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IN VITRO EVALUATION OF THE ANTIBACTERIAL EFFICACY OF COMBINED HERBAL EXTRACTS AGAINST *STREPTOCOCCUS PYOGENES*

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ABSTRACT

Objectives:

Herbal extracts including ginger (*Zingiber officinale),* turmeric (*Curcuma Longa*) *and* Saffron (*Crocus sativus)* are known for their antibacterial activities. *Streptococcus pyogenes* is the predominant bacterial pathogen responsible for pharyngitis in both paediatric and adult populations. Over the years, the incidence of antibiotic resistance has been on the rise. Consequently, relying on a single herbal extract is inadequate for preventing bacterial infections. Additional studies are required to investigate the potential benefits of combining various herbal extracts with similar actions to identify bacteriostatic or bactericidal agent. This research aims to determine the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), and *Crocus sativus* (Saffron) against Streptococcus pyogenes.

Methods: Different combinations of herbal extract test solutions were used in Mueller Hinton broth (Different combination of extracts with concentration about 11.00%, 14.10%, 17.20%, 20.30% and 23.50%) and positive control. Mueller-Hinton broth loaded with 1 ml bacterial suspension was used to determine the MIC (Minimum Inhibitory Concentration). The MBC (Minimum Bactericidal

Concentration) was estimated by culturing solutions from different extract dosages onto blood agar plates.

Findings: The MIC (Minimum Inhibitory Concentration) for the combination of herbal extracts against *Streptococcus pyogenes* remains undetermined. The combined extracts exhibited a Minimum Bactericidal Concentration (MBC) of around 23.50%. This indicates that the combination of these herbal extracts has significant antibacterial activity against *S. pyogenes*.

Novelty: Combination of herbal extracts including ginger (*Zingiber officinale),* turmeric (*Curcuma Longa*), *and* Saffron (*Crocus sativus)* showed antibacterial activity against *S. pyogenes*.

Keywords: Herbal extracts, *Curcuma Longa*, *Zingiber officinale, Crocus sativus, Streptococcus pyogenes*, sore throat

1. INTRODUCTION

Pharyngitis, commonly referred to as a sore throat, is characterized by inflammation of the pharynx at the back of the throat. This condition can lead to discomfort, scratchiness, and pain, particularly during swallowing. The common cold is frequently caused by viral infections. Moreover, bacterial infections, particularly those caused by Streptococcus pyogenes, can also be responsible. Symptoms of pharyngitis include swallowing difficulty, a sore or scratchy throat, swollen lymph nodes, fatigue, headache and fever. This condition often leads to a dry, scratchy, and painful sensation, making it uncomfortable to swallow or talk **[1]**. Pharyngitis can result from a variety of causes, including viral infections like the common cold, influenza, or COVID-19, as well as bacterial infections such as strep throat. Other causes of pharyngitis include allergies, irritants such as smoke, and acid reflux. While viral pharyngitis often resolves on its own, bacterial pharyngitis may require antibiotic treatment. Maintaining hydration, getting adequate rest, and consulting a healthcare professional if symptoms continue or deteriorate is crucial. Diagnosis typically involves a physical examination, throat culture, and sometimes blood tests. Treatment varies depending on the cause: viral pharyngitis usually resolves on its own with symptom relief measures, while bacterial pharyngitis requires antibiotics. Practicing good hygiene and avoiding close contact with infected individuals are essential preventive measures. While pharyngitis is typically mild and resolves within a week, it is important to seek medical advice if symptoms persist or worsen. **[2]**

Group A Streptococcus (GAS), also known as *Streptococcus pyogenes*, is a bacterium frequently associated with causing pharyngitis, which is the inflammation of the pharynx. This condition is symptomized

by sore throat, difficulty swallowing, and fever. These bacteria are extremely contagious and can be transmitted via respiratory droplets when an infected person sneezes or coughs. Prompt diagnosis and antibiotic treatment are crucial to prevent complications like rheumatic fever or kidney-related issues **[3]**.

Curcumin, the active compound in turmeric *(Curcuma longa)*, has gained recognition for its powerful antimicrobial properties. It possesses antibacterial properties that are effective against a broad spectrum of both gram-negative and gram-positive bacteria. Curcumin provides antibacterial mechanisms by destroying bacterial cell wall, reducing the production of bacterial virulence factors, and preventing biofilm formation. Additionally, curcumin induces oxidative stress in bacterial cells, further enhancing its antibacterial effects. Studies have demonstrated that curcumin can be particularly effective against certain bacterial strains, such as *Streptococcus pyogenes* and methicillin-sensitive *Staphylococcus aureus*. Its potential as an antibacterial agent is enhanced when used in combination with conventional antibiotics, often resulting in synergistic effects. Despite its promise, challenges such as low bioavailability and stability need to be addressed to fully harness curcumin's antibacterial potential **[4]**.

Ginger, scientifically known as *Zingiber officinale*, is renowned for its potent antibacterial properties. Ginger's antibacterial effectiveness is mainly due to its bioactive constituents, including gingerols, shogaols, paradols, and zingerone. These compounds work synergistically to disrupt bacterial cell membranes, inhibit protein synthesis, and induce oxidative stress within bacterial cells. Ginger has proven effective against a broad spectrum of bacterial pathogens, including both gram-negative and gram-positive bacteria. Its ability to combat antibiotic-resistant strains further highlights its potential as a natural alternative or complement to conventional antibiotics. Despite its promising antibacterial capabilities, challenges such as optimizing its bioavailability and stability remain areas of ongoing research **[5]**.

Saffron, derived from the stigmas of *Crocus sativus*, is renowned not only for its culinary and medicinal uses but also for its potent antibacterial properties. Saffron contains bioactive substances like crocin, crocetin, and safranal. They demonstrate substantial antimicrobial activity against wide range of pathogens. These compounds act by invading bacterial cell wall, obstructing protein biosynthesis and preventing biofilm formation. Studies indicate that saffron is effective against both kind of bacteria,

highlighting its versatility as an antibacterial agent. Moreover, the antibacterial effectiveness of saffron is boosted when incorporated into nanoemulsions, enhancing its stability and bioavailability. However, more research is required to fully comprehend the mechanisms and optimize its application in clinical environments **[6]**.

2. MATERIALS AND METHODS

The research was conducted in September 2024 until February 2025, in the Department of Microbiology, Zydus Wellness Ltd, Ahmedabad. Turmeric with more than 95% curcuminoids were procured from Konark Herbals & Healthcare. Ginger with more than 10% total gingerols were procured from Vidya Herbs. Saffron with more than 30% safranal content procured from Gangwal Healthcare Pvt. Ltd.

Table 1: Plant extracts with marker potency and dosage information

Plant Name	Biological Name	Marker (Active) Compound Name	Marker Potency as per vendor COA	Minimum Dosage of dried plant as per AP^
Turmeric	*Curcuma Longa*	Curcuminoids	NLT 95%	1000 mg
Ginger	*Zingiber officinale*	Gingerols	NLT 10%	1000 mg
Saffron	*Crocus sativus L.*	Safranal	NLT 30%	25 mg

^ According to the Ayurvedic Pharmacopoeia, the recommended therapeutic doses are 1 to 3 grams of turmeric powder, 1 to 2 grams of ginger powder, and 25 to 50 milligrams of dried saffron powder

Table 2: Plant extracts with marker dosage calculation

Plant Name	Marker Content in plant as per literature $	Dosage as per Literature	Dosage per Day per purity of marker compound	Dosage (3x a day) #	Dosage as per 100 % formulation
Turmeric	3.1-3.4%	34 mg	36 mg	12.0 mg	12.0%
Ginger	0.040 - 0.48%	0.05 mg	5 mg	10.0 mg	10.0%
Saffron	0.073-0.078%	0.80 mg	3.0 mg	1.50 mg	1.50%

$ Marker compound content in dried basis of plant as per literatures.
 # Dosage considering three times a day (Either tablet or any fast-dissolving dosage form) [7]
We have made different combinations of Dosages concentrations in Mueller hinton broth

Table 3: Different dosage combinations for MIC & MBC study

Plant Name	D1	D2	D3	D4	D5	Negative Control
Turmeric	6.0%	7.5%	9.0%	10.5%	12.0%	18.0%
Ginger	4.0%	5.5%	7.0%	8.5%	10.0%	15.0%
Saffron	1.00%	1.10%	1.20%	1.30%	1.50%	3.0%
Total	11.00%	14.10%	17.20%	20.30%	23.50%	36.00%

This study utilized five different concentrations along with two control tubes. The positive control was prepared by adding a micro streaker in a few colonies of S. pyogenes to 1 mL of Mueller Hinton broth **(see Figure 1)**. The negative control consisted of a mixture of turmeric, ginger, and saffron, with concentrations of 18% curcuminoids, 15% gingerols, and 3% safranal (totalling 36%) in Mueller Hinton broth (1 mL quantity). In this exercise, serial dilutions of ginger, turmeric, and saffron were prepared in Mueller Hinton broth. The dilutions, containing varying concentrations of the combined extracts, were labelled as D1, D2, D3, D4, and D5, as detailed in the

Table 3. The combinations of extracts, with concentrations of approximately 11.00%, 14.10%, 17.20%, 20.30%, and 23.50%, were prepared using a similar method. It is processed to mix 1 mL of these combination extracts with 1 mL of Mueller Hinton broth in the subsequent tube for each available tube. Once all the extract combinations were prepared, 1 mL of bacterial suspension was added to each tube. The tubes were then incubated atleast 24 hours at 37°C. To ensure high accuracy, the experiment was replicated four times **[8]**.

Figure 1: Positive control for *S. pyogenes* on Mueller Hinton broth

The MIC was identified as per visual inspection, showing that the lowest concentration that could prevent bacterial growth, indicated by a clear solution. The MBC was determined by streaking samples from the previous dilution tests onto a blood agar plate and incubating them atleast 24 hours at 37°C. The MBC was identified as the lowest concentration that eliminated bacteria, indicated by no growth on the media **[9]**.

3. RESULTS & DISCUSSION

Following a 24-hour incubation period, the cloudiness of each tube was evaluated. **Table 1** presents the MIC (determined through four replications) of the combined extracts (ginger, turmeric and saffron) against *Streptococcus pyogenes.*

During the study, the MIC of the combined extracts (ginger, turmeric, and saffron) against *Streptococcus pyogenes* could not be clearly determined due to the colour of the extracts and the turbidity formed at different dosages (see **Figure 2**). The MBC of all the combined extracts against *Streptococcus pyogenes* was estimated by streaking all designed concentration onto a blood agar plate.

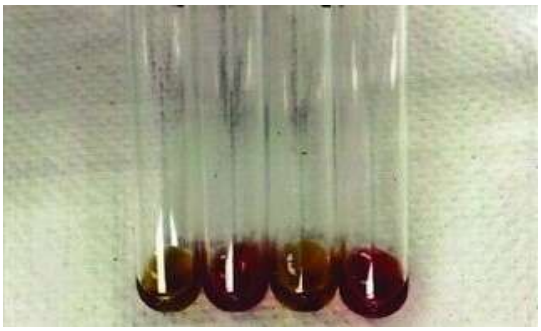

Figure 2: Colour of extracts combination turns red & turbid during MIC study

After a 24-hour incubation period, the streaking results onto a blood agar plate indicated that at D5 (Dosage 5) with 23.50% of total combination of extracts, showing no bacterial growth in any of the replications (see **Figure 3**). Other dosage replications consistently showed the same results. Therefore, MBC required in the study with the combinations of extracts (ginger, turmeric and saffron) against *Streptococcus pyogenes* was approximately 23.50%.

Table 4: MIC of different combination of extracts dosages against *S. pyogenes*

Replication	Positive control	Negative Control	Combination of extracts concentration				
			D1	D2	D3	D4	D5
			11.00%	14.10%	17.20%	20.30%	23.50%
I	++++	--	U.D.	U.D.	U.D.	U.D.	U.D.
II	++++	--	U.D.	U.D.	U.D.	U.D.	U.D.
III	++++	--	U.D.	U.D.	U.D.	U.D.	U.D.
IV	++++	--	U.D.	U.D.	U.D.	U.D.	U.D.

Description:
U.D. : Undecided
+++ : high-dense colony growth
-- : no colony of *Streptococcus pyogenes*

Table 5: MBC of different combination of extracts dosages against *Streptococcus pyogenes*

Replication	Positive control	Negative Control	Combination of extracts concentration				
			D1	D2	D3	D4	D5
			11.00%	14.10%	17.20%	20.30%	23.50%
I.	++++	--	+++	++	++	+	--
II.	++++	--	+++	++	++	+	--
III.	++++	--	+++	++	++	+	--
IV.	++++	--	+++	++	++	+	--

Description:
++++ : Extremely dense colony growth due to positive control
+++ : high-dense colony growth
++ : Dense colony growth
+ : Low-dense colony growth
-- : Not a visible colony growth

Figure 3: Incubated plate (24 hrs) with five different dosages against *Streptococcus pyogenes*
Description:
D1 – Dosage 1 : Maximum colony growth of *Streptococcus pyogenes*
D2 – Dosage 2 : Visible colony growth of *Streptococcus pyogenes*
D1 – Dosage 3 : Small colony growth of *Streptococcus pyogenes*
D1 – Dosage 4 : Slight colony growth of *Streptococcus pyogenes*
D1 – Dosage 5 : No colony growth of *Streptococcus pyogenes*

Ginger (*Zingiber officinale*), Turmeric (*Curcuma Longa*) and Saffron (*Crocus Sativus*) exhibits antibacterial activity against *Streptococcus pyogenes*. This activity is attributed to various bioactive compounds present in these therapeutically active compounds. Particularly oleoresins and essential oils. Ginger comprises several key antimicrobial compounds, including gingerol, geraniol, limonene, zingiberol, linalool, citral etc. **[10]** Curcumin includes Curcumin I (diferuloylmethane), Curcumin II (desmethoxycurcumin) and Curcumin III (bisdemethoxycurcumin) **[11]**. Additionally, Saffron includes Safranal, Crocin, Crocetin & Picrocrocin **[12]**.

These active markers exert their antimicrobial effects by denaturing bacterial proteins and disrupting the cytoplasmic membrane. They act by compromise the structural integrity of bacterial cell membranes. Additionally, they induce microbial protein denaturation. as an antimicrobial agent through two mechanisms: it alkylates nucleophilic groups and denatures microbial proteins, leading to enzyme inactivation within the bacteria. All extracts work together synergistically to combat bacteria, either inhibiting their growth or killing them outright. Together, these extracts can work more effectively against bacterial pathogens, potentially offering a natural alternative to conventional antibiotics **[13]**. The study found that the MBC for a mixture of *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric), and *Crocus sativus* (Saffron) against *S. pyogenes* was consistently 23.50% across all trials. The combination of extracts from *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric), and *Crocus sativus* (Saffron)

demonstrated the ability to eradicate *Streptococcus pyogenes* at a concentration of 23.50%, achieving a 100% kill rate. These experimental results suggest that these combinations have significant potential as natural antibacterial agents against *S. pyogenes*. Further research is necessary to explore different combinations of extracts to uncover additional antibacterial properties and minimize bacterial resistance.

4. CONCLUSION

The research concluded that determining the MIC (Minimum Inhibitory Concentration) was challenging due to the natural turbidity of the combined extracts of *Zingiber officinale* (Ginger), *Curcuma longa* (Turmeric), and *Crocus sativus* (Saffron). This turbidity affected the results of different extract combination Dosages. The MBC (Minimum Bactericidal Concentration) of combined extracts effective for *Streptococcus pyogenes* was found to be approximately 23.50% for combined extracts. These findings suggest that the combination of extracts from Ginger, Turmeric, and Saffron exhibits potential antibacterial activity against *S. pyogenes*.

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