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RESEARCH ARTICLE

Exam the Synergistic Potency of Tea Leaves Extract (*Camellia Sinensis*) and Moringa Leaves (*Moringa Oleifera*) as an Inhibitor of the Growing of Some Bacteria that Cause Food Poisoning

Ibtisam Fareed Ali Karm[1*]

[1*]*Department of Commodity Evaluation and Service Performance, Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq. E-mail: ibtisam77@mracpc.uobaghdad.edu.iq*

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ABSTRACT

Issues of antibiotic-resistant microorganisms have become a genuine wonder and needs to discover arrangements viable with the peril it addresses. Therefore, scientists have arisen calling to go towards regular other options and sources given commonly by nature. The study focused on the synergistic effectiveness of the cold (E1) and hot (E2) aqueous extracts of the leaves of two plants, Camellia sinensis, and Moringa oleifera, compared with (E3) which is control treatment, subsequent to researching some dynamic and active mixtures in the two plants separates utilizing phytochemical investigation and infrared spectroscopy analysis (FTIR). In addition, their utilization as anti-infection agents to delay the growth of some positive and negative isolated pathogenic bacteria that cause food contamination, by measuring the inhibition zone for bacteria growth, and determine the MIC assay for concentrates.

The outcomes showed that the main mixtures combinations that were subjectively analyzed by FTIR were phenols, polyphenols, alkaloids, flavonoids, and other significant mixtures. Results showed that there were significant differences among the bacterial species towards each concentrate, just as clear critical differences among the pre-arranged concentrates towards every one of the confined bacterial species.

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Introduction

Clinical plants have begun to think about a fundamental source in treating/forestalling different sorts of sickness. Regular wellsprings of treatment that are harmless to the ecosystem can be utilized as antimicrobial specialists, some people have for quite some time been sow them as a protected option in contrast to ordinary, engineered, medicine, and anti-infection agents to stay away from their entanglements (Afroz et al., 2020).

Each the plant comprises of a few significant fixings that can be utilized in the clinical field, and can be associated with the advancement of various types of medications, there is extraordinary potential for future disclosures from plants and other normal items which, accordingly, offer immense potential in inferring valuable data about novel synthetic constructions and their new kinds of exercises identified with new medication advancement (Mohammed, 2019; Yuan et al., 2016).

Numerous investigations can be found in the writing detailing the medical advantages of a few Camellia groups, specifically *C. sinensis, C. oleifera*, and *Camellia japonica*. These species have been featured as having antimicrobial (antibacterial, antifungal, antiviral) and antitumoral movement and just like an immense well spring of polyphenols especially catechins, epicatechin, and particularly epigallocatechin-3-gallate, the major

*Corresponding author: ibtisam77@mracpc.uobaghdad.edu.iq

polyphenols of green tea underpinning properties and natural movement (Teixeira and Sousa, 2021). *Moringa oleifera* and *Moringa stenopetala* are underutilized types of tropical trees that may assume a significant part in dietary expansion and add to the mitigation of stowed away yearning in less created tropical and subtropical nations, numerous investigations propose that *Moringa spp*. leaves have incredible potential in the improvement of food additives and anti-infection drugs (Seleshe and Kang, 2019).

Synergism happens when at least two mixtures interface in manners that commonly upgrade, enhance or potentiate each other's impact more fundamentally than the straightforward amount of these ingredients. Herbal grown therapies have the advantage of benefit of consolidating their dynamic segments with numerous different substances which may seem to be inactive unconventionally, other than being protected and more effective when contrasted with their separated dynamic mixtures (Sebastian et al., 2019).

The study aimed to enhance the effective role of the synergy between plant extracts and analyze the most important compounds in addition to taking caution in using the lowest concentrations to inhibit the growth of bacterial pathogens.

Materials and Methods

Plant Collecting and Formulating

The two plants leaves gathered are the Camellia and Moringa from the nearby business sectors and general stores spread in the zones of Baghdad. From that point forward, the leaves were dried and processed with an electric processor gadget to become powdered, and afterward the powder of the plant's leaves was set up to make removes from them (Ayoola et al., 2018).

The Aqueous Extracts Preparing

1. **Cold aqueous extract:** At cold extract, 50 grams of fresh leaves powders of each plants were weighed out and dipped into 500 ml cold distilled water for 24 hour with occasional shaking at room temperature then Filtered and stored into a clean conical flask and marked E1.
2. **Hot aqueous Extract:** Adding 50 grams of studied plants to 500 ml hot boiling aqueous solvent using shaking incubator, for 1hr, after gradually cooling it filtered then stored for farther use and named E2. Both extracts were evaporated then obtained to be stored at 4°C for biological activity test (Akueshi et al., 2002).

Table 1. Treatments of plants leave extracts prepared in study

treatments	Aqueous ext.	Plants leave ext.	Ratio
E1	Cold extract	*Camellia sp.* ext. + *Moringa sp.* ext.	1:1
E2	Hot extract	*Camellia sp.* ext. + *Moringa sp.* ext.	1:1
E3	Aqueous solution	The antibiotic ciprofloxacin	---

Pathogenic Poising Bacteria

Tests of microscopic pathogenic organisms were gathered from swabs for a supplies utilized by some patients in medical clinics, and some bacteria were distinguished for the investigation, two of isolates were positive for Gram stain and the other were Gram negative (Karm, 2019).

Determine the Active Groups

The phytochemical analysis and FT-IR (Fourier transform infrared spectroscopy) analysis were performed using standard methods (Rajiv et al., 2017).

Biological Activity

Measure of Inhibition Zone

Firstly the supplement agar were spread with 100 µL of specific culture of pathogenic microscopic organisms, antibacterial effects were affirmed by utilization of disc diffusion technique. The leaves extracts and the anti-microbial medication ciprofloxacin were filled in at specific points. All plates incubated for 48 hours, finally measure the inhibition zone (Rajaa and Nahed, 2017).

Determine the MIC Assay

Work has been done to decide the MIC of every one of the concentrates towards the segregated and pathogenic microscopic organisms, as a response, stocks were additionally made for it and the tested concentration were 10,20,40,80 and160 µg \ml for E1, E2 and E3and furthermore to decide the MIC of the anti-infection, which will be the examination factor to decide the activity of the plant extricate as an elective treatment to chemical antibiotics (Rahman et al., 2007).

Statical Analysis

Factual Statistical analyses of outcomes were finished by the program SAS that was utilized to examine the outcomes acquired. Least significant difference (LSD) probability test was done with level of ($P < 0.05$), all date were triplicate (SAS, 2012).

Results and Discussion

Pathogenic Isolated Bacteria

A great deal of pathogenic microorganisms was noticed and four species of genera bacteria were chosen to be tested due to their connection with human poisoning. The bacteria were diagnosed according to morphology and biochemical tests (CLIS, 2007; Abdul Samad et al., 2018). The two *Staphylococcus spp*. were gram positive bacteria. While *Shigella sp*. and *Pseudomonas sp*. were Gram-negative.

Analysis of Active Groups and Compounds

After the concentrates were acquired from the leaves of the two plants, a few mixtures were blessed to clarify the main dynamic composites present in each concentrate independently. It was tracked down that the two concentrates contain some significant impact constituents like alkaloids, phenols, tannins, flavonoids, oils particularly volatile oils (Ayoola et al., 2018), and this likewise was affirmed by a subsequent test, which is Fourier Transform Infrared Spectroscopy (FTIR) examination which is quite possibly the main tests depends on the standard of momentary exchange infrared spectroscopy to decide the dynamic gatherings in the concentrates arranged in present test (Berthomieu and Hienerwade, 2009).

The effective groups that fall within the range of peaks at 1000 to 3500cm^{-1} include groups of phenols , alkaloids in addition to aromatic compounds at general, tests of the treatment (E1) recorded the peaks (1039.63, 1145.72, 1236.37, 1369.46, 1456.26, 1514.12, 1649.14, 2926.01 and 3425.58) cm^{-1} also the treatment (E2) showed some peaks (1055.06, 1242.16, 1315.45, 1433.11, 1548.84, 1651.07, 2954.95 and 3415.93) cm^{-1} symbolizes the presence of functional groups such as (C-O) alcohols, ethers, esters carboxylic acid, anhydrides, alcohols, phenols (O-H), sulfonic and sulfonyl chlorides (S=O), groups of alkanes (C-H), (- CH_3 bend) refers to amines (C-N) and (C-C) and amide group (C=O) can due to Flavonoids, Polyphenols and catechins. figure (1, 2).

These result were in agree with a study by Altemimi et al., 2017 they found that infrared spectroscopy can essentially be described as a vibrational spectroscopy. Different bonds have diverse vibrational frequencies if these kinds of bonds are present in an organic molecule (Altemimi et al., 2017). Other study by Senthilkumar et al 2017 infers that FT-IR investigation might be utilized in a basic manner to quickly appraise the cell antioxidant possibilities of plant extracts (Senthilkumar et al., 2017).

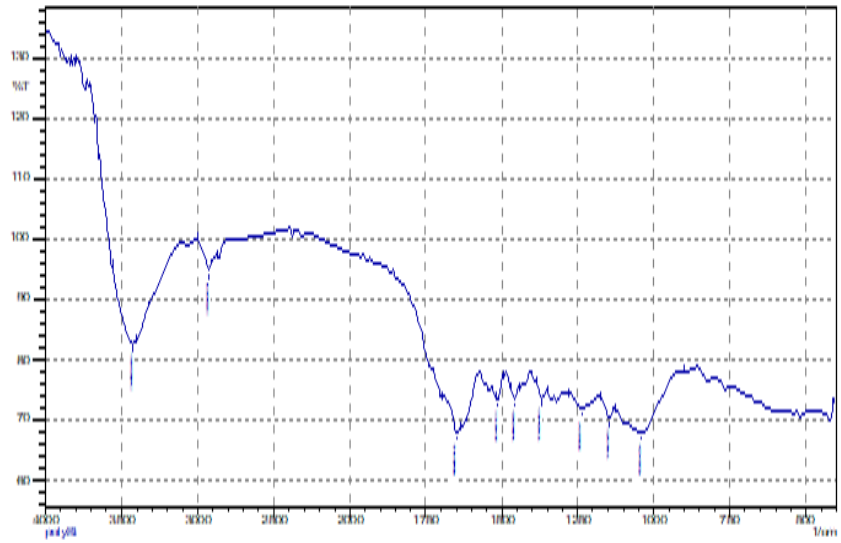

Figure 1. FTIR analysis of E1

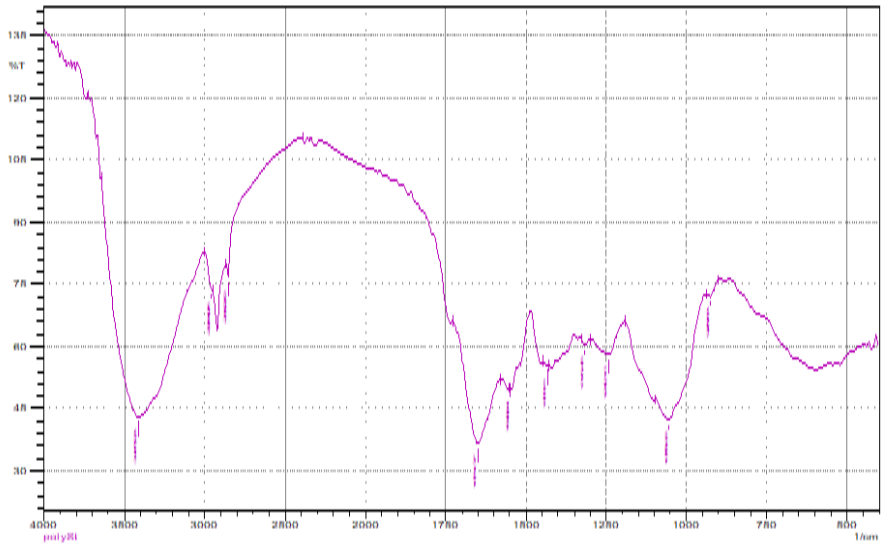

Figure 2. FTIR analysis of E2

In Vitro Antibacterial Activity

The results of the research showed that the response towards individual extracts could differ depending on the type of bacteria and their interaction with the active compounds present in the extract, two ways were depended upon:

1. Disc Diffusion Method (Determine the Inhibition Zone)

The antibacterial activity of E1 and E2 treatments showed clear effect that was seen in both treatments of aqueous leaves extract, so E1 ext. on *S. aureus, S. epidermidis, Shigella sp. and Pseudomonas sp.* with inhibition zone 9.3, 11.0, 10.7 and 11.6 mm respectively. While E2 ext. inhibitions results were 12.0, 15.6, 15.5 and 16.4. There were significant differences among all data of each tested bacteria treated with the extracts E1, E2 and E3 as control. Additionally significant differences were showed at all tested bacteria towards treatments E1, E2 and E3, Table (2).

Table 2. Inhibition zone (mm) for tested bacteria growth treated with extracts

Ext.	*S. aureus*	*S. epidermidis*	*Shigella sp.*	*Pseudomonas sp.*	LSD
E1	9.3	11.0	10.7	11.6	2.06 *
E2	12.0	15.6	15.5	16.4	2.84 *
E3(C)	10.5	16.0	16.2	18.5	3.58 *
LSD	1.87 *	2.66 *	2.71 *	2.95 *	---

*(P≤0.05). The inhibition growths measured by mm, all data are mean of three replications.

2. Determine the MIC

The results of the minimum inhibitory concentration (MIC) of both treatments E1 on *S. aureus, S. epidermidis, Shigella sp.* were 320, 160, 320 and 640 Mg \ml respectively. In addition E2 ext. results on the same tested bacteria species were 80, 160, 80 and 230 Mg \ml. There were critical differences among all results of each tried bacteria organisms treated with the concentrates E1, E2, and E3 as control. Also, huge significant differences were founded at all tried microbes towards treatments E1, E2, and E3, Table (3).

Table 3. MIC Mg \ml of plants leaves extracts against tested bacteria

Ext.	*S. aureus*	*S. epidermidis*	*Shigella sp.*	*Pseudomonas sp.*	LSD
E1	320	160	320	640	35.07 *
E2	80	160	80	320	29.88 *
E3(C)	80	80	40	160	22.06 *
LSD	32.79 *	27.22 *	34.63 *	45.72 *	---

* (P≤0.05). The MIC determined by Mg \ml, all data are mean of three replications.

There were several works reach agreement with present study thus results by Radji et al., (2013) concluded that leave quotation for Camellia might be suitable in opposing initial drug resistance caused by MRSA and *Pseudomonas aeruginosa*, other investigation by Teixeira and Sousa, (2021), give highpoints for *camellia spp*. As a featured that has antimicrobial (antibacterial, antifungal antiviral) and antitumoral action and just like a massive basis of polyphenols like the catechins. Unegbu et al., (2020) lab work were in bargain with present fallouts, contracted effects for *Moringa oleifera* leaf as energetic composites to break the development of some bacteria such as *Staph spp.* and *E coli*, as of some secondary metabolites existing.

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