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Assessment of the Antimicrobial and Antioxidant Activities of Green Tea and Black Tea

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Tea and coffee have various biological properties including antimicrobial activity against human pathogens. Green tea and black tea were collected, and extracts were obtained using different solvents. These extracts were tested for their antimicrobial activity against human test pathogens using well-diffusion and disc-diffusion methods. The extracts were then subjected to partial purification by thin layer chromatography (TLC), column chromatography, and HPLC, and screened for the presence of active phytochemical compounds. The results demonstrated that the antimicrobial and antioxidant properties of the extracts from green tea performed well in all attributes, followed by black tea. The good performance of the tea extracts was primarily the result of their high polyphenol concentrations, which are present as a series of chemicals called catechins, including gallocatechin (GC), epigallocatechin (EGC), epicatechin (EC), and epigallocatechin gallate (EGCG). In conclusion, the present work, the methanolic extracts of green tea showed greater antioxidant activity compared to black tea. These purified compounds showed broad antimicrobial and antioxidant activity against all tested human pathogens and are worthy of further study.

Key words: Green tea, Black tea, Microbial pathogens, Antimicrobial activity, HPLC, Antioxidant activity, Phytochemical compounds.

The increasing occurrence of antibiotic resistance in microbial strains has led to renewed interest in traditional treatments for infections including the use of herbal medicine. Spices and herbs are generally used in foodstuffs to enhance the flavor or color attributes of food. Moreover, these materials have antimicrobial and antioxidant activities[1]. Today, tea is the most popular beverage worldwide, second only to water, and the per capita worldwide consumption is approximately 40 liters per year. Approximately 3 billion kilograms of tea are produced and consumed annually, and this value is growing at a rate of 2.1 percent per year.

Tea is typically made from the *Camellia sinensis* plant, which is the source of all non-herbal teas. Commercial teas come in three major categories: unfermented green fully fermented black and semi-fermented oolong. Most of the tea consumed in the world is black tea, with green tea accounting for 20 percent. Tea contains more than 4,000 chemical compounds that may affect the human body, but most attention has been focused on tea's polyphenolic compounds. Both black and green teas are derived from the same source, but the leaves that eventually become black tea are first broken up and exposed to air. This process promotes oxidation and deactivation of the valuable polyphenols. Tea is rich in polyphenolic compounds, which are bonded benzene rings with multiple hydroxyl groups. Polyphenols are classified by structure into flavonoids and non-flavonoids, and the polyphenols in tea are primarily flavonoids. Tea polyphenols are strong antioxidants.

The three major types of commercially produced coffee beans (*Coffea* spp.) are *Arabica,*

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Robusta, and *Liberia. Coffee's* bioactive profile contains many of the most important constituents known to exist within functional foods, including flavonoids (catechins and anthocyanins), caffeic acid, and ferrulic acid. In the human diet, coffee is the major source of caffeoyl-quinic acids, which are also known to be powerful antioxidants.

Both tea and coffee have various biological properties including antimicrobial activity showed that the moderate daily consumption of green tea killed *Staphylococcus aureus* and other harmful bacteria[2]. Subsequently, the antibacterial activity of Turkish tea against infection by *Vibrio cholerae* O1 has also been reported[3].

Few studies on the antimicrobial activity of coffee-based solutions are found in the literature. Toda et al. reported the effects of coffee on microbial species such as *S. aureus, Salmonella typhi A, Shigella dysenteriae, Vibrio cholerae,* and *Yersinia enterocolitica* and attributed its antibacterial effects to tannic acid[2]. The present study was therefore initiated with the purpose of studying the antimicrobial and antioxidant activity of two important beverages, green tea & black tea.

MATERIALS AND METHODS

Plant collection

For this study, black tea (broken Orange Pekoe) and green tea (silver tips) were collected from the United Planters Association of Southern India (UPASI), Coonoor.

Sample pretreatment

The leaves and seeds were excised and subjected to a three-step surface sterilization procedure consisting of a 60-second wash in 99% ethanol, a 6-minute wash in a 1% sodium hypochlorite solution, a 30-second wash in 99% ethanol, and a final rinse in sterile water[4].

Disc preparation

The solvent extracts were used in a disc-diffusion assay to test for antimicrobial activity. The discs were prepared using Whatman filter paper No.1; 0.005 ml of extract was added to each disc, and the discs were then dried[5].

Bacterial and fungal test strains

The test pathogens used in this study included *S. aureus* (Methicillin-Resistant *S. aureus* -MRSA), (Vancomycin-Resistant *S. aureus* - VRSA), *Bacillus subtilis, Escherichia coli, Salmonella paratyphi A, Shigella dysenteriae, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio* spp. and *Candida albicans.*

Preparation of aqueous extracts

The aqueous extracts of green tea and black tea were prepared using the method described[6]. Approximately 2 gm of each sample was crushed in 1 ml of distilled water. The extract was filtered using Whatman filter paper No. 1. The filtrates were collected in sterile vials, and antimicrobial susceptibility tests were conducted using both well-diffusion and disc-diffusion methods.

Extraction of active compounds using solvents

Solvent extracts of green tea and black tea were prepared using different solvents such as n-hexane, di-chloromethane, chloroform, ethyl acetate, acetone, methanol, and water.

Partial purification of selected solvent extracts

Based on the determined antibacterial and antifungal activity, several potential solvent extracts of green tea and black tea were selected for further study using thin-layer and column chromatography.

Bioautography

A bioautography procedure was used for the detection of active compounds separated by TLC. Chromatograms were developed as described above and were placed in a sterile bioassay Petri dish and overlaid with 10 ml of molten nutrient agar seeded with 0.2 ml of *E. coli* as a test organism. Plates were incubated at 37°C for 24 hours (overnight). Reference chromatograms were also prepared. The Rf of the inhibition zones on each test chromatogram was compared with the Rf of the reference chromatogram. The corresponding spots demonstrating antibacterial activity were scraped, collected, and used for further studies.

UV-Vis spectral analysis of active fractions

UV-Vis spectral analysis was performed using the procedure described [7]. Briefly, 1 ml of each active fraction was placed in a quartz cuvette and used for multi-wavelength scanning from 190 nm to 900 nm in a UV-Vis spectrophotometer (Shimadzu).

High-performance liquid chromatography (HPLC) analysis

The suspected polyphenolic compounds

present in methanol extracts of green tea and black tea were analyzed using HPLC[8]. Samples were analyzed at the UPASI Tea Research Institute. The HPLC system consisted of binary pumps combined with a diode array detector and a high-pressure gradient mixer, an automatic injector with a 20-µl sample loop and a C 18 150 × 2.1 mm column (serial number: 00G-4257-E0 Luna 5 µm phenyl hexyl). The column pressure was maintained at approximately 400 bars. The sensitive ranges were observed between 190 nm and 400 nm. Analytical HPLC was performed, and the results were tabulated.

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extracts

The antioxidant activities of the plant extracts and the standard were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity using a modified method. Diluted working solutions of each of the extracts were prepared in methanol. Ascorbic acid was used as a standard in 1-100 µl/ml solutions. DPPH (0.002%) was prepared in methanol, and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in the dark for 30 minutes, and the optical density was then measured at 517 nm using a Cecil-Elect spectrophotometer. Methanol (1 ml) containing the DPPH solution (0.002%) was used as a blank. The optical density was recorded, and the % inhibition was calculated using the following formula:

Percent (%) inhibition of DPPH activity = (A-B)/ (A*100), where A is the optical density of the blank and B is the optical density of the sample.

Phytochemical screening of active compounds

Chemical tests were conducted using the aqueous extracts from plants and/or the powdered specimens using standard procedures to identify the constituents, as described[9]. Tests for alkaloids, carbohydrates, phytosterols, phenolic compounds, and tannins were performed.

RESULTS

Determination of the antimicrobial activity of aqueous extracts

The aqueous green tea extract showed a maximum 14 mm inhibition zone against *Salmonella paratyphi A* and *E. coli*, an 18 mm zone of inhibition against *Shigella dysenteriae*, and a 12 mm inhibition zone against *Pseudomonas aeruginosa*. The aqueous black tea extract showed a 10 mm inhibition zone against *E. coli, Bacillus subtilis,* and *Pseudomonas aeruginosa.*

Determination of the antimicrobial activity of solvent extracts

Varied resistance to the solvent extracts was observed among the test pathogens. *Proteus vulgaris* and *Vibrio* spp. showed resistance to green tea extracts in methanol solvent (Fig. 1). *Bacillus* spp., *Pseudomonas aeruginosa*, and *Klebsiella* spp. showed resistance to green tea extracts in acetone solvent (Fig. 2). *E. coli, Bacillus* spp., and *Pseudomonas aeruginosa* showed resistance to green tea extracts, whereas *S. aureus* showed resistance to both green tea and black tea extracts in ethyl acetate solvent (Fig. 3).

TLC analysis of selected solvent extracts

Methanolic solvent extracts showed good results. Therefore, the methanolic extracts were partially purified from green tea and black tea. The compounds were separated, and the Rf values were calculated. All spots had an RF value of 0.768.

Column chromatography of selected solvent extracts

In total, 30 fractions were collected for each methanolic extract. Each fraction was further assessed for antimicrobial activity using the disc-diffusion method. Of the 30 fractions obtained, fractions 11 through 17 showed antimicrobial activity against *E. coli* on MHA plates (Fig. 4).

UV-Vis spectral analysis of active fractions

Methanolic extracts from green tea and black tea were analyzed using UV-V is spectral analysis, and various peaks were observed.

Table 1. HPLC analytical report of methanolic extracts from green tea and black tea

Sample	Catechins	Area	Conc./ml
GT	EC	546.598	1.546
	EGCG	140.338	0.397
	ECG	258.02	0.73
BT	EGCG	973.258	2.752
	ECG	638.79	1.806

GT- Green tea, BT- Black tea, EC-Epicatechin, EGCG-Epigallocatechingalate, EGC-Epigallocatechin.

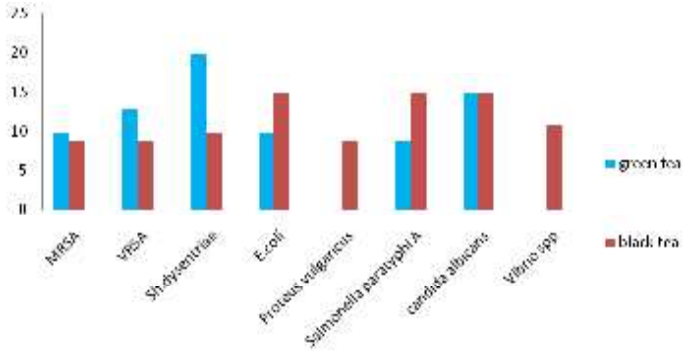

Fig. 1. Antimicrobial activity of methanol extracts of green tea and black tea usingdisc diffusion method.

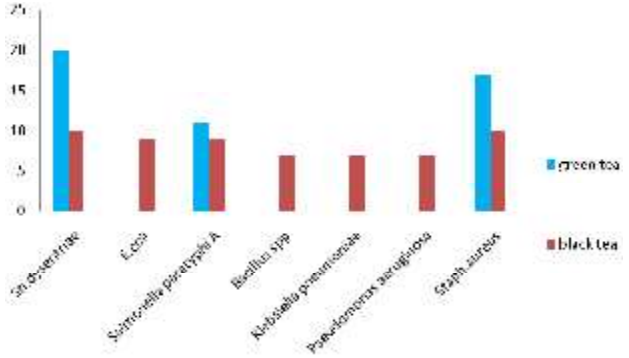

Fig. 2. Antimicrobial activity of acetone extracts of green tea and black tea using disc diffusion method.

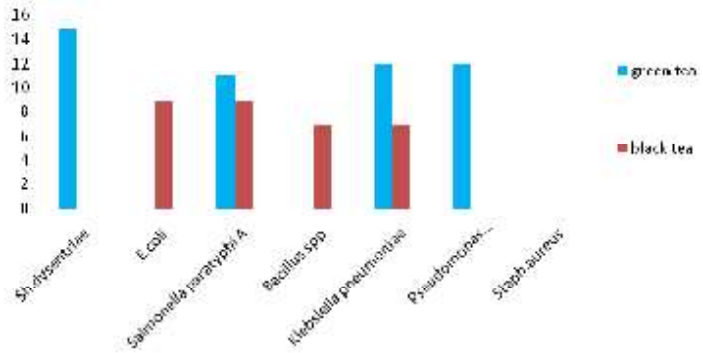

Fig. 3. Antimicrobial activity of ethyl acetate extracts of green tea and black tea using disc diffusion method.

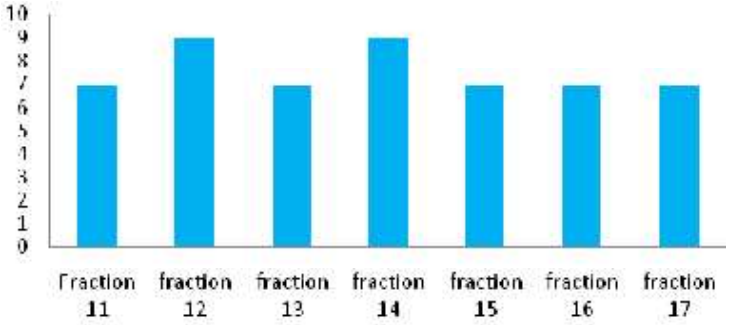

Fig. 4. Column Extract of green tea in MHA plates

HPLC analysis

The methanolic extracts from green tea and black tea were analyzed using analytical HPLC techniques (Table 1).

Detection of antioxidant activity

Periodic optical densities were observed and were compared with the standard, ascorbic acid. Green tea and black tea had antioxidant activity.

Phytochemical analysis of green tea and black tea

Detection of polyphenolic compounds

Both samples tested, green tea and black tea showed positive results for the presence of polyphenolic compounds by forming a dark green color.

Detection of flavonoids

All two samples tested showed yellow color formation, which is a positive result that indicates the presence of flavonoids.

Detection of terpenoids

Layers of reddish-brown color formed in positive samples. Green tea and black tea showed this characteristic change, thus demonstrating the presence of terpenoids.

DISCUSSION

Methanolic green tea extracts showed maximum antibacterial activity with a 20 mm zone of inhibition against *Shigella dysenteriae* followed by black tea extracts. Similar antibacterial activity of tea was recognized 90 years ago[10]. This action is manifested both directly as bacteriostatic and bactericidal effects and indirectly as the inhibition of certain bacterial enzymes[11].

Methanolic green tea extracts inhibited *S. aureus,* methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant *S. aureus* (VRSA) with inhibition zones of 16, 14, and 13 mm, respectively. Water extracts showed antibacterial activity of 14, 10, and 13 mm for *S. aureus*, MRSA, and VRSA, respectively.

Similar supporting evidence was reported in[12], where efficacy was observed between green tea extracts against MRSA. Significant synergy was found for all 40 MRSA strains tested at 40- to 200-fold dilutions of the tea extracts. A similar finding was made by[2], as a cup of tea containing approximately 3 mg of solids per ml inhibited methicillin-resistant *S. aureus.*

The antibacterial effects of catechins primarily include acting on and damaging the bacterial membranes of *S. aureus* and *E. coli.* The antibacterial activity of catechins is predominantly related to the gallic acid moiety and the hydroxyl group member[13]. The mechanism of catechin action involves inducing the rapid leakage of small molecules entrapped in the intraliposomal space and liposomal aggregation.

In a previous study, only water extracts of coffee showed antibacterial activity against *E. coli, Bacillus* spp., *Salmonella paratyphi A, Shigella dysenteriae,* and *Pseudomonas aeruginosa*, with a maximum zone of inhibition of 13 mm, which is similar to the zones, reported by[14]. Methanol extracts from green tea and black tea were analyzed using analytical HPLC to compare their major polyphenolic profiles, particularly catechins, as they are likely responsible for the observed antimicrobial activity. Among which, the obtained peaks were observed in different major of peaks in the green tea at a retention rates of time 6.305,6.793,44.739 respectively to compare to the standard.

In the future, the compounds can be separated and characterized using IR and NMR spectrophotometry. This will allow the chemical nature of the compounds to be determined. Similar results for both green tea and black tea extracts obtained by HPLC, in their study, extract concentrations of 12.5 mg/ ml^{-1} and 25 mg/ ml^{-1} completely inhibited the growth of *Streptococcus pyogenes* after 7 hrs and 3 hrs, respectively[8].

This study demonstrated that the methanolic extracts of green tea showed greater antioxidant activity compared to black tea. The catechins, epicatechins, and epigallocatechins contained within green tea and black tea are all within the flavan-3-ols class. Additionally, the tea extracts contained phenolic compounds.

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