. The second most sensitive strain was *M. luteus*, followed by *P. aeruginosa*. In contrast, there was poor inhibition of *E. coli* observed for few extracts (white, green tea). <sup>[31]</sup>

The green tea aqueous extract was assessed against the standard strains of *E. coli*, *S. aureus*, and *P. aeruginosa*. There was no activity observed against any concentration for *E. coli* and *S. aureus* with no Zone of Inhibition (ZoI) on MHA, whereas *P. aeruginosa* showed good activity with ZoI ranging from 16 to 9mm for 200 – 12.5 $\mu$ g/mL, whereas no activity was seen for 6.25 $\mu$ g/mL. <sup>[35]</sup>

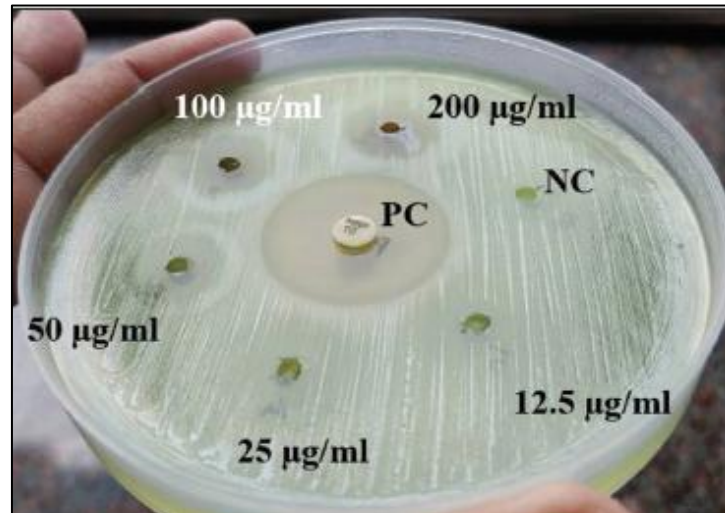


Figure 2 – Zone of Inhibition for *P. aeruginosa*

The Time Kill Assay performed on Hibiscus extracts depicted that autoclaved extracts were more effective against *E. coli* and *S. aureus* than filtered extracts. This may be because autoclaving may have produced additional compounds in the extracts that were more effective against these pathogens. [39] The rate of growth inhibition was higher in autoclaved extracts than filtered extracts. Cider strain was considered as a control. It was observed that the growth of *S. aureus* was inhibited at very low concentrations whereas *E. coli* was inhibited in a comparatively higher concentration. Polyphenols were said to have antimicrobial properties. To test this, the anthocyanin and phenolic content was measured in Hibiscus extracts (autoclaved and filtered). Cyanidin-3-glucoside is an anthocyanin therefore, the anthocyanin content is measured in milligrams of cyanidin-3-glucoside equivalents per liter.

This method is based on the fact that higher the polyphenol content, the higher is the antimicrobial activity. This along with the previous method – Time kill assay concluded that autoclaved extracts have a higher antimicrobial activity than filtered extracts because autoclaved extracts had a higher anthocyanin content than filtered extracts.

Conventional antimicrobials were used as control as their MIC is already known. The Rooibos extracts were tested individually as well as in combination with the conventional antimicrobials. There was no noteworthy antimicrobial activity observed in organic as well as aqueous extracts when *A. linearis* was accessed individually. But the best antimicrobial activity was observed against *E. coli* by both aqueous and the organic extracts.

Generally, organic extracts show better antimicrobial activity than aqueous extracts, but this study shows that they had a very insignificant difference. This might be because Rooibos tea is more commonly known for its antimutagenic and antioxidant activity, rather than for its antimicrobial activity. This study has indicated that rooibos tea when taken in combination with conventional antimicrobials results in interactions that are largely indifferent and additive in nature.

In the case of aqueous green tea extracts, *E. coli*, *S. aureus*, and *P. aeruginosa* were used to determine the MIC. Percolated extract in *P. aeruginosa* was the only strain to show activity because the total phenolic contents, especially EGCG, exerts antimicrobial activity on *P. aeruginosa*.

Rooibos extracts were evaluated for Fractional inhibitory concentration assessment against Gram-positive, Gram-negative bacteria and yeasts. EGEG is a recurring compound found in many teas, which has a synergistic effect with the  $\beta$ -lactam antibiotics. When *A. linearis* was combined with Penicillin G, the combination was most synergistic against *S. aureus* maybe because one of the constituents of the extracts could have potentiated the uptake and antimicrobial effect of the antibiotic. The studied teas showed a better antimicrobial activity against gram-positive bacteria compared with the others.

The antimicrobial activities of various herbal teas, alone and in combination with antibiotics, showed that herbal teas have antimicrobial activities against gram-positive and gram-negative bacteria and yeast when they were used alone. The combinations of herbal teas with antibiotics demonstrated synergistic, additive, or antagonistic effects, depending on the antibiotic or type of tea.

Disk diffusion is performed as it is a very simple, feasible test to figure out which tea extract has antimicrobial properties or has the ability to inhibit microbial growth. This is observed by the formation of the Zone of Inhibition (ZoI). In most of the cases, the ZoI was observed except in the case of aqueous green tea extract.

There was no activity observed against any concentration for *E. coli* and *S. aureus* with no Zone of Inhibition (ZoI), whereas *P. aeruginosa* showed good activity with ZoI. Rooibos infusion had a low antimicrobial activity against



*B. cereus* as it does not contain catechins, but it contains various other compounds (Aspalathin, Isoorientin, Orientin and Rutin) that could contribute to the antioxidant and antimicrobial activities. It was also observed that the clinical isolates were more sensitive to tea extracts than the standard strains

## V. DISCUSSION

Several products claiming to have antioxidant properties are marketed in the stores. This study helped us evaluate the properties promised to us by companies. A general trend was observed with the results with green tea having the highest flavonoid and phenolics content it also showed the highest antioxidant activity in DPPH assay, ABTS radical assay and FRAP assay which can also be due to the natural presence of Ascorbic acid in green tea. Black tea showed a higher antioxidant activity than green tea in case of BCB method and a lower activity than rooibos tea in DPPH method. Hibiscus tea showed lowest antioxidant activity in all the four assays and had the lowest flavonoid and phenolics content. This is common with floral teas.

Since the type of solvent used heavily affects the flavonoid and polyphenol extraction from a plant material the choice between aqueous and alcoholic extracts has to be made at the very beginning. While alcohol does extract more carotenoids, water extracts more polyphenols. This is because most of the polyphenols are polar and so are better soluble in water hence there is a better extraction in shorter time which is better suited for the present study.<sup>[9]</sup>

DPPH is an easy, valid, economically feasible method to evaluate the radical scavenging properties of antioxidants found in food. The results are highly reproducible and can be compared to other methods, but it is important to note that the absorbance produced by a DPPH assay is decreased by exposure to light, oxygen and depends on the pH and type of solvent used and its absorbance reduces in methanol.<sup>[40, 41]</sup>

BCB method has been one of the oldest methods for measuring antioxidant activity but the lack of standardization makes it difficult to have reproducible results. As the system used for antioxidant activity assay by the  $\beta$ -carotene method contains  $\beta$ -carotene/Linoleic Acid water emulsion, and so the type of solution the sample is made in influences the solubility of the emulsion, which in turn influences the reaction kinetics. Also, the presence of natural acidic components in the plant extracts heavily influence the assay.

Normally the antioxidant will react with peroxy radicals and form hydroperoxides, but in the presence of metal ions (which are common in plant extracts) the hydroperoxides are converted back to peroxy radicals.<sup>[42]</sup>

DPPH and ABTS assays are based on electron and H atom transfer, while the FRAP assay is based on electron transfer reaction.<sup>[43]</sup> ABTS and DPPH suffer from the difficulties related to the formation and stability of the colored compounds whose absorbance needs to be measured and if the initial sample is colored then it could interfere with results. In case of FRAP there is a gradual increase in absorbance with time in case of some flavonoids that could provide differences in results depending on if there is a delay between the incubation and measurement of absorbance.<sup>[41]</sup>

In Time Kill Assay, the effects of autoclaving Hibiscus extracts on their antimicrobial activity and phenolic and anthocyanin content were examined. Autoclaved extracts were more effective against both *E. coli* and *S. aureus* than filtered extracts in a microbiological medium.

Autoclaving may have produced smaller or additional compounds in the extracts that were more effective against these pathogens. It was found that heating *H. sabdariffa* at 60°C and 100°C for 60 minutes did not affect the antimicrobial activity of the Hibiscus extract.<sup>[39]</sup>

In another study, *H. sabdariffa* extract was heated to 75°C and 100°C against *S. aureus*, *E. coli* and other microbes, it was found that the antimicrobial activity was significantly reduced in aqueous extracts whereas if ethanol extracts were heated to 50°C and 75°C, the antimicrobial activity was unaffected.<sup>[44]</sup> But in this study, autoclaved extracts showed enhanced antimicrobial activity as compared to filtered extracts may be because in this study the extracts were autoclaved following the entire extraction process while other studies heated the extracts prior to concentrating them.<sup>[32]</sup>

Dried calyces of *H. sabdariffa* are rich in both anthocyanins and phenolic compounds. Autoclaving did not affect total phenolic content but, it caused a significant degradation of anthocyanins as they are not heat stable. Anthocyanins in heat-treated Hibiscus beverages degrade over time during storage.<sup>[45]</sup> In this study, the anthocyanin content of the filtered extract (14.27mg of cyanidin-3-glucoside equivalents per liter) is lesser than that shown in other published

studies. Anthocyanins in autoclaved extracts forms complexes with other compounds such as tannins which give a polymeric color. This showed that it had approximately 20% more polymerization than the filtered extract. In this study, the extract was dialyzed and lyophilized, which could have concentrated the phenolic compounds. <sup>[32]</sup>

Minimum Inhibitory Concentration (MIC) Assays for both the aqueous and organic extracts displayed the best antimicrobial activity against *E. coli* with an MIC value of 1.50mg/mL. <sup>[34]</sup>

In this study, the antimicrobial activity of green tea extract on *E. coli*, *S. aureus* and *P. aeruginosa* was tested. According to the study by Jazani et al., the MIC of the water-soluble green tea extract against all strains of *P. aeruginosa* (of which 55.8% were MDR strains) was lower than this study. <sup>[49]</sup> Studies conducted by Tiwari et al., 2005 and Lee et al., 2003 implied that green tea extracts showed activity against MDR *P. aeruginosa*, while the activity was also demonstrated against MRSA and methicillin-sensitive *S. aureus*. <sup>[50-52]</sup>

Chan et al., evaluated various green, black, and herbal tea extracts against three Gram-positive organisms, namely *Micrococcus luteus*, *S. aureus*, and *Bacillus cereus*, where green tea inhibited all the three Gram-positive bacteria and three Gram-negative bacteria such as *E. coli*, *Salmonella typhi*, and *P. aeruginosa* and found that none of the tea extracts showed any activity against these. <sup>[53]</sup>

The infusion extract did not show any activity against any of the standard strains tested in the present study. Daljit et al. (2009) reported that the 5% aqueous green tea extract can also inhibit the growth of *P. aeruginosa* because the total phenolic contents, especially EGCG, exert antimicrobial activity on *P. aeruginosa*. <sup>[54, 55]</sup> This variation might be due to different methods of extraction of tea, species of tea, their seasonal variation and collection process, and the process of drying. <sup>[35]</sup>

Rooibos tea is recognized for its antimutagenic and antioxidant activity, rather than antimicrobial activity. Therefore, it was expected that there would be no noteworthy antimicrobial activity demonstrated by *A. linearis*. <sup>[46]</sup> There has been very little research conducted on the antimicrobial properties of rooibos tea. The two noteworthy studies that investigated the antimicrobial activity of extracts of *A. linearis* concluded that rooibos had an inhibitory effect on *E. coli*, *S. aureus* and *B. cereus* growth after 12 hours. <sup>[47, 48]</sup>

When *A. linearis* was combined with Penicillin G, the combination was most synergistic against *S. aureus* as one of the constituents of the extracts could have potentiated the uptake and antimicrobial effect of the antibiotic. Epigallocatechin-3-gallate (EGCG) is a recurring compound found in many teas, which has a synergistic effect with the  $\beta$ -lactam antibiotics, such as Penicillin, and their derivatives, when tested against methicillin-resistant *S. aureus* (MRSA). <sup>[57-59]</sup>

In the current study, Antagonistic interactions were minimal, with only 4 antagonistic  $\Sigma$ FIC values identified when *A. linearis* along with Ciprofloxacin, Gentamicin, and Amphotericin B. Antagonism was not observed against the tested Gram-positive pathogens. In a study by Tiwari et al. (2005), the combined antimicrobial activities of green and black tea extracts with Chloramphenicol, Gentamicin, Methicillin or Nalidixic acid were mostly synergistic against enteropathogens. This indicates that tea drinkers experience an enhanced therapeutic effect of an antimicrobial agent, thereby recovery from an infection may be more rapid. <sup>[50, 34]</sup>

In this study, the antimicrobial activities of 31 different herbal teas, both individually and in combination with chemical antimicrobials were examined. Rosehip, rosehip bag, pomegranate blossom, thyme, wormwood, mint, echinacea bag, cinnamon, black, and green teas were observed to be effective against most of the studied microorganisms.

In general, the studied teas demonstrated a better antimicrobial activity against gram-positive bacteria as compared to others. It was assumed that the differences in the antimicrobial activities of the various teas would depend on either the type of microbial strain or the tea. According to Hu et al. (2001), Ampicillin sulbactam and EGCG combinations were synergistic against MRSA strains. <sup>[60]</sup> Lee et al. (2005) demonstrated that Ciprofloxacin and catechin combinations were synergistic against *E. coli*. <sup>[61]</sup> Although Ampicillin or Nystatin combinations were synergistic, most of the Ciprofloxacin, Erythromycin, Cefuroxime, or Amikacin and various tea combinations, especially with the rosehip, rosehip bag, and pomegranate blossom teas, were found to be antagonistic against several studied bacteria. Similarly, Hu et al. (2002) observed that EGCG showed antagonistic interactions with Vancomycin, Teicoplanin, Or Polymyxin B against MRSA. <sup>[62, 33]</sup>

In this study, the antimicrobial activities of 31 different herbal teas, both individually and in combination with antimicrobials were examined and these showed that herbal teas have antimicrobial activities against gram-positive and



gram-negative bacteria and yeast when they were used alone. The combinations of herbal teas with antibiotics showed synergistic, additive, or antagonistic effects, depending on the antibiotic or type of tea.<sup>[33]</sup>

The tea extracts inhibited the food-borne bacteria but not the intestinal bacteria studied. The yeast *C. albicans* was inhibited by extracts from green and white tea and from some commercial teas. This yeast was more resistant than the strains of *Bacillus*, *Micrococcus* and *Pseudomonas* assayed.<sup>[31]</sup>

In this study, the antimicrobial activity of green tea extract on *E. coli*, *S. aureus*, and *P. aeruginosa* was evaluated by disk diffusion method. There was no activity observed against any concentration for *E. coli* and *S. aureus* with no ZoI on MHA, whereas *P. aeruginosa* showed good activity.

From a total of 30 isolates, 21 isolates showed an enhancement in average increase in the zone size of 4.80mm. In a study conducted by Maksum Radji et al., in 2013, the zone size inhibition with green tea extract was found to be  $18.970 \pm 0.287$  and  $19.130 \pm 0.250$  for strains of *S. aureus*, whereas for strain of *P. aeruginosa* and multidrug-resistant (MDR) *P. aeruginosa*, it was found to be  $17.550 \pm 0.393$  and  $17.670 \pm 0.398$ , respectively.<sup>[63, 35]</sup>

## VI. CONCLUSION

The consumption of beverages with antioxidant and antimicrobial properties has become a huge consumer attraction. The analysis of the teas proved that it has a high antioxidant content and can be of great benefit to the human health if consumed in diet. There is a noticeable relationship between the phenolics content and the oxidant inhibition and radical scavenging property of the teas, meaning these properties are mainly because of the polyphenols.

A raising issue in the field of medicine is the presence of Multidrug Resistant (MDR) Strains which makes it difficult to eliminate microbes as they are becoming resistant to wide range of antibiotics. The analysis of teas proved that it also has a higher antimicrobial property which can neutralize this issue. It was found that autoclaved tea extracts showed higher antimicrobial content than filter sterilized tea extracts.

Normally, teas are rich in polyphenols which facilitate the increase in antimicrobial activity which was proved by the MIC and FICI methods. The antimicrobial property was also assessed by the Disk Diffusion method by observing the inhibition zones.

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