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Research Article

Killing Kinetics/Death Dynamics of Green Tea Extract Using Visible (Vis) Ultra-Violet Spectrophotometric Protocol against Enteric Organisms Isolated from Patient's Fecal Samples

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Abstract

The main objectives of this research work are to isolate, identify, and characterized the Enteric organisms isolated from Patient's Fecal Samples, to determine the antimicrobial efficacy of various brands of green tea extracts on the isolated enteric organism, and to determine the killing kinetics/Death dynamics of the green tea extracts using Visible (Vis) Ultra-Violet Spectro photometric Protocol. Enteric organisms, such as *Escherichia coli* and *Salmonella enterica*, are a major cause of foodborne illnesses and health challenges worldwide. Antibiotic resistance among these organisms is increasing, making it difficult to treat nosocomial infections. Therefore, alternative antimicrobial agents are needed, to control infections caused by these organisms. Green tea extract has been an alternative source of antimicrobial agents, The enteric bacteria used in this study were obtained from Patient's Fecal Samples obtained from the University's Health Centre Adekunle Ajasin Unversity, Akungba Akoko, Ondo state, using the pour plate method and they were identified and characterize base on the cultural and biochemical characteristics. The following bacteria were isolated *Escherichia coli* (T4), *Pseudomonas aeruginosa* (OK3*), Escherichia coli* (T10), *Serratia odorifera* (IK4), *Klebsiella terrigena* (IK16), *Moraxella bovis* (T3), *Enterobacter agglomerans* (IK1), *Pasteurella multocida* (T19), *Burkholderia pseudomallei* (T31), and *Mannheimia haemolytica* (T22). In this study, the antimicrobial efficacy of different green tea brand extracts against enteric bacteria was investigated using the agar well diffusion method at different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. The results of this study showed that green tea extract has antimicrobial activity against all tested enteric organisms. The zone of inhibition ranged from 2mm to 30 mm. Killing time/Death dynamics assay were done via Visible (Vis) Ultra-Violet spectrophotometry protocol against the enteric organisms showing that green tea can inhibit the growth rate of the enteric organisms at Wavelength 620λ and the death dynamics with the addition of Green tea at 48th Hour interval at Wavelength 620λ. *Serratia odorifera* and *Pasteurella multocida* showed significant inhibition of growth in the presence of green tea. In conclusion, the results of this study suggest that green tea may be a potential natural alternative for the control of enteric organisms.

Keywords: Killing Kinetics; Green Tea Extract; Visible (vis); Ultraviolet Spectrophotometric; Enteric Isolates

Introduction

Green tea is often made from the *Camellia sinensis* plant, which is grown in over 30 countries in specific tropical and subtropical regions. There are four main types of tea produced, including white, green, Oolong, and black tea, which are distinguished by the degree of fermentation and drying processes. White tea undergoes the least processing and uses very young leaves and leaf buds, whereas green tea is produced from more mature leaves with no

fermentation. Oolong tea is partially fermented, and black tea is fully fermented. The popularity of tea types varies depending on the country, with green tea being most commonly consumed in China, Japan, and Korea, while black tea is more popular in the US and the UK. Various studies have been conducted on different aspects of tea, including its chemical composition, health benefits, and processing methods, which have been documented in research articles [1-5].

Camellia sinensis, also known as the tea plant, has been traditionally used for its medicinal properties in many cultures. One of its most notable properties is its antibacterial activity, which has been extensively studied in recent years. *Camellia sinensis* contains various bioactive compounds, including catechins, theaflavins, caffeine, and theobromine, which are responsible for its antibacterial properties [6].

Moringa oleifera is a fast-growing, drought-resistant tree that is native to the sub-Himalayan regions of northern India, but is now widely distributed in tropical and subtropical regions around the world. It is also known as the drumstick tree, horseradish tree, or ben oil tree, and is valued for its medicinal, nutritional, and environmental benefits [7]. *Moringa oleifera* is considered a superfood due to its high nutritional value. It is an excellent source of vitamins A, C, and E, calcium, potassium, and protein. The leaves, flowers, seeds, and roots of the tree are all used for medicinal purposes in traditional medicine systems in many cultures [8].

One of the most well-known benefits of *Moringa oleifera* is its potential to lower blood sugar levels. Research has shown that it contains compounds that can help to lower blood glucose levels and improve insulin sensitivity, making it a promising natural treatment for diabetes [9]. *Moringa oleifer*a has also been shown to have anti-inflammatory and antioxidant properties. The leaves and seeds of the tree contain high levels of antioxidants, which can help to protect against oxidative stress and reduce inflammation in the body. This makes it a potentially useful treatment for a range of conditions, including heart disease, cancer, and arthritis [10].

Peppermint (*Mentha piperita*) is an aromatic plant that belongs to the mint family. It is widely used as a flavoring agent in food and beverages, as well as in medicinal preparations. Peppermint leaves have been traditionally used in folk medicine for their antispasmodic, analgesic, and anti-inflammatory properties [11] Peppermint (*Mentha piperita*) is a popular herb used for various purposes, including medicinal, culinary, and cosmetic applications. One of the most common uses of peppermint is in tea, where the dried leaves or fresh leaves are steeped in hot water to create a refreshing and soothing drink. Peppermint tea is enjoyed for its minty flavor and aroma, as well as for its potential health benefits, including antibacterial properties [12].

According to research, green tea possesses several properties, including anticarcinogenic, anti-inflammatory, antimicrobial, and antioxidant, making it beneficial for various conditions such as cardiovascular disease, diabetes, obesity, and neurologic and oral health [13-18]. The anticarcinogenic properties of green tea help regulate cell proliferation, apoptosis, and angiogenesis in tumor cells [4,16-19].

Additionally, green tea's anti-inflammatory properties help decrease protein denaturation and increase the production of anti-inflammatory cytokines [3,4,20]. The antioxidant properties of green tea help limit free radicals by binding to reactive oxygen species, increase basal levels of antioxidant enzymes, and improve their activity [22]. Green tea can also help prevent atherosclerosis, reduce total lipid levels, and improve the LDL/HDL ratio [21].

According to Reygaert (2018), Green tea is a rich source of polyphenols, particularly flavonoids, and catechins are the most significant flavonoids. Catechins constitute 80-90% of the flavonoids and approximately 40% of the water-soluble solids in green tea. The four main catechins present in green tea are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin-3-gallate, and (-)-epigallo catechin-3-gallate, with (-)-epigallocatechin-3-gallate being the most abundant (~60%), followed by(-)-epigallocatechin (~20%), (-)-epicatechin-3-gallate (~14%), and (-)-epicatechin (~6%). Although (-)-epigallocatechin-3-gallate is the most researched catechin concerning health benefits, (-)-epigallocatechin and (-)-epicatechin-3-gallate have also been studied. Although standardized extracts of green tea with consistent catechin levels are available for supplement use, the amount of catechins in a specific green tea beverage can vary widely [22]. Enteric microorganisms are microorganisms that typically inhabit the intestines of animals and humans. These microorganisms can either be harmless, such

as gut flora or microbiota, or pathogenic. The intestinal tracts of all humans and animals are colonized by a large number of enteric microorganisms, as stated by Horta-Baas *et al*. [23]. The majority of enteric bacteria are beneficial and helps maintain a healthy intestinal tract, and they are commonly referred to as gut flora or human microbiota. Other notable Enterobacteriaceae members include *Klebsiella, Proteus*, and *Enterobacter*.

Additionally, *Salmonella, Shigella, Yersinia, Campylobacter jejuni* (*C. jejuni*), and *Clostridium difficile* (*C. diff*), which are Gram-positive bacteria, are examples of other pathogenic enteric bacteria. Among the *Shigella* species, *Shigella boydii* (*S. boydii*), Shigella *dysenteriae (S. dysenteriae), Shigella flexneri* (*S. flexneri*), and *Shigella sonnei* (S. *sonnei*) are known to have many pathogenic subtypes. Enteric bacteria are a significant cause of human infections, and the human gut plays a crucial role in the development and spread of multi-drug resistant organisms, as indicated by Wallace *et al*. [24].

Materials and Methods

Sample collection and location

The green tea (Twining of London, Dilmah green tea, Legend grea tea, Qualitea green tea and Vita green tea) used for this study were obtained from local patients medicine sellers in Ikare-Akoko, Ondo State, which shares boundary with neighboring states; on east- Edo and Delta, on west – Ogun and Osun, on the north – Ekiti and Kogi and south – bright Atlantic Ocean. Ondo state is located on the latitude 5° 45° and 7° 52° and longitude 4° 20° and 6° 05° E.

Collection of enteric organisms isolated from patient's fecal samples

Five (5) different fecal samples from sick individual were collected aseptically in sterile universal bottles from the Microbiology section of the University's Health Centre at Adekunle Ajasin University, Akungba-Akoko in Ondo State. The samples were then promptly transferred to the laboratory within 15 minutes for microbial analysis.

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Plate 1: Showing the Green teas Used for this Study; (1) Twining of London, (2) Legend Green Tea, (3) Vita Green Tea, (4) Qualitea and (5) Dilmah Green Tea.

Isolation, identification and characterization of enteric organisms isolated from patient's fecal samples

Preparation of culture

9 ml of sterile physiological saline was aseptically dispensed into 5 sterile test tubes and the mouth was corked with cotton wool wrapped with aluminum foil, each test tube was then labeled as 10^{-1}- 10^{-5} respectively. Fecal samples were dispensed into 9 ml of sterile physiological saline. 1 ml of the stock culture was serially transferred to other 9 ml of sterile physiological saline in other test tubes in an aliquot manner up to the fifth dilution [22,23].

Bacteriological analysis of enteric organisms isolated from patient's fecal samples

Pour plate method of inoculation was used for the enumeration of bacteria; 0.5 ml of the five -fold dilution of $10^{-3}$and 10^{-5} fecal samples (inoculum) was put into sterile Petri dishes. MacConkey agar (55 grams of the Agar into 1 liter) and Eosin Methylene Blue (EMB) agar(37.50 grams of agar into 1 liters) were prepared according to manufacturer prescription into sterile conical flask,

corked with cotton and aluminum foil and then homogenized to dissolve. It was sterilized in an Autoclave at a temperature of 121°C for 15 minutes. After the sterilization, the medium was allowed to cool to about 45°C. 20 ml of the sterilized media were then poured into different sterile Petri dishes containing the 0.5 ml of the inoculums aseptically and allowed to set. Then the plates were incubated at 37°C for 24hrs. After 24hrs, the cultural characteristics on the plates were studied and recorded. Resultant colonies were sub-cultured on fresh Nutrient agar and then incubated for 24hrs. Pure isolates were preserved on a double strength nutrient agar slant for further studies [22,23].

Identification of isolates

Microscopic examination of enteric organisms isolated from patient's fecal samples

Cultural and microscopic examinations were performed to determine the identity of the isolated enteric microorganisms. The cultural characteristics of the isolates were examined; the creamy pigmentation, round and slightly elevated shape, irregular and thread-like some of which are swarmy, with no distinct colony, and cellular morphology characteristics. Furthermore, conventional identification of the isolates was carried out using various biochemical tests such , Indole, Motility, Gram staining, fermentation of sugars (Sucrose, Lactose, Dextrose), Urease, Hydrogen sulphide, gelatin liquefaction and nitrate reduction test [25].

Gram staining of enteric organisms isolated from patient's fecal samples

Sterilized inoculating loop was used to make a smear of the culture onto a clean, grease-free slide. The slide was then labeled with each isolate code and heat-fixed. The smear was flooded with crystal violet (primary stain) for 60 seconds and rinsed with water. Lugol's Iodine was applied to the slide as a mordant and left to stay for 1 minute. After that, it was rinsed with water and left for 30 seconds. 70% ethanol was used to decolorize the smear for 15 seconds, and it was immediately rinsed off in gently running tap water to remove the ethanol effect. The slide was then counterstained with safranin for 60 seconds, rinsed with water, and blot dried. It was reported that the slides were viewed under the microscope using oil immersion (×100). It was observed that Gram-positive cells appeared purple since they retained the purple color of the primary stain (crystal violet) as they were not decolorized by alcohol. In contrast, Gram-negative cells appeared pink as the alcohol removed the crystal violet-iodine complex [25].

Biochemical tests of enteric organisms isolated from patient's fecal samples

The isolates were identified by conventional methods. Briefly, for the identification of test isolates, using a sterile wire loop a drop of normal saline was put on the center of grease-free slide and a portion of the colony was picked and emulsified into the center of a glass slide and allowed to air dry before fixing. To gram stain, crystal violet was then applied after 3min. It was then replaced with a gram's iodine for one (1) minute, prior to rinsing with water and application of 95% alcohol until no color appeared on the flow. Slides were then rinsed with water and safranin was applied for 1-2min. this was followed by rinsing and air-drying before being observed microscopically under ×100 oil immersion lens. Growth was interpreted as described by [22,23]. Where interpreted that purple and blue color indicated the presence of Gram-positive bacteria and pink or red color identify the presence of gram-negative bacteria. Fungal isolates were identified based on cultural and morphological characteristics with reference to the standard atlas [22,23]. All slants of test organisms were kept at -4°C prior to the bioassay of the extracts. Extensive series of biochemical tests were carried out to further confirm all the test bacterial strains. Biochemical tests done includes; Indole test, Catalase, Citrate, Methyl Red-Voges Proskaeur (MR-VP), Triple Sugar Iron (TSI), Urease, Motility Test, Oxidase Test [26].

Preparation of green tea for antimicrobial susceptibility test against enteric organisms isolated from patient's fecal samples

From each of the green tea sample, 1g of the powdered green tea were dissolved into 10ml of sterile distilled water making it 100 mg/ml.

Preparation and standardization of inoculum suspension

Direct colony suspension technique was used for the standardization and preparation of inoculum. Pure cultures of the test organisms were transferred into sterile screw-capped

McCartney bottles containing normal saline (0.90% w/w) using a flamed inoculating loop. A suspension with a turbidity equivalent to 0.5 McFarland standard was also prepared at the same time to serve as a reference for turbidity. To achieve equal turbidity, both the reference and inoculum suspensions were placed against a white card with black stripes. Turbidity was observed with the unaided eye. Standardized inoculums were refrigerated [22,23].

Antimicrobial assay of green tea against enteric organisms isolated from patient's fecal samples

Antimicrobial susceptibility testing (AST) was carried out using Mueller-Hinton agar and the organisms were seeded on the Mueller-Hinton agar using sterile swab-sticks. Sterile swab-sticks were used to pick inoculum from standardized inoculum suspension to sterile Petri-dishes containing molten Mueller-Hinton agar. Agar well diffusion method was used to dispense antimicrobials (green teas). A sterile cork borer of 5min diameter was used to bore wells on the agar medium that has been seeded with test organisms. Using syringe, the graded concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) of the green tea extract were gently dispensed into each designated wells. Positive controls (Erythromycin) were also incorporated into one of the designated well. After 24 hours the results were taken by observing, measuring and recording the zone of inhibitions formed around the agar wells [27].

Measurement of growth dynamic/death rate of isolates using ultra violet spectro photometer against enteric organisms isolated from patient's fecal samples

This procedure used by Osuntokun (2019) was used during this test. Growth dynamic test was done to determine the rate of growth of the isolates as well as their killing time in due time. A loopful Colony was picked from the stocked culture slant and inoculated into 9ml nutrient broth which was incubated for 24hours at 37^0C. A loopful of the each organism was picked from the broth culture into another 9ml nutrient broth in three sets which are labebelled as set A, B, C and D respectively. The growth rate was measured using ultraviolet (uv-vis) spectrophotometer; the spectrophotometer was set at 620λ wavelength, warmed up for 15 minutes and calibrated; during the determination of the growth rate, sterilized nutrient broth was used to calibrate the spectrophotometer for set A. During the determination of the killing rate sterilized nutrient broth, which erythromycin has been incorporated into was used to calibrate spectrophotometer for set B. During the determination of the killing rate sterilized nutrient broth, in which the green tea to be assay has been incorporated into, was used to calibrate spectrophotometer for set C. The first was reading was taken at zero hour, eighth hour and it continues after every 12 hours for 6 times. At the 4^{th} reading for set B, which is the 48^{th} hour of set B, 1 ml of 0.5 mg/ml of ciprofloxacin was added to determine up the rate of kill. Also at the 4^{th} reading set C, which is the 48^{th} hour, 1 ml of the most effect green tea was added to determine the killing time [28,29].

Results

Table 1 shows the production date, expiration date, location of production, and the total number of green teas used for this study. A total of five (5) different green teas were obtained from patent sellers in Ikare-Akoko. All the green teas used in this study were unexpired.

Green tea	Production date	Expiry date	Location
Twining of London	Jul-20	Jul-23	London
Dilmah green tea	Jan-20	Jan-23	Sri Lankan
Legend grea tea	Jan-20	Jan-23	Sri Lankan
Qualitea green tea	Oct-20	Oct-23	Sri Lankan
Vita green tea	December 2020	December 2025	Sri Lankan

Table 1: The List of Green Teas Obtained From Patient Store, Production Date, Expire Date and Location of Production.

Table 2 shows the gram staining result of isolated enteric bacteria. The following isolates include seven isolates with rod-shaped morphology, namely T4, Ok3, T10, Ik4, Ik16, Ik1, and T31. The rest of the isolates include three isolates with cocci-shaped morphology, namely T3, T19, and T22. All the isolates are Gram-negative based on the Gram staining result.

Table 3 displays the cultural characteristics of the isolated enteric bacteria. It is noteworthy that all isolates have pink colonies,

Isolate Code	Gram Staining	Shape
T4	Negative	Rod-shaped
Ok3	Negative	Rod-shaped
T10	Negative	Rod-shaped
Ik4	Negative	Rod-shaped
Ik16	Negative	Rod-shaped
T3	Negative	Cocci
Ik1	Negative	Rod-shaped
T19	Negative	Cocci
T31	Negative	Rod-shaped
T22	Negative	Cocci

Table 2: Gram Staining of Enteric Organisms Isolated From Patient's Fecal Samples.

with the exception of Ik1, which has a greenish metallic sheen. The table indicates that T4, Ok3, T3, and T31 isolates have a flat elevation, while the others have a raised elevation. Furthermore, all the isolate colonies were opaque, except for Ik1, which is translucent. All the isolates have an entire margin, and their shape is predominantly round, except for T22, which has an entire shape.

Isolate Code	Colony Colour	Elevation	Opaque/Translucent	Margin	Colony Shape
T4	Pink	Flat	Opaque	Entire	Round
Ok3	Pink	Flat	Opaque	Entire	Round
T10	Pink	Raised	Opaque	Entire	Round
Ik4	Pink	Raised	Opaque	Entire	Round
Ik16	Pink	Raised	Opaque	Entire	Round
T3	Pink	Raised	Opaque	Entire	Round
Ik1	Greenish Metallic Sheen	Flat	Translucent	Entire	Round
T19	Pink	Raised	Opaque	Entire	Round
T31	Pink	Flat	Opaque	Entire	Round
T22	Pink	Raised	Opaque	Entire	Round

Table 3: Sample Code and Morphological Characteristics of Enteric Organisms Isolated From Patient's Fecal Samples on MacConkey and EMB agar.

Figure 1 Shows the number of colonies for samples with their respective dilution factors. The number of colonies is a measure of the microbial growth on MacConkey culture, and the dilution factor is used to determine the concentration of the original culture. The table can be sorted by the number of colonies to identify the highest and lowest values. The sample with the lowest number of colonies is Ik4, with only 21 colonies observed at a dilution factor of 10^5. The sample with the highest number of colonies is T31, with 224 colonies observed at a dilution factor of 10^3.

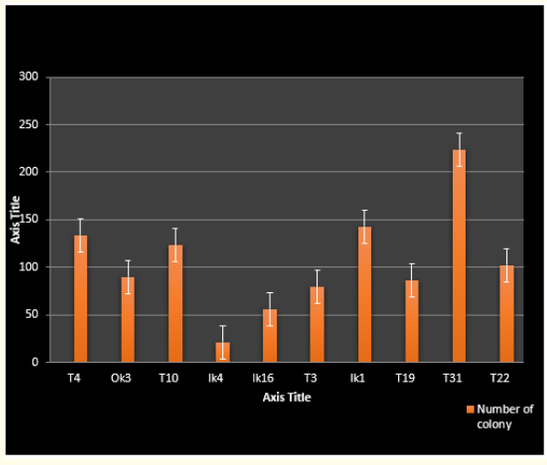

Figure 1: Dilution Factors and number of colony found on MacConkey Agar, Eosin Methylene Blue Agar of Enteric Organisms Isolated From Patient's Fecal Samples.

Table 4 presents the results of preliminary biochemical tests carried out on ten Enteric Bacteria Isolates, namely T4, Ok3, T10, Ik4, Ik16, T3, Ik1, T19, T31, and T22. The tests performed include Indole, Motility, Lactose, Sucrose, Glucose, Arabinose, Maltose, H2S, Gelatin liquefaction, Nitrate reduction, Mannitol, and probable Isolates. The results reveal that of T4 and T10 were positive for Indole, Lactose, Sucrose, Glucose, Arabinose, Maltose, H_2S, and Nitrate reduction. Ok3 was positive for Indole, Motility, Lactose, Glucose, Arabinose and Maltose. IK4 was positive for Sucrose, Glucose, Arabinose, Maltose. Ik16 was positive for Sucrose, Glucose, Arabinose, and Mannitol. T3 was positive for Sucrose. Ik1 was positive for Motility, Arabinose, and Mannitol. T19was positive for Sucrose, and is suspected to be the isolate for.T31was positive for Lactose and Arabinose. T22 was positive for Maltose.

ISOLATE CODE	Indole	Motility	Lactose	Sucrose	Glu-cose	Arabinose	Maltose	H_2S	Gelatin liquefaction	Nitrate reduction	Mannitol
T4	+	_	+	+	+	+	+	_	_	+	+
Ok3	+	+	+	_	+	_	+	_	+	+	+
T10	+	+	+	+	+	+	+	_	_	+	+
Ik4	_	_	_	+	+	+	+	_	_	+	+
Ik16	_	_	+	+	+	+	+	_	_	_	+
T3	_	_	_	+	+	_	+	_	+	_	+
Ik1	_	+	_	+	+	+	+	_	_	_	+
T19	_	_	_	+	+	_	+	_	_	_	+
T31	_	_	+	_	_	+	+	_	_	_	_
T22	_	_	_	+	_	_	+	_	_	_	+

Table 4: Preliminary Biochemical Tests of Enteric Organisms Isolated From Patient's Fecal Samples.

Table 5 Using Bergey,s Manual *Moraxella bovis*, *Escherichia coli, Pseudomonas aeruginosa, Serratia odorifera, Klebsiella terrigena, Enterobacter agglomerans, Pasteurella multocida, Bukholderia pseudomallei* and *Mannheimia haemolytica* were identified.

Isolate Code	Probable Isolates
T4	*Escherichia coli*
Ok3	*Pseudomonas aeruginosa*
T10	*Escherichia coli*
Ik4	*Serratia odorifera*
Ik16	*Klebsiella terrigena*
T3	*Moraxella bovis.*
Ik1	*Enterobacter agglomerans*
T19	*Pasteurella multocida*
T31	*Bukholderia pseudomallei*
T22	*Mannheimia haemolytica*

Table 5: List of Enteric Isolates Characterize Using Bergey's Manual of Determinative Bacteriology.

Figure 2 shows Antimicrobial Screening of Qualitea green tea, Vita green tea, Twining of London, Dilmah green tea and Legend grea tea at 100 mg/ml Erythromycin as Control. The results in this table indicate that Qualitea green tea exhibited the highest zone of inhibition against *Serratia odorifera* (T5) with a value of 28 mm, while the lowest zone of inhibition was observed against *Mannheimia haemolytica* (T22) with a value of 15 mm. Vita green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 28 mm, whereas the lowest zone of inhibition was observed against *Escherichia* coli (T4). Similarly, Twining of London green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 24 mm, while the lowest zone of inhibition was observed against *Pseudomonas aeruginosa* (OK3) with a value of 13 mm. Dilmah green tea exhibited the highest zone of inhibition against *Moraxella bovis* (T5) with a value of 24 mm, while the lowest zone of inhibition was observed against *Bukholderia pseudomallei* (T31) with a value of 15 mm. Finally, Legend green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1)

with a value of 30 mm, while the lowest zone of inhibition was observed against *Bukholderia pseudomallei* (T31) with a value of 15 mm.Erythromycin showed the highest zone of inhibition against *Escherichia coli* (T10) with a value of 48 mm, whereas the lowest zone of inhibition was observed against *Moraxella* sp. (IK1) at 15 mm.

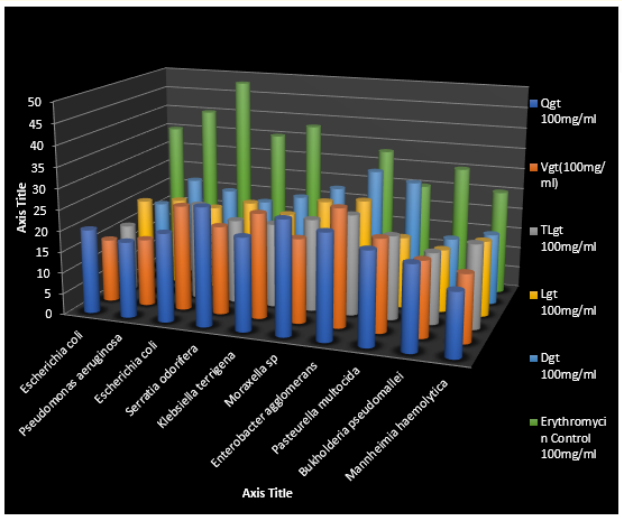

Figure 2: Measuring the Inhibition Zone of Enteric Organisms Isolated From Patient's Fecal Samples Against Different Brands of Green Tea at 100 mg/ml Concentration.

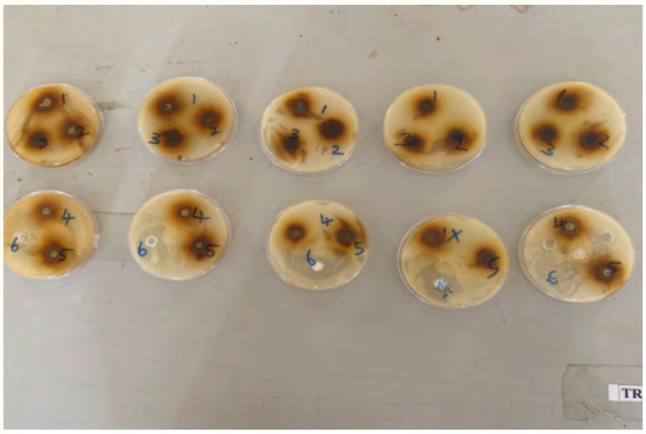

Plate 2: Showing the *In-vitro* Antimicrobial Assay of the Five Different Brand *Green teas* against Enteric Organisms Isolated From Patient's Fecal Samples.

Figure 3 shows Antimicrobial Screening of Qualitea green tea, Vita green tea, Twining of London, Dilmah green tea and Legend grea tea at 50 mg/ml Erythromycin as Control. The results in this table indicate that Qualitea green tea exhibited the highest zone of inhibition against *Serratia odorifera* (IK4) with a value of 15 mm, while the lowest zone of inhibition was observed against *Bukholderia pseudomallei* (T31)and *Mannheimia haemolytica* (T22) with a value of 7 mm. Vita green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 14 mm, whereas the lowest zone of inhibition was observed against *Escherichia* coli (T4) at 7 mm. Similarly, Twining of London green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 12 mm, while the lowest zone of inhibition was observed against *Pseudomonasaeruginosa* (OK3) with a value of 6 mm. Dilmah green tea exhibited the highest zone of inhibition against *Moraxella bovis* (T5) with a value of 12 mm, while the lowest zone of inhibition was observed against *Bukholderia pseudomallei* (T31) with a value of 7 mm. Finally, Legend green tea showed the highest zone of inhibition against *Pasteurella multocida* (T 19) with a value of 14 mm, while the lowest zone of inhibition was observed against *Bukholderia pseudomallei* (T31) with a value of 7 mm.Erythromycin showed the highest zone of inhibition against *Escherichia coli* (T31) with a value of 23 mm, whereas the lowest zone of inhibition was observed against *Enterobacter agglomerans* (IK1) at 4 mm.

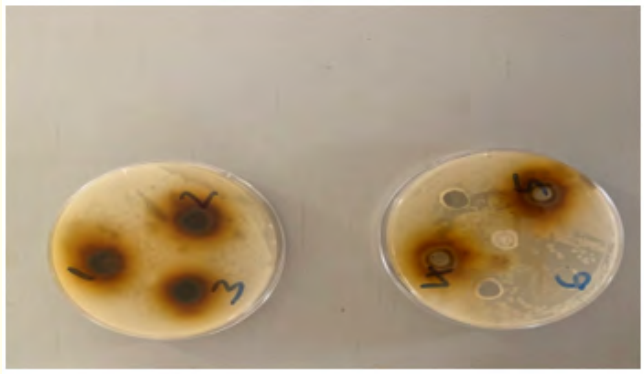

Plate 3: Showing the *In-Vitro* Antimicrobial Assay of the Green Teas against Enteric Organisms.

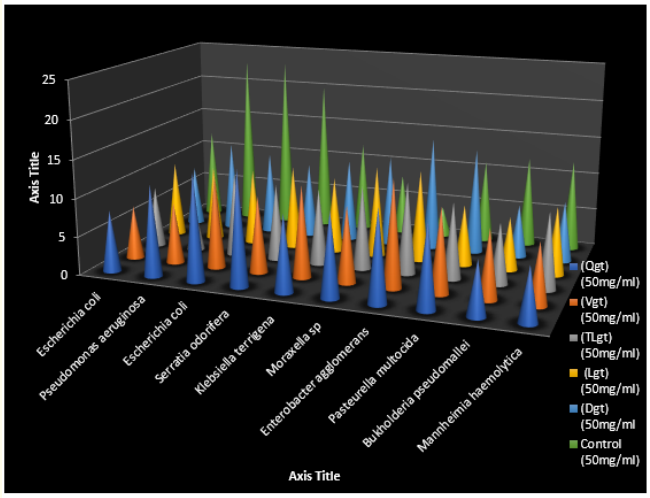

Figure 3: Measuring The Inhibition Zone of Enteric Organisms Isolated From Patient's Fecal Samples Different Brands of Green Tea at 50 mg/ml Concentration.

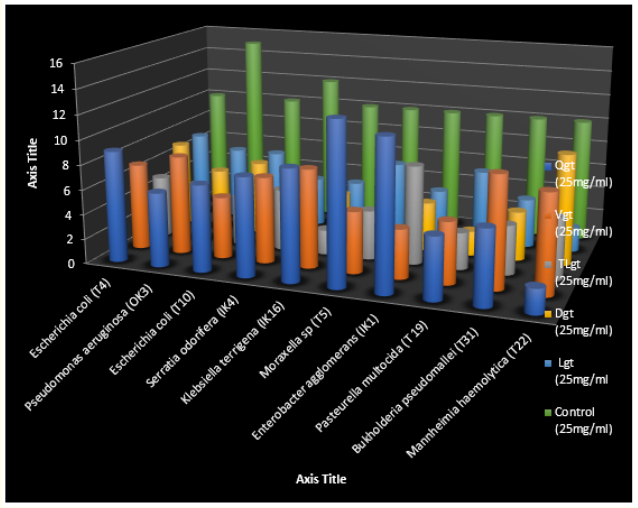

Figure 4: Measuring The Inhibition Zone of Enteric Organisms Isolated From Patient's Fecal Samples Different Brands of Green Tea at 25 mg/ml Concentration.

Figure 4 shows Antimicrobial Screening of Qualitea green tea, Vita green tea, Twining of London, Dilmah green tea and Legend grea tea at 25 mg/ml, Erythromycin as Control. The results in this table indicate that Qualitea green tea exhibited the highest zone of inhibition against *Moraxella* sp (T5) with a value of 13 mm, while the lowest zone of inhibition was observed against *Mannheimia haemolytica* (T22) with a value of 2 mm. Vita green tea showed the highest zone of inhibition against *Bukholderiapseudomallei* (T31) with a value of 9 mm, whereas the lowest zone of inhibition was observed against *Enterobacter agglomerans* (IK1) at 4 mm. Similarly, Twining of London green tea showed the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 12 mm, while the lowest zone of inhibition was observed against *Pseudomonas aeruginosa* (OK3) with a value of 6 mm. Dilmah green tea exhibited the highest zone of inhibition against *Mannheimia haemolytica* (T22) with a value of 9 mm, while the lowest zone of inhibition was observed against *Pasteurella multocida* (T 19) with a value of 2 mm. Finally, Legend green tea showed the highest zone of inhibition against *Escherichia coli* (T4) with a value of 7 mm, while the lowest zone of inhibition was observed against *Mannheimia haemolytica* (T22) with a value of 2 mm.

Figure 5 shows Antimicrobial Screening of Qualitea green tea, Vita green tea, Twining of London, Dilmah green tea and Legend grea tea at 12.5 mg/ml, Erythromycin as Control. The results in this table indicate that Qualitea green tea exhibited the highest zone of inhibition against *Enterobacter agglomerans* (IK1) with a value of 6 mm, while the lowest zone of inhibition was observed against *Escherichia coli* (T10) with a value of 3 mm. Vita green tea showed the highest zone of inhibition against *Pasteurella multocida* (T19) and *Klebsiella terrigena* with a value of 5 mm, whereas the lowest zone of inhibition was observed against *Mannheimia haemolytica* (T22) at 2 mm. Similarly, Twining of London green tea showed the highest zone of inhibition against *Escherichia coli* (T4) and *Serratia odorifera* with a value of 5 mm, while the lowest zone of inhibition was observed against *Enterobacter agglomerans* (IK1) and *Mannheimia haemolytica* (T22) with a value of 3 mm. Dilmah green tea exhibited the highest zone of inhibition against *Mannheimiahaemolytica* (T22) with a value of 9 mm, while the lowest zone of inhibition was observed against *Pasteurella multocida* (T19) with a value of 2 mm. Finally, Legend green tea showed the highest zone of inhibition against *Moraxella* sp (T5) with a value of 6 mm, while the lowest zone of inhibition was observed against *Enterobacter agglomerans* (IK1) and *Escherichia coli* (T4) with a value of 2 mm.

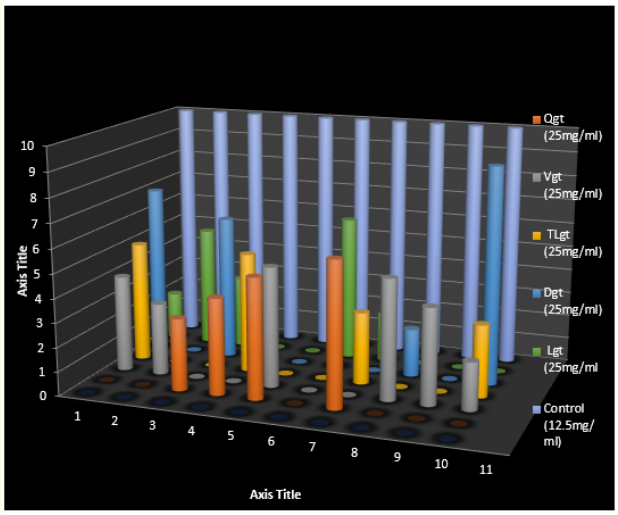

Figure 5: Measuring The Inhibition Zone of Enteric Organisms Isolated From Patient's Fecal Samples Different Brands of Green Tea at 12.5 mg/ml Concentration.

Key

Qualitea green tea (Qgt), Vita green tea (Vgt), Twining of London Green tea (TLgt), Dilmah green tea (Dgt), Legend green tea (Lgt). Zone of inhibition in milimetre (mm)
control- Erythromycin. 100,50,25, 12,5 mg/ml concentration.

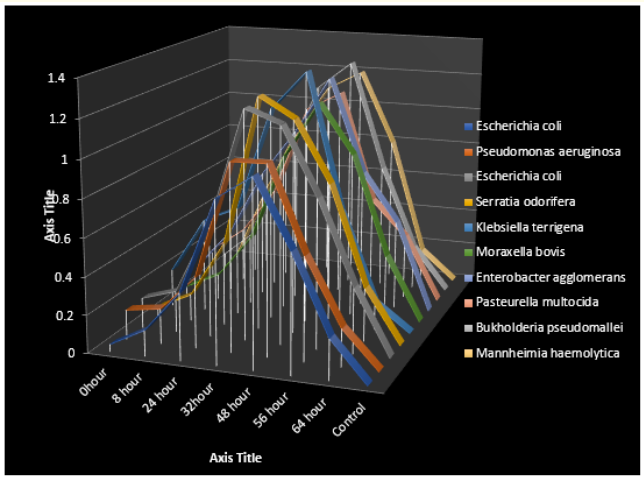

Figure 6: Growth Dynamic of Enteric Organisms Isolated From Patient's Fecal Samples Using Ultraviolet Spectrophotometer With Wavelength 620λ.

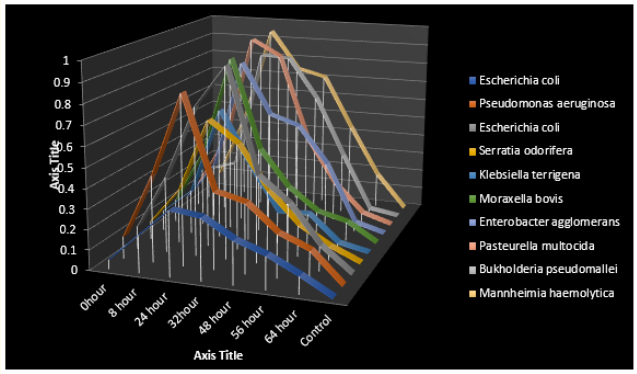

Figure 7: Growth Dynamic and Killing Time of Enteric Organisms Isolated From Patient's Fecal Samples with Addition of Ciprofloxacin Antibiotic at 24th Hour Using Ultraviolet Spectrophotometer With Wavelength 620λ.

Figure 6 shows the growth dynamics of enteric bacterial isolates using a UV spectrophotometer with 620λ. In this table, it is observed that at 0 hours, *Klebsiella terriger* had the highest growth rate of 0.205λ, while *Escherichia coli* had the lowest growth rate of 0.039λ. At the 64th hour, *Enterobacter agglomerans* had the lowest death rate of 0.481λ, whereas *Klebsiella terriger* had the highest death rate of 0.139λ.

Figure 7 shows the growth dynamics and killing time of enteric bacterial isolates with the addition of ciprofloxacin antibiotic at 24 hours, using an ultraviolet spectrophotometer. In this table, it is observed that at 0 hours, *Pasteurella multocida* had the highest growth rate of 0.124λ, and Escherichia coli had the lowest growth rate of 0.047λ. At 64 hours, *Escherichia coli* had the highest death rate of 0.085λ, and *Pseudomonas aeruginosa* had the lowest death rate of 0.151λ.

Figure 8 shows the growth dynamics and killing time of enteric bacteria isolates with the addition of Qualitea green tea at the 24th hour, using ultraviolet spectrophotometer. The table reveals that *Moraxella bovis* had the highest growth rate of 0.191λ at 0 hour, while *Bukholderia pseudomallei* had the lowest growth rate of 0.034λ. At 64th hour, *Escherichia coli* had the highest death rate of 0.087λ, while *Serratia odorifera* had the lowest death rate of 0.243λ.

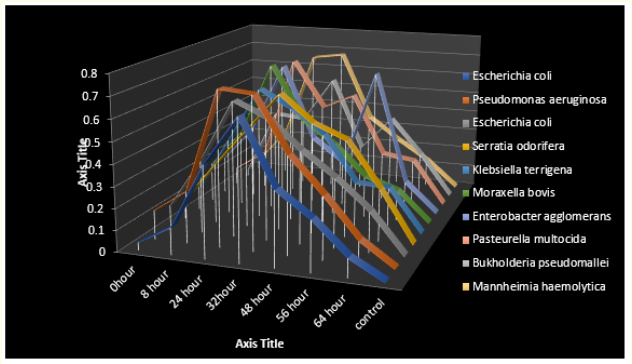

Figure 8: Growth Dynamic and Killing Time of Enteric Organisms Isolated From Patient's Fecal Samples with Addition of Green tea (Qualitea green tea) at The 48th Hour Using Ultraviolet Spectrophotometer With Wavelength 620λ.

Discussion

The research work determined the antimicrobial efficacy of different brands of green tea extract against enteric organisms isolated from patients' fecal samples. Enteric organisms are bacteria that typically inhabit the gastrointestinal tract, and they can cause various infections, such as diarrhea, dysentery, and food poisoning. The rise of antibiotic-resistant strains of enteric organisms has made it imperative to explore alternative antimicrobial agents basically from medicinal plants and in advanced research, nano-particle may be very useful (30).

Green tea has been widely recognized for its health benefits, especially its potential antimicrobial properties against different strains of microbes. This research work shows the antimicrobial efficacy of different brands of green tea against enteric organisms isolated from patient's fecal samples by measuring the zone of inhibition of five different green tea brands: Qualitea green tea (Qgt), Vita green tea(Vgt), Twining of London Green tea(TLgt), Dilmah green tea(Dgt), Legend grea tea(Lgt) and Concentration of 100,50,25, 12,5 mg/ml, Using Erythromycin as control It was observed that all five brands exhibited antimicrobial potency with the zones of inhibition ranging between 15 and 30mm respectively against the tested enteric organisms which include *Escherichia coli, Pseudomonas aeruginosa, Serratia odorifera, Klebsiella terrigena, Moraxella bovis, Enterobacter agglomerans, Pasteurella multocida, Bukholderia pseudomallei*, and *Mannheimia haemolytica.* this results was corroborated by (31,32).

The zone of inhibition refers to the clear zones around the agar well or antimicrobial well. It indicates the degree of antimicrobial activity of the green tea extract against the test organism. The larger the zones of inhibition, the more effective the green tea extract, in inhibiting the growth of the organism. In this study, the zone of inhibition ranged from 15 to 30 mm, with *Enterobacter agglomerans* being the most susceptible to green tea extracts and *Mannheimia haemolytica* being the least susceptible. This is related to zones of inhibition result obtained by Archana and Abraham (33) in a Comparative analysis of the antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens, where *E.coli*, *Enterococcus faecalis, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Vibrio cholera* were all sensitive to the green tea against test organisms..

When comparing the results of the green teas to the control (Erythromycin), it was observed that erythromycin generally had a higher zone of inhibition values than the green teas for most bacterial species. However, some green teas showed significant antimicrobial potency against certain bacterial species. For example, Qualitea green tea (100 mg/ml) had a zone of inhibition of 28 mm against *Serratia odorifera* (IK4), which was higher than the control (Erythromycin). Also, when comparing the results obtained from the growth dynamics and killing time of the bacterial isolates, are different in the presence of Ciprofloxacin antibiotic and green tea Ciprofloxacin antibiotic and the results of green tea (Qualitea)**.** it was observed that the bacterial growth was significantly inhibited by the presence of ciprofloxacin antibiotic, as indicated by the low OD (optical density) values for most isolates at the different time intervals. On the other hand, the effect of green tea on bacterial growth varied depending on the isolate. Some isolates, such as *Serratia odorifera* and *Pasteurella multocida*, showed significant inhibition of growth in the presence of green tea, while others, such as *Klebsiella terrigena* and *Moraxella bovis*, were less affected by the green tea treatment (34).

It was also observed that all the green teas, show some antibacterial potency on the isolated bacteria at 50 mg/ml, at 25 mg/ml, and 12.5 mg/ml, some organisms were not susceptible to the green teas as some of them has no zone of inhibition to the different brands green teas. At 25 mg/ml 48% of the organisms did not have a zone of inhibition to the enteric organisms. At 12.5

mg/ml 68% showed no zone of inhibition to the green teas. The antimicrobial activity of the green teas used in this study may be attributed to their polyphenolic compounds, mainly catechins (35). Catechins have been reported to have broad-spectrum antimicrobial activity against various microorganisms, including bacteria, fungi, and viruses (35). The antimicrobial activity of catechins is primarily due to their ability to disrupt the bacterial cell membrane and inhibit bacterial growth. Green tea catechin contains, epigallocatechin gallate (EGCG), which has been shown to disrupt the cell membrane and inhibit bacterial growth (36). Catechins, Epigallocatechin gallate (EGCG), has been widely studied for their antimicrobial properties against bacteria and virus (36).

EGCG is a polyphenolic compound found in high concentrations in green tea, and it has been shown to have a broad range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. Several studies have shown that EGCG has potent antimicrobial activity against various microorganisms. The mechanism of action of EGCG against bacteria is thought to involve disruption of the bacterial cell membrane and inhibition of bacterial growth and by binding to lipids in the membrane and causing structural changes that lead to membrane damage and leakage of intracellular contents. This disruption of the cell membrane can lead to bacterial death or inhibition of bacterial growth (37). (38). They also act to inhibit the activity of DNA gyrase, an enzyme that is essential for bacterial DNA replication, and dihydrofolate reductase, an enzyme that is necessary for bacterial folate metabolism (39).

Green tea has limitless potential for bacteria and viruses making them stand out, as a natural product, it has been postulated that Epicatechin is cost-effective, highly biocompatible, and has low toxicity (29). Epicatechin (EC) helps in the inhibition of HIV-1 reverse transcriptase *in vitro* and binds directly to CD4 molecule with consequent inhibition of gp120 binding (40). Epigallocatechin (EGC) inhibited plaque forming activity of influenza A and B virus whereas EC exhibited little inhibition. A polyphenol mixture is more efficient than a single compound in plaque inhibition and epicatechin had no antiviral effect and only a little inhibition on plaque formation, epicatechin had no antiviral effect and only a little inhibition on plaque formation (36). Epigallocatechin (EGC) increases lysosomal acidification, and regulates autophagy and lipid clearance in the liver due to its antisteatotic property (41). EGC, in a dose-dependent manner can reduce the release of cytokines/chemokines responsible for inflammation showing anti-inflammatory property(42). Its antiallergic property strongly inhibits the activation of mast cells and the expression of high-affinity IgE receptors, which produces an allergic reaction on exposure to certain foreign antigens (36).

In addition, green tea has been shown to have antioxidant and anti-inflammatory properties (43). The whole results of this study suggest that green tea may be a potential natural alternative to synthetic antimicrobial agents, as a formidable control of enteric organisms and its related infection. However, further studies are needed to determine the optimal concentration and formulation of green tea extracts, as an antimicrobial agent. In addition, the potential toxicity and side effects of green tea extracts should also be evaluated.

CONCLUSION

Based on the results obtained from this study, it can be concluded that green tea has a potential antimicrobial effect against enteric organisms. The antimicrobial screening of five different green tea brands (Qualitea, Vita, Twining of London, Dilmah, and Legend) showed that all of them had moderate to high inhibitory effects against the tested organisms. The growth dynamic and killing rate of the bacterial isolates, after incorporation of green tea (Qualitea) also demonstrated that green tea can inhibit bacterial growth, as shown by the lower optical density values at 24 hours. Therefore, it can be inferred that green tea can potentially be used as an adjunct therapy for bacterial infections..

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Competing Interests

Authors have declared that no competing interests exist.

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