

In Vitro Comparison of Antimicrobial Efficacy of Garlic, Curcumin and the Combination (Turmeric Garlic Composite) Against Common Oral Pathogens

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ABSTRACT:

Background: Medicinal plants have been used traditionally to cure numerous diseases. Recently, there has been a sudden increase in the use of herbal extracts as an alternative approach to modern day medicines due to the advantages of being safe besides being affordable and ease of procurement. Garlic and Curcumin possess antimicrobial properties against oral pathogens. **Aim:** To evaluate the antimicrobial efficacy of Garlic, Curcumin, and the composite of Garlic-Curcumin against oral pathogens and to compare the efficacy of these extracts with the standard antimicrobials Amoxycillin and Nystatin. **Methodology:** The fresh forms of Turmeric, Garlic and Turmeric garlic composite were powdered and extracts were obtained by Soxhlet's extraction process. The antibacterial efficacy was assessed by broth dilution and disc diffusion assay. **Results:** The Turmeric-Garlic composite exhibited the maximum antibacterial efficacy with the highest zone of inhibition against oral pathogens followed by Garlic and the difference between these were statistically significant

INTRODUCTION:

Herbology is the study of plants for medicinal purpose, which is of great interest in medicine and dentistry in the current era¹. Plants are natural sources of antibacterial agents. Plant-derived medicines have been a part of our traditional health care system and the antimicrobial properties of these compounds are well documented. Herbal medicines are more effective and less harmful as they have negligible side effects^{2,3}. They exhibit low mammalian toxicity as compared to synthetic medicines which excludes hypersensitivity, immune-suppression and allergic reactions^{4,5}. The adverse effects associated with antimicrobial agents and the continuous evolution of bacterial resistance has necessitated the search for novel and effective antimicrobial compounds from natural sources like plants and herbs⁶. Spices are the flavor of life. Many spices used have antimicrobial and medicinal properties¹⁰. Turmeric (*Curcuma longa*), which is commonly and widely used as a home remedy, has been in focus in the field of medicine since long. Turmeric is a member of the Zingiberaceae family. Curcuminoids are the main component of turmeric which contains curcumin (diferuloylmethane), demethoxy-curcumin, and bisdemethoxycurcumin. Amongst them curcumin is the main component of turmeric responsible for biological properties. Isolation of curcumin was first done by Vogel and Pelletier in

1815. J Milobedzka and V Lampe in 1910 determined its chemical structure⁷. Garlic, botanically known as *Allium sativum* L is prophylactic as well as therapeutic medicinal plant. The word garlic is derived from the old English word garleac. The 'gar' means spear (referring to spear shaped leaves) and 'leac' means leek. It is common ingredient of many dietary medicinal formulations of Ayurveda¹¹. Though there is extensive literature stating the antibacterial effects of garlic and turmeric against oral pathogens, no research has stated the effectiveness of the combination. Hence we formulated a composite (garlic+ turmeric) to harness their synergistic effect. This study aimed to compare the antibacterial efficacy of garlic, curcumin and the combination against the common oral pathogens against the standard antimicrobials Amoxycillin and Nystatin.

MATERIALS AND METHODS :

PREPARATION OF EXTRACTS OF GARLIC CURCUMIN COMPOSITE :

The fresh forms of garlic cloves were collected, made into pieces, dried at room temperature and finely grounded to powder. The fresh forms of Curcumin were collected, made into pieces, dried at room temperature and finely grounded to powder. The Turmeric Garlic

Composite was prepared by combining the powders of Curcumin and garlic in the ratio of 1:1 (20gm). This was subjected to infusion method of Soxhlet extraction at 60 degree celsius for 6 hours. The solvents thus obtained were evaporated and the dried extracts were collected and stored in sterile containers. The standard antibiotic Amoxycillin and anti fungal Nystatin were procured for comparative analysis.

MICROBIOLOGICAL ANALYSIS:

The bactericidal activity was determined by agar well diffusion method. Nutrient agar and Nutrient broth was used for the four bacterial cultures whereas the Potato Dextrose agar and broth was be used for *Candida albicans* (fungi). Standard fungal and microbial cultures were sourced from Microbial Test Collection Centre Chandigarh. The organisms under the study were *Streptococcus mutans* (MTCC890), *Enterococcus faecalis* (MTCC 439), *Lactobacillus acidophilus* (MTCC10307), *Escherichia coli* (MTCC44), *Candida albicans* .(MTCC227).

DETERMINATION OF MIC:

Bacterial Strains were Grown overnight on MHA Plates at 37°C before being used. The antimicrobial activity of extract was examined using the standard broth dilution method. The MIC was determined in MHA broth using serial two-fold dilutions of extract and the measurements were obtained in triplicates to confirm the value of MIC for each tested bacteria.

DISC DIFFUSION ASSAY:

Agar plates were inoculated with a standardized inoculum of the test microorganism and filter paper discs

(about 6 mm in diameter), containing the test compound at a desired concentration, were placed on the agar surface using sterile forceps. The antimicrobial agent diffused into the agar and inhibited germination and growth of the test microorganism and the diameters of inhibition growth zones were measured using a millimeter scale. The antibiotic disc (Amoxycillin) was kept as control against the bacteria and Nystatin against *Candida* and incubated. Reading was taken at 600 nm using ELISA plate reader (TECAN -MODEL)

STATISTICAL ANALYSIS:

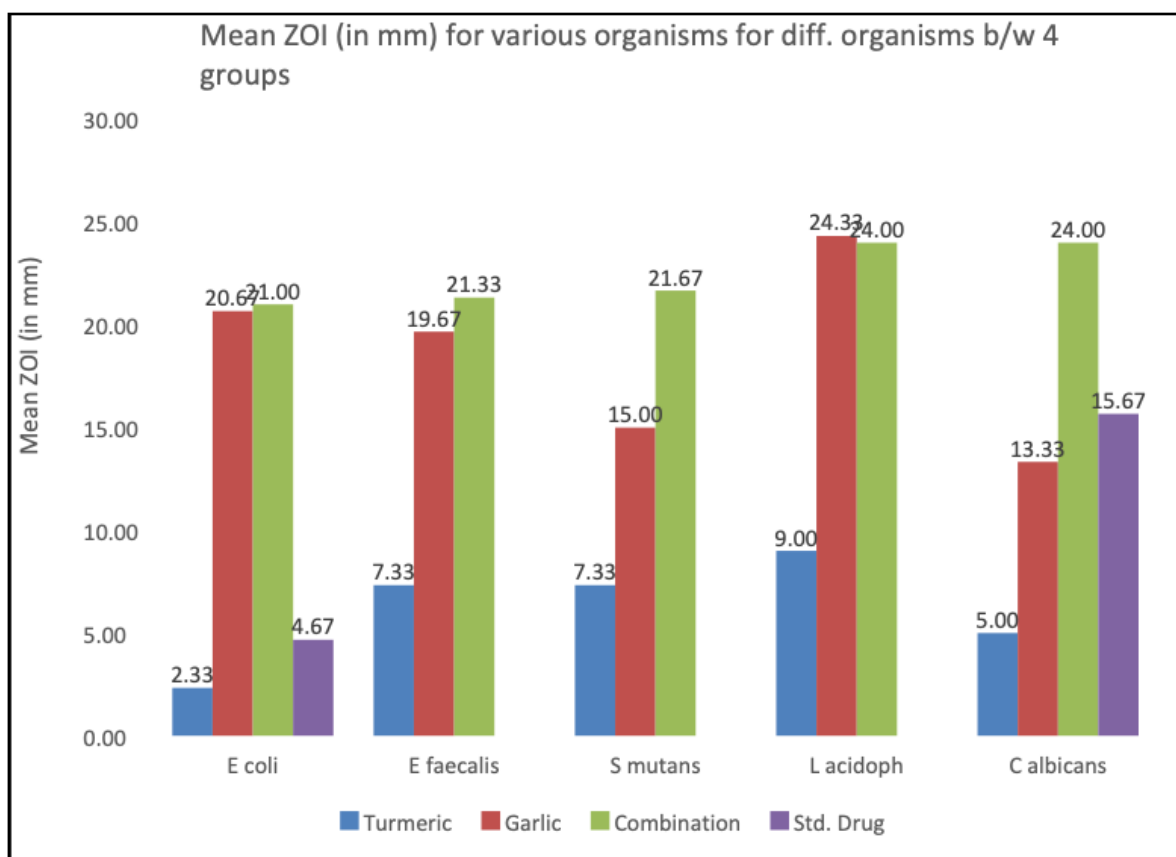
Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Zone of Inhibition was expressed in terms of Mean & SD for each study group. Kruskal Wallis Test followed by Dunn's Post hoc test was used to compare the mean ZOI between different groups against study organisms. The level of significance was set at $P < 0.001$.

RESULTS:

Table 1 shows the mean zones of inhibitions for garlic, curcumin, combination and the standard antimicrobials Amoxycillin and Nystatin against *S mutans*, *L acidophilus*, *E faecalis*, *E coli* and *C albicans*. The zone of inhibition of Turmeric-Garlic composite was significantly higher than Turmeric and standard antimicrobials Amoxycillin and Nystatin. The Turmeric-Garlic composite was more effective than Garlic against *S mutans* and *C albicans* which was statistically significant. This study also showed that Amoxycillin was not effective against *E faecalis*, *S mutans* and *L acidophilus*.

TABLE 1:

Comparison of mean ZOI (in mm) for various organisms b/w different study groups using Kruskal Wallis Test									
Bacteria	Turmeric		Garlic		Combination		Std. Drug		p-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
E coli	2.33	4.04	20.67	1.53	21.00	3.00	4.67	0.58	<0.001*
E Faecalis	7.33	0.58	19.67	1.53	21.33	1.53	0.00	0.00	<0.001*
S Mutans	7.33	0.58	15.00	1.00	21.67	1.53	0.00	0.00	<0.001*
L Acidophilus	9.00	1.00	24.33	1.53	24.00	2.00	0.00	0.00	<0.001*
C Albicans	5.00	4.36	13.33	0.58	24.00	1.00	15.67	1.53	<0.001*



DISCUSSION:

Herbs are a source of medicinal compounds since ancient times. These herbal extracts are used in different systems of medicine for the treatment of various ailments, as well against viral and fungal infections. The extracts of curcumin and garlic possess antibacterial and antioxidant properties and can be used in the prevention and cure of diseases. They can be used in place of chemotherapeutics due to their natural constituents and have been investigated in different parts of the world. The present study showed that garlic was very effective against all the tested micro organisms. This was in accordance with the study conducted by Mansour amin et al, Motamayel et al. It was much better than the standard drug Amoxicillin with a statistically significant difference. Garlic in combination with curcumin was more effective than the individual components as well as the standard antibiotics. A statistically significant difference was seen in relation to S mutans and C albicans. The genetic and structural differences of the bacterial strain may play a role in the bacterial susceptibility to the constituents like garlic, curcumin and the combination. Garlic is a strong antimicrobial agent and acts as an inhibitor on both gram-positive and gram-negative bacteria. Allicin (allyl 2-

propenethiosulfinate or diallyl thiosulfinate) is the principal bioactive compound present in garlic. When garlic is chopped or crushed, allinase enzyme is activated and produce allicin from alliin (present in intact garlic) which is responsible for the antimicrobial action¹⁶.

Turmeric is considered as a disinfectant since ages. Components of turmeric are named curcuminoids (curcumin or diferuloyl methane, demethoxycurcumin and bisdemethoxycurcumin). These components are polyphenols and possess strong antioxidant activity¹⁴. The stability and assembly of FtsZ protofilaments as a crucial factor for bacterial cytokinesis are introduced as a possible drug target for turmeric as an antibacterial agent. Curcumin suppresses the cytokinetic Z-ring formation without significantly affecting the segregation and organization of the nucleoids. It was demonstrated that curcumin reduces the bundling of FtsZ protofilaments associated with the binding ability to FtsZ with a dissociation constant of 7.3 μM . It was shown that curcumin via inhibition of assembly dynamics of FtsZ in the Z-ring can possibly suppress the bacterial cell proliferation as one of the probable antibacterial mechanisms of action¹⁵.

The antimicrobial efficacy of the combination was more compared to the other groups and the standard antibiotic

Amoxicillin and anti fungal Nystatin especially against *S mutans* and *C albicans*. This can be attributed to the synergistic effect of antibacterial effects of curcumin and Garlic.

Several studies have shown that curcumin exhibits synergistic antibacterial effects when combined with traditional antibacterial agents other natural products which is in accordance the results of our study²⁷.

This combination can be used in the formulation of mouth rinses, root canal irrigants, medicaments, anti fungal, anti inflammatory gels and ointments. However, there is a need for more studies to substantiate this.

CONCLUSION:

- Turmeric and garlic extracts have substantial antibacterial effect and their combination increases its antibacterial efficacy or zone of inhibition against all the tested oral pathogens.
- Amoxicillin was not effective against any of the tested microorganisms except *E coli*.
- Turmeric-Garlic combination showed significantly higher antibacterial efficacy against *E.coli* and *S.mutans*

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