



ANTIMICROBIAL ACTIVITY OF COMBINED EXTRACTS OF *CAESALPINIA PULCHERRIMA* AND *ZINGIBER OFFICINALE*

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ABSTRACT

The rise of multidrug-resistant (MDR) microorganisms has driven the search for novel plant-derived antimicrobial agents. This study evaluated the in vitro antimicrobial potential of *Caesalpinia pulcherrima* leaf and *Zingiber officinale* rhizome extracts, individually and in combination, against pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus*. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, glycosides, phenols, triterpenoids, proteins, and reducing sugars in both the extracts. Using agar disc diffusion and minimum inhibitory concentration (MIC) assays, the combination of both extracts demonstrated synergistic activity, yielding larger zones of inhibition and lower MIC values than those of the individual extracts, approaching the efficacy of ciprofloxacin. These findings suggest that the combined plant extracts could serve as promising candidates for novel antimicrobial therapies.

Keywords: *Caesalpinia pulcherrima*, *Zingiber officinale*, antimicrobial activity, phytochemistry, medicinal plants, synergistic effect.

1. INTRODUCTION

Microbial pathogenesis and additional viral illnesses have been managed for a long time through the consumption of publicly accessible antibiotics. The widespread consumption of medicines has led to the development of multiple drug resistance (MDR) in numerous infections caused by bacteria. The most important impediment to effective contagious disease therapy and microbial pathogen management is the rising antibiotic resistance. Similarly, substances like sulfates, nitrates, nitrites, and antimicrobial agents pose risks to human health,

potentially leading to headaches, nausea, weakness, cognitive impairments, epilepsy, cancer, and malnutrition. The growth of resistance to drugs in infections, as well as greater consumer curiosity in healthy food, has prompted the search for novel antibacterial medicines. Environmental compounds are an essential supplier for novel organic medications, so their application as complementary therapies in the management for a variety of disorders has grown in the past few years. Compared to manufactured medications, spices and herbs offer less adverse reactions. These are additionally cheaper, have greater customer acceptance, & remain readily accessible for those in the lower social strata. Herbs and spices have been increasingly popular in the past few years, both in emerging and wealthy nations, due to their beneficial properties. Herbs' antibacterial effect is attributable to unique phytonutrients or oils that are essential. The primary variables influencing the effectiveness of antibiotics include the kind and content of the spices, the quantity utilized, the sort of microbe, the chemical makeup of the food being consumed, the pH level, and the temporal conditions of the surroundings. A number of studies are being released detailing the antifungal and antibacterial effects of different spices and herbs. Nevertheless, there's currently not much information regarding the exact process of their antibacterial activity. The current investigation sought to identify and contrast the antibacterial effects of plant extracts of *Caesalpinia pulcherrima* as well as rhizome of the medicinal plant *Zingiber officinale* to that exhibited by conventional antimicrobial agents, demonstrating that a mixture of *Caesalpinia pulcherrima* and *Zingiber officinale* produces operational antibacterial properties in laboratory antibacterial studies. A disease occurs when microorganisms invade a living thing's internal organs, multiply, & host cells respond with the pathogens & poisons that create. An infectious health condition, additionally referred to as a transferable or communicable illness, is an ailment caused by a viral infection. A variety of microorganisms may trigger diseases, among the most common of which being viruses and bacteria. Guests are able to combat diseases via their natural defenses. Mammal hosts respond to pathogens via an intrinsic reaction, which frequently includes irritation, subsequently followed by a response that is adaptive. Examples include: *Mycobacteria TB*, *E. coli*, *Staphylococcus aureus*, *botulinum Clostridium botulinum*, and *Salmonella* species. Viruses as well as associated organisms like the viroid. (E.g., AIDS, and HIV Rhinoviruses, Viral infections such as Herpes virus, Ebolavirus & severe acute pneumonia corona virus). Ascomycota includes yeast cells like Candidiasis (also known as the biggest cause of fungus infections), fungal filaments like *Aspergillus*, pneumonia species, & dermatological fungi which attack epidermis as well as surface elements in people. Basidiomycota, which includes the dangerous genus the *Cryptococcus*. Single-celled microorganisms (e.g., malaria, *Toxoplasma*, *Babesia*) - Macroparasites (bugs or helminths), including as parasite round worms as well as the pinworms, worms called tape (cestodes), and fluke-like (trematode like schistosomes). Helminth-caused illnesses are frequently referred to as diseases, but they can also be termed illnesses. The symptoms and indications of an illness vary according to the kind of sickness. Some symptoms associated with infection affect the entire physique, including weariness, diminished hunger, losing weight, high temperatures, sweating throughout the night, shivers, & discomfort. Some are limited to particular physical regions, like rashes on the skin, wheezing, or running noses. In some situations, viral illnesses can remain undiagnosed throughout the majority, if not all, of their duration in an individual carrier. In the other situation, the sickness may only be classified as an "ailment"(which signifies illness) in individuals whom became unwell upon coming into touch via an unaffected carrier. A parasitic infection cannot be considered the same as a disease that is transmissible, because certain bacteria do not produce illness in the host.

2. MATERIALS AND METHODS

2.1 Identification and Collection of plant material

Caesalpinia pulcherrima leaves and *Zingiber Officinalis* rhizomes were obtained from the local

markets of Gudlavalleru and Gudivada. The recovered leaves and rhizomes were carefully washed in distilled water to eliminate contaminants before drying at room temperature.

2.2 Preparation of extract by using Maceration method

The drained leaves and rhizomes of *Caesalpinia pulcherrima* and *Zingiber Officinale* were ground to a coarse powder and extracted separately in methanol at ambient temperature with periodic shaking for a period of four days. The macerate and filtrate were dried at lower temperatures, forty to fifty degrees Celsius, using vacuums.

2.3 Phytochemical analysis

Phytochemical examination of the extraction was performed using several methods to detect the existence of alkaloids, glycosides, flavonoids, tannins, saponins, steroids, terpenoids, and other compounds.

2.4 Tests for alkaloids

1. To conduct the Dragendorff's test (Potassium Bismuth Iodide), add 1 ml of the reagents to the extract. An orange-red precipitation suggests the existence of an alkaloid.
2. The Wagnerian test (Iodine Solution in Potassium Iodide) consisted of introducing Wagner's reagent to the extraction. Reddish-brown precipitate indicates the existence of an alkaloid.
3. To conduct the Mayer's test (Potassium Mercuric Iodide), add 1 or 2 ml of the reagent to the extract. A dull white precipitate shows an abundance of alkaloids.
4. To perform the Hager's test (saturated picric acid solution), add 3 ml of Hager's reagent to the extraction. Yellow colour precipitates suggest the existence of an alkaloid.

2.5 Screening for sugars and carbohydrates

1. Molisch test: Add 1 ml of α naphthol solution to the extract, then add concentrated sulfuric acid down the edges of the test tube. The existence of carbohydrates is indicated by the purple or reddish violet tint at the point of intersection of the two liquids.
2. To do Fehling's test, add equal amounts of Fehling's solution A and B to the extraction. When heated slightly, a brick red precipitate forms, indicating the existence of polysaccharides.
3. Benedict's test: Add eight drops of the test solution to 5 ml of Benedict's reagent, stir, and boil for 2 minutes before cooling. A red precipitate suggests the existence of carbohydrate^[15]

2.6 Tests for proteins

1. Biuret test: To the concentrate, adding one milliliter of forty percent concentrated sodium hydroxide as well as two drops of one percent sulphate of copper solution. A purple hue shows the presence of proteins.
2. Xanthoproteic test: One milliliter of powerful nitric acid solution was introduced to the extracts, producing a white precipitate. The fact that was subsequently heated & chilled. Then twenty percent sodium hydroxide or ammonia had been introduced. The orange hue indicates the existence of heterocyclic amino acids in the body.
3. To conduct the lead acetate test, add 1 ml of the solution to the extract. A white precipitate shows the existence of peptides.

2.7 Test for amino acids Ninhydrin test

Two heaping teaspoons of freshly prepared 0.2% ninhydrin reagent, a reagent were stirred into the resulting solution & warmed. The increase of blue hue indicates the presence of proteins, peptides, or amino acids. [16].

2.8 Evaluate for steroid & the sterols

1. The Libermann Burchard test consisted of dissolving the test extract in 2 ml of chloroform in a dry test tube. An amount often teaspoons of acetic anhydride & a couple of drops of highly concentrated sulfuric acid were mixed. The solution's hue changes to red, blue, and finally bluish green, indicating the presence of steroids.
2. Salkowaski test: The resulting mixture was immersed in chloroform, and a comparable amount of strong sulfuric acid was added. The layer composed of chloroform is blue red to cherry red in hue, yet the layer of acid exhibits a prominent fluorescence of green that shows the presence of steroids.

2.9 Tests for glycosides

1. Legal test: The substance being extracted has been rendered alkali by soaking it in pyridine and then adding sodium nitroprusside. The presence of glyco can be detected by the hue pink or red.
2. Baljet test: Add sodium picrate solution to the extract. A yellow to orange hue indicates the presence of glycosides.
3. Borntrager's test: Incorporate a few a milliliter of mild sulfuric acid into the experiment's solution. Boil, filter, and then extract the filtrate using ether or chloroform. The organic layer was separated and treated with ammonia. Glycosides are visible in the colors pink, red, and violet.
4. Keller Killiani test: The specimen was immersed in acetic acid containing residues of ferric chloride & was placed on the top layer of a concentrated sulfuric acid solution. The joint created a reddish brown.

2.10 Test for flavonoids Shinoda test

Magnesium turnings have been added to the extract before being treated with strong hydrochloric acid. A crimson hue indicates the presence of flavonoids.

2.11 Tests for tannins

1. Ferric chloride was used to treat the extracts. A deep blue or greenish black hue indicates the occurrence of tannin.
2. The resulting substance was processed with a potassium dichromate solution. Precipitation indicates the existence of tannins.

2.12 Test for triterpenoids

Two or three tin grains were placed into the test container tube and dissolved in 2 mL of thionyl chloride solution. Then the test solution was added. Pink coloration indicates the presence of triterpenoids.

2.13 Tests for fixed oils

1. Spot assessment: A tiny quantity of extracts had been squeezed between two filter sheets. Oil stains on papers indicate the presence of persistent oils.

2. Saponification Test: To the extracted substance, add 0.5 N alcoholic potassium hydroxide and phenolphthalein. The resulting mixture was boiled in a water bath for approximately 1-2 hours. The creation of soap or partial neutralization of alkali indicates the existence of fixed oils. [17]

2.14 Anti-microbial activity Agar Disc Diffusion

The sensitivity of a microbe to antimicrobial agents as well as chemotherapy treatments can be assessed using the paper-based plates technique. The current investigation was conducted to assess the sensitive pattern of several *Caesalpinia pulcherrima* chemicals using a wide range of pathological & that are not pathogenic bacteria, including Bacteria are classified as Gram + (*Staphylococcus aureus*) or a gramme- [18]. The microorganisms were obtained from the Division of Microbiology. The existing cultures were maintained on the Mueller-Hinton agar medium slope [19]. To study the susceptibility patterns of these species to *C. pulcherrima* compounds, overnight cultures of selected organisms in Mueller Hinton broth were utilized as sample. The antimicrobial capacity was tested using Kirby-Bauer's approach, with some slight changes. [20]. The samples were diluted in dimethyl sulfoxide (DMSO) at an ultimate concentration of 20 mg/mL. Pure DMSO was utilized as a negative control. The efficacy of the drug was compared with conventional medications Amoxycillin and Ciprofloxacin at a concentration of 10µg/disc. Bacteria medium consisted of Mueller Hinton (Himedia) agar.[21] It was made accordance to the manufacturer's instructions and sterilized using an autoclave. Each petri dish received 20 mL of sterile media, which was then permitted to harden. The collection of platters was labelled & inoculated with the experiment's microorganism utilizing a cotton swab that had been wrapped in a sustained condition of nightly developing colonies that streaked the surface of the plate & reveal development of grass after incubating. The cellulose discs (5mm) were manufactured utilizing Whatmann filter paper as the substrate following the standard processes [22]. The sterilized discare equally dispersed on the inoculation plates.

A micropipette was used to transfer 20µl of each extract from the original solution (20mg/mL) to the plates. Every surface received 400 µg of raw extraction. Following appropriate extracts penetration through the medium, these dishes were left to incubate for 24 hours at 37°C in aerophilic conditions. The restricted zone has been determined in millimeters using a ruler. The investigation was conducted in three independent cases, and the mean outcomes were provided [23]. Lowest inhibitory level (MIC) The minimal inhibitory concentrations were determined by a solution dilution test. The extracted chemicals were prepared at several concentrations (20mg/mL, 15mg/mL, 10mg/mL, 5mg/mL, and 2.5mg/mL). For every 9mL of nutrient, 0.1mL of each concentration was added. The broth contained 0.1mL of a standardized test microorganism of bacterial cells [24]. The test tubes were incubated at 370 degrees Celsius for 24 hours. Positive control groups were formed equally employing solvents and testing organisms that did not contain extracts. The concentration with the weakest inhibitory effect was determined by selecting the tube with the least amount of extract that did not grow after incubated [25].

3. RESULTS AND DISCUSSION

3.1 Early phytochemical evaluation.

Biochemical evaluation of ethanol extraction of *Caesalpinia pulcherrima* leaves showed that presence of compounds, Flavonoids, saponins, Tannins. (Table.1)

Table.1: Phytochemical analysis

S. No	Phytonutrient	Test	Results
1	Flavonoids	NaOH test	-
2	Tannins	FeCl ₃	+
3	Saponin	Froth test	+
4	Glycosides	Kellerkiliani	+
5	Steroids	Liebermann-Burchard	—
6	Alkaloids	Dragondroff' test	-
7	Phenols	Ferric chloride test	+
8	Triterpenoids	Thionyl chloride	+
8	Anthraquinone	Molisch test	+
9	Proteins	Biuret test	+
10	Reducing sugar	Saponification test	+

3.2 Early Phyto-chemical evaluation.

Biochemical evaluation of ethanol extraction of *Zingiber officinalis* rhizomes showed that presence of compounds, Flavonoids, saponins, Tannins (Table.2)

Table.2: Phytochemical analysis

S. No	Phytonutrient	Test	Results
1	Flavonoids	NaOH test	-
2	Tannins	FeCl ₃	+
3	Saponin	Froth test	+
4	Glycosides	Kellerkiliani	+
5	Steroids	Liebermann-Burchard	—
6	Alkaloids	Dragondroff' test	-
7	Phenols	Ferric chloride test	+
8	Triterpenoids	Thionyl chloride	+
8	Anthraquinone	Molisch test	+
9	Proteins	Biuret test	+
10	Reducing sugar	Saponification test	+

3.3 Antimicrobial activity by Agar disk diffusion method:

S. No	Groups	Zone of Inhibition (mm)	
		Escherichia coli	Staphylococcus aureus
1	<i>Caeselpinia pulcherrima</i>	15.8	16.96
2	<i>Zingiberofficinale</i>	20.3	21.5
3	Combination (CP+ZO)	24.8	23.3
4	Standard	27.6	28.9

3.4 Minimum Inhibitory concentration Plant extract

S. No	Groups	Concentration(mg/ml)	
		Escherichia coli	Staphylococcus aureus
1	<i>Caeselpinia Pulcherrima</i>	10	15
2	<i>Zingiberofficinale</i>	5	5
3	Combination (CP+ZO)	5	2.5
4	Standard (Ciproflacin)	2.5	2.5

The synergistic effect observed may result from the complementary mechanisms of the phytoconstituents present in the two plants. Flavonoids can disrupt microbial membranes and inhibit nucleic acid synthesis, while phenolics can denature proteins and inactivate enzymes. The lower MIC values for the combination indicate enhanced potency, consistent with previous studies demonstrating the benefits of combining plant extracts for antimicrobial therapy.

4. CONCLUSION

The findings give evidence supporting the ongoing utilization of *C. pulcherrima* (L) as a form of conventional medicine. Additional study is required to identify the chemical that causes *C. pulcherrima* (L)'s antibacterial properties. In the present investigation, the 70% ethanol extract of the leaves has been shown to inhibit microbial growth in vitro against bacteria that cause illnesses. The present research demonstrated that ginger extracts have beneficial effects and activity against bacteria, as well as the reduction of growth of bacteria was dose dependent. Additionally, the combination of both medicinal plants showed a significant increase in antimicrobial activity, and higher concentrations showed more microbial activity.

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