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Synergistic Antibacterial Effects of Garlic, Onion and Ginger Polyherbal Extract with Azithromycin Against Periodontal Pathogens: A Promising Approach to Combat Resistant Infections

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Abstract

Microbial infections pose a significant challenge to modern medicine. This study evaluated the antibacterial properties of Garlic (Allium sativum), Onion (Allium cepa), and Ginger (Zingiber officinale, individually and as a polyherbal extract (GOG), alone and with azithromycin, to assess synergistic effects. Antibacterial activity was tested against gram-negative anaerobic oral pathogen Prohormones gingival is, Treponema DeNicola, and Annarella forsythia common causes of periodontitis. Using the MIC method, the combination of GOG and Azithromycin showed inhibition rates of 89.76%, 85.89%, and 78.02% for T. forsythia, P. gingival is, and T. DeNicola, respectively, at 320 µg/ml. Phytochemical analysis revealed bioactive compounds, including steroids, alkaloids, flavonoids, tannins, and glycosides. These findings suggest that GOG and azithromycin offer a promising alternative for treating resistant bacterial infections in periodontal disease.

Keywords: gog extract; antibacterial activity; synergism; mic; azithromycin

Introduction

Periodontitis, a chronic inflammatory disease affecting the tissues surrounding the teeth, is primarily caused by a complex biofilm of anaerobic bacteria. Traditional antibiotic treatments often face challenges such as antibiotic resistance and adverse side effects. Plant-based polyherbal extracts have gained attention due to their potential antibacterial properties and minimal side effects (Kwon et al., 2021). Herbs have been used medicinally for a very long time; written records go back to 2800 BC. Today, herbal therapy is still widely used in many nations. Herbs have a few benefits over prescription medications, one of which is that they often carry a lesser risk of adverse effects and long-term safety issues. In addition, compared to pharmaceutical drugs, herbs are frequently more readily available and less expensive (Tamura, 2005). The phenomenon of antibiotic resistance has existed since the discovery of antibiotics. These drugs are derived from natural substances produced by fungi and certain bacteria as a defines mechanism. However, the bacteria that produce these antibiotics have also evolved ways to protect themselves. The increasing menace of antibiotic-resistant bacteria has become a global health concern. Intensive care specialists consider it a major challenge in inpatient treatment. As a result, there has been renewed interest in exploring the potential of herbs as an anti-microbial agent against resistant strains of bacteria.

Garlic, Onion, and Ginger are common food items with known medicinal properties. The text highlights the growing interest in herbal medicines as a potential solution to the problem of antibiotic resistance. It specifically mentions garlic, onion, and ginger as three common food items with recognized medicinal properties. These plants have been shown to have various health benefits, including cardiovascular health, diabetes management, and cancer prevention (Zine & Nilesh, 2015). The study focuses on the potential synergistic effects of plant extracts and antibiotics. It aims to investigate if combining extracts from garlic, onion, or ginger with traditional antibiotics could enhance their effectiveness against certain bacteria. The growing worry about the emergence of antibiotic-resistant bacteria, which poses a serious risk to public health, inspired this study.

Review of literature

Garlic

The perennial bulbous plant Allium sativum, popularly referred to as garlic, is a member of the

Amaryllidaceae family. Its flat, linear leaves, range in width from 0.5 to 1 inch, giving it a height of up to 1 meter. In the Northern Hemisphere, the plant has bisexual flowers that bloom in pink or purple during the summer and are pollinated by insects such as moths, butterflies, and bees. Garlic can be produced in harsh northern climates like Alaska if the right circumstances are met. Its bulb contains 10 to 20 cloves and is shielded by thick leaves (El-Saadony et al., 2024). Garlic's cultural and therapeutic importance is reflected in its many names, including Ail, Ajo, Camphor of the Poor, Nectar of the Gods, and Stinking Rose. Originating from its bulb, it belongs to the kingdom Plantae, whose taxonomic hierarchy includes Sativum and the genus Allium. A number of garlic's health advantages are attributed to its abundance of sulphur-containing chemicals, particularly allicin. Polysaccharides, phenolic chemicals like β-reconcile acid, and saponins, which are particularly abundant in purple garlic, further influence its pharmacological activities. Due to these bioactive components, garlic is a highly prized plant in both conventional and alternative medicine, with a variety of uses in health and well-being (Fejes et al., 2024).

Onion

One of the most well-liked and extensively grown vegetables in the world is the onion (Allium cepa), a member of the Amaryllidaceae family. Its unique flavour, culinary adaptability, and commercial significance make it extremely valuable. Onions have long been used for their therapeutic qualities, but despite their extensive use and possible health advantages, little thorough research has been done on their traditional and pharmacological characteristics. Studies have looked into the bioactive ingredients in onions and their possible health effects, especially their antibacterial qualities, to close this gap. Even though the first results are encouraging, more research is necessary to completely comprehend and utilize onions' therapeutic potential. Onions, often called eschalot, top onion, or Japanese leek, are the biological source of their dried bulbs. According to botany, they belong to the kingdom Plantae, which includes the division Monocot, the super division Angiosperms, the subkingdom Tracheophytes, and the order Aspergils, which includes the species cepa and the genus Allium (Elattar et al., 2024). Bioactive chemicals such as quercetin, gallic acid, and ferulic acid are among the many phenolic compounds found in onions. The type of onion affects the content of these substances. Notably, red onions are rich in anthocyanins, which are pigments that give them their colour and certain health advantages. These anthocyanins, which add to onions' medicinal potential, include cyanidin, peonidin, and special derivatives such as carboxypyranocyanidin. All of these substances point to onions as a pharmacologically promising and nutritionally significant vegetable that merits more study to optimize its advantages (Kiran et al., 2024).

Ginger

One of the most popular spices and therapeutic herbs in the world is ginger (Zingiber officinale), a rhizomatous perennial plant in the Zingiberoside family. Ginger, a native of Southeast Asia, is well known for its culinary uses, fragrant qualities, and wide range of traditional medical benefits. It has a long history of being used as a remedy for several conditions, such as respiratory infections, nausea, inflammation, and digestive issues. Even with its wide-ranging traditional use, more thorough research is still required to completely understand its pharmacological potential and mechanisms of action. According to studies, ginger has a strong bioactive profile, especially anti-inflammatory, antioxidant, and antibacterial qualities that support its wide range of medicinal uses (Zeeshan et al., 2024). Asian ginger, Jamaica ginger, and real ginger are some of the synonyms for ginger, which is derived from the rhizome of Zingiber officinale. Classified botanically under the kingdom Plantae, its taxonomic hierarchy includes the division Monocot, the class Zingiberales, the order Zingiberaceae, the genus Zingiber, the species officinale, the subkingdom Tracheophytes, and the super division Angiosperms. Gingerols, shogaols, and paradols are the most common bioactive chemicals found in ginger, while there are many others. It has strong anti-inflammatory and anti-cancer properties, and its distinctive pungency is due to gingerols, especially [6]-gingerol. Heat or drying transforms gingerols into shogaols, which have stronger anti-inflammatory and antioxidant qualities. Further contributing to its aromatic and medicinal qualities are essential oils including bisabolene, curcumin, and zingiberone. These chemicals highlight the therapeutic value of ginger and make it a promising option for additional study and advancement in the pharmacological and functional food sectors (Ahmed Abdelmawgood et al., 2024).

Methodology

Collection, identification and extraction of plant materials

Fresh plant material was obtained from the local vegetable market in Davangere, Karnataka, India, and verified by registered botanist Dr. Halesh C. of the Department of Botany, Davanagere University, and Davanagere, based on morphological features (Figure 2). To get rid of extra moisture, and dirt the gathered plant parts were cleaned with sterile distilled water and patted dry with a fresh cloth. Each 30 grams of raw material was chopped into tiny pieces for effective extraction. Fresh juice was extracted from the prepared material by pressing it with a sterile muslin cloth. It was then promptly transferred to a sterile container, carefully sealed, and subjected to a preliminary phytochemical property analysis.

Preliminary Phytochemical Investigation

Preliminary phytochemical inquiry provides an overview of the phytochemicals found in a crude drug extract and serves as a foundation for additional research by consulting standard literature (Khandelwal KR, Kokate CK, Pawar AP, n.d.).

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is a critical parameter in microbiology that quantifies the lowest concentration of an antimicrobial agent (e.g., antibiotic, disinfectant, or natural product) that inhibits the visible growth of a particular microorganism. It is a fundamental tool for understanding the susceptibility of microorganisms to antimicrobial agents and for guiding therapeutic decisions.

Test organisms

The antimicrobial activity was assessed against key oral pathogenic test organisms, including T. forsythia, P. gingivalis, and T. denticola, which are anaerobic, gram-negative bacteria associated with periodontal diseases.

Sample Preparation and Standardization

A 32 mg sample was dissolved in water. The CLSI guidelines were followed in the preparation of the bacterial inoculum. The McFarland turbidity of 0.5 (10.8 CFU/mL) was found by adjusting the OD600 value, as supported by a calibration curve specific to each microbe.

Inoculum and Sample Preparation

Bacterial cultures cultivated on LB broth were used to create a cell suspension. The concentration of the cells was brought down to 1-2 x 10^8 cells/ml. After making stock solutions weighing 320 µg, these were diluted twice in broth to yield concentrations of 160, 80, 40, 20, and 10 µg/ml.

Drug and Sample Spike Concentration

A combination of 320 µg of sample and 30 µg of Azithromycin was prepared and diluted twofold in broth, resulting in concentrations of 160-15, 80-7.5, 40-3.25, 20-1.25, and 10-0.625 µg/mL of sample with Azithromycin. LB broth was inoculated with the respective culture without the addition of any test compounds as a control.

Procedure

A 96-well plate was filled with 10 µL of bacterial inoculum (1–2 × 10^8 CFU/mL) and 90 µL of test samples at different concentrations. 10 microlitres of the inoculum were mixed with 90 microlitres of drug-free LB broth as the control. The plates were incubated for 24 hours at 37 °C. After that, 30 µL of resazurin (0.015%) was added to each well. Colour shifts were noticed during an additional two to four hours of incubation. Those wells with concentrations exceeding the MIC threshold were detected as having no colour change (blue resazurin remained constant). As a sign of bacterial viability, active bacterial metabolism changed the colour of resazurin from purple-blue to pink or colourless (Figure 1) (Saquib et al., 2019). Spike recovery was calculated as per formula which mentioned below. Spike Recovery = % Inhibition of spike- % Inhibition of sample (Synergism effect).

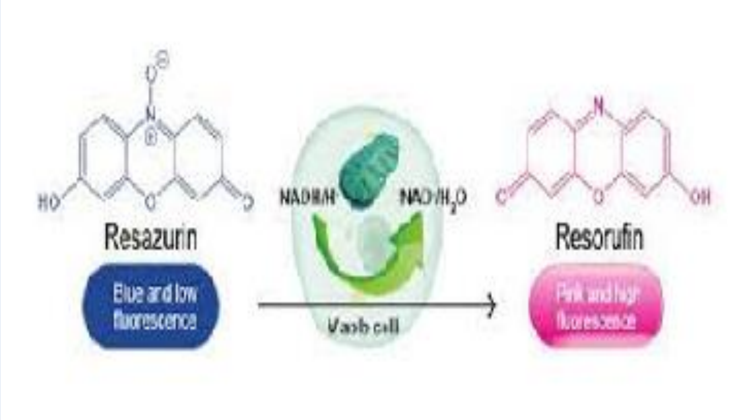

Figure 1: General chemical reaction involved in antimicrobial activity

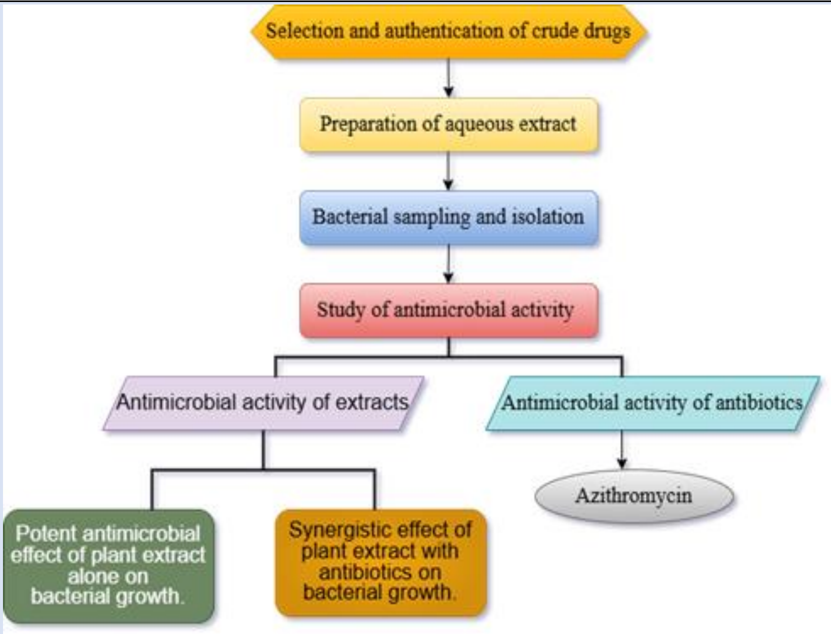

Figure 2: Schematic representation of the antimicrobial activity of individual extracts and GOG

Result and Discussion

The phytochemical profiles of garlic, ginger, onion, and their polyherbal combination extract (GOG) were examined in the study using conventional qualitative methods. Alkaloids, glycosides, proteins, carbohydrates, tannins, flavonoids, and steroids were found in all extracts except ginger, according to the results. A wide variety of bioactive components were present in the GOG extract, demonstrating its varied phytochemical profiles.

Table 1: Results of preliminary phytochemical investigation.

Sl. No	Chemical constituents	Photochemical tests	Ginger Extract	Onion Extract	Garlic Extract	GOG Extract
1	Alkaloids	a. Dragendroff's test	+VE	-VE	-VE	+VE
		b. Hager's test	+VE	+VE	-VE	+VE
2	Glycosides	a. Keller Killiani	+VE	-VE	+VE	+VE
		b. Modified Brontrager's	-VE	-VE	+VE	+VE
3	Carbohydrates	a. Molish test	-VE	+VE	+VE	+VE
		b. Fehling's test	+VE	-VE	-VE	-VE
		c. Benedict's test	+VE	-VE	-VE	+VE
		d. Barfoed's test	-VE	-VE	+VE	-VE
4	Proteins	a. Million's test	-VE	-VE	-VE	-VE
		b. Xanthoprotein test	-VE	-VE	-VE	-VE
5	Tannins	Lead acetate test	+VE	-VE	+VE	+VE

		Dilute Iodine Solution	+VE	-VE	+VE	+VE
6	Flavonoids	Shinoda test	+VE	+VE	-VE	+VE
7	Steroids	Salkowski test	+VE	-VE	-VE	+VE

To assess the test compounds' synergistic antibacterial efficacy against T. forsythia, the study used sample recovery and spike recovery percentages at various doses (10–320 µg/mL) was represented in Figure 3 & 6). At 10 µg/mL, the synergistic impact was minimal, but it enhanced as concentrations increased. While spike recovery was modest at 3.84%, sample inhibition rose to 22.04% at 20 µg/mL. Sample inhibition reached 32.68% and spike recovery reached 21.84%, indicating significant inhibition at 40 µg/mL and higher. At 80 µg/mL, the trend persisted, with sample and spike inhibitions rising to 42.46% and 35.90%, respectively. At the maximum dose of 320 µg/mL, spike recovery decreased to 30.77% and inhibition reached 58.99%. Potential synergistic interactions between test chemicals and the target organism are suggested by the findings (Table 2).

Table 2: Minimum inhibitory activity (MIC) of tested compound against T. 5orsythia

T.Forysthia	Conc µg/ml	OD @517 of sample	OD @517 of Spike	%Inhibition of spike	% Inhibition of Sample	Spike Recovery	Outcome
	Control	0.7803	0.781	0.00	0.00	0.00	Synergism
	10	0.642	0.618	20.94	17.72	3.21	
	20	0.6083	0.579	25.88	22.04	3.84	
	40	0.5253	0.355	54.52	32.68	21.84	
	80	0.449	0.169	78.35	42.46	35.90	
	160	0.3896	0.094	87.97	50.07	37.90	
	320	0.32	0.080	89.76	58.99	30.77	

The investigation used optical density measurements at 517 nm to assess the test drugs' synergistic effectiveness against P. gingivalis. The compounds had a dose-dependent impact, with higher concentrations exhibiting more antibacterial activity, according to the data (Figure 4). The chemical mixture's improved inhibitory potential was further highlighted by the low spike recovery percentages, particularly at higher doses. This points to a potential use of the investigated chemicals in P. gingivalis-targeting treatment approaches. A notable rise in synergistic effects was seen in the suppression of spike and sample recovery across different doses. A robust synergistic effect was shown by the modest inhibition of spike and sample recovery. According to the study, the chemicals may be employed in P. gingivalis-targeting therapy techniques (Table 3).

Table 3: Minimum inhibitory activity of the tested compound against P. Gingivalis

	Conc µg/ml	OD @517 of sample	OD @517 of Spike	% Inhibition of spike	% Inhibition of Sample	Spike Recovery	Outcome
P. Gingivalis	Control	0.7903	0.815	0.00	0.00	0.00	Synergism
	10	0.692	0.709	13.01	12.44	0.57	
	20	0.6183	0.628	22.94	21.76	1.18	
	40	0.5353	0.472	42.09	32.27	9.82	
	80	0.469	0.302	62.94	40.66	22.29	
	160	0.4293	0.212	73.99	45.68	28.31	
	320	0.376	0.115	85.89	52.42	33.47	

The findings revealed a little 5.82% inhibition and no synergistic impact at 10 µg/mL. The inhibition rose to 10.64% and the spike inhibition to 21.24% at 20 µg/mL. At 10.60%, the spike recovery stayed constant. Significant synergistic effects were seen at higher dosages, with sample inhibition rising to 23.03%, spike recovery to 11.83%, and spike inhibition to 34.86%. The effects were most noticeable at 160 µg/mL and 320 µg/mL. Sample inhibition was 35.05%, spike inhibition was 65.75%, and spike recovery was 30.70% at 160 µg/mL. Sample inhibition was 41.76% and spike inhibition peaked at 78.02% at 320 µg/mL (Figure 5). Although substantial suppression is shown at higher dosages, these results point to a concentration-dependent synergistic impact (Table 4).

Table 4: Minimum inhibitory activity of the tested compound against T. Denticola

T. denticola	Conc µg/ml	OD @517 of sample	OD @517 of Spike	% Inhibition of spike	% Inhibition of Sample	Spike Recovery	Outcome
	Control	0.789	0.7717	0.00	0.00	0.00	Syne gism
	10	0.743	0.6369	16.55	5.82	10.73	
	20	0.705	0.5987	21.24	10.64	10.60	
	40	0.607	0.4877	34.86	23.03	11.83	
	80	0.571	0.3283	54.40	27.58	26.82	
	160	0.512	0.2359	65.75	35.05	30.70	
	320	0.459	0.1359	78.02	41.76	36.27	

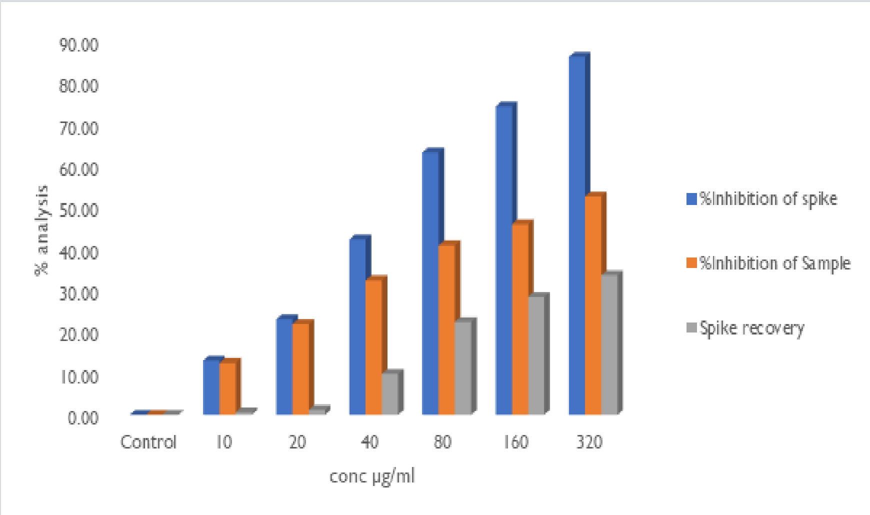

Figure 3: Graphical Representation of MIC values of P. gingivalis

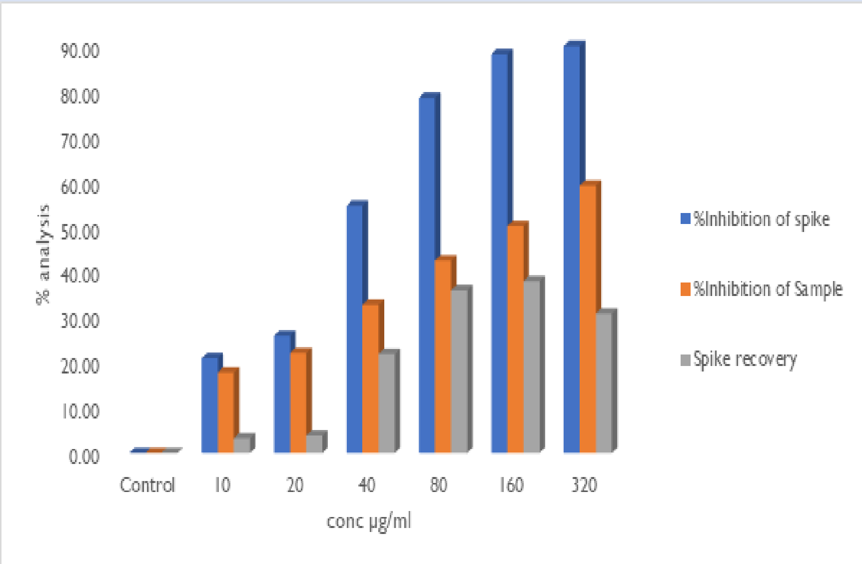

Figure 4: Graphical Representation of MIC values of T. forysthia

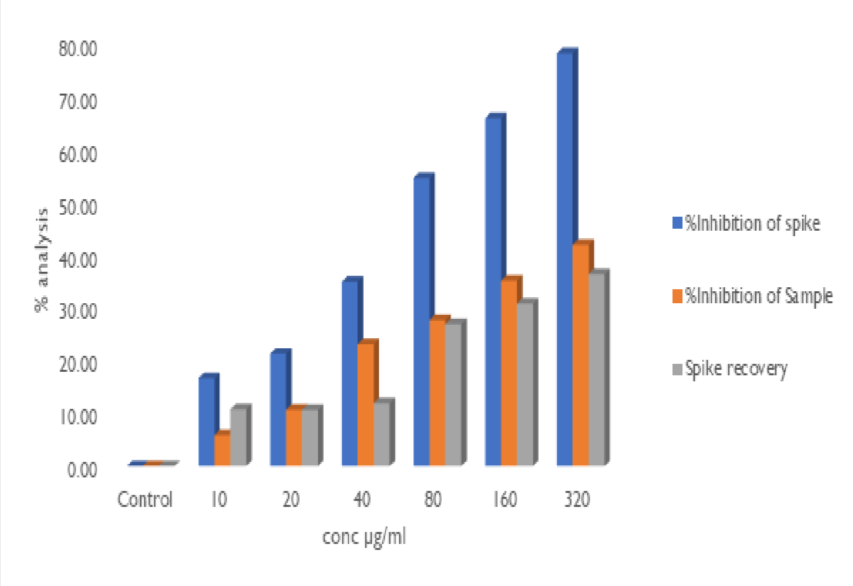

Figure 5: Graphical Representation of MIC values of T. denticola

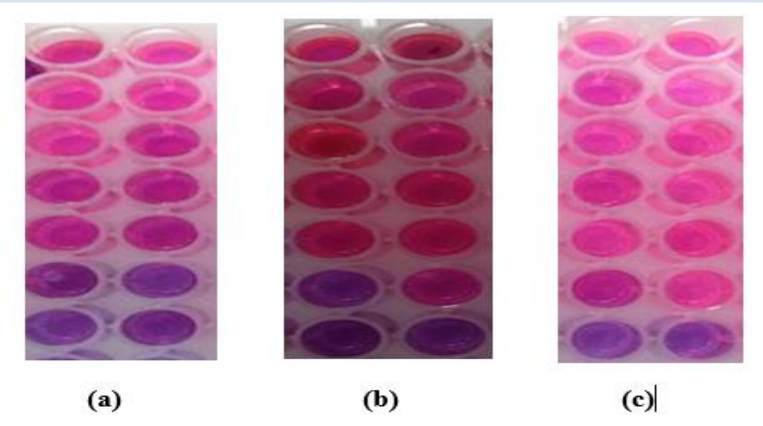

Figure 6: Results of antimicrobial activity of GOG extracts against (a) T. forsythia (b) P. gingivitis and (c) T. denticola

Conclusion

The polyherbal combination (GOG) extract of ginger, garlic, and onion has a wide range of bioactive components, including alkaloids, glycosides, tannins, flavonoids, and steroids, according to a study on the phytochemical profiles of these substances. The GOG extract demonstrated strong inhibitory effects against P. gingivalis, with spike inhibition reaching 85.89% at the highest dose, and significant synergistic antibacterial efficacy against T. forsythia, P. gingivalis, and T. denticola, with inhibition increasing to 58.99% at 320 µg/mL. The study suggests that the GOG extract and its components have significant antimicrobial potential, with enhanced synergistic effects at higher concentrations.

Declarations

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

Debayan Bhattacharjee conceptualized and designed the study, contributed to data analysis, and supervised the research process. Mohit K was responsible for conducting the experiments, collecting data, drafting the manuscript, and assisting in data interpretation. Prathibha G S provided critical revisions to the manuscript, drafted the manuscript, and contributed to validating findings. All authors reviewed and approved the final manuscript.

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