

Antimicrobial effect of garlic on oral microbes – An *in vitro* study

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ABSTRACT

Introduction: There are many different treatment modalities for treating the microorganisms present in the oral cavity. Due to the frequent use and misuse of the therapeutic agents has led to the occurrence of adverse effects and development of resistant strains. Hence, the need for natural medications is in higher demand. Garlic and ginger are the medicinal plants which are known for their antimicrobial effects against the various pathogens. Garlic and ginger have a wide variety of properties such as antibacterial, anti-inflammatory, and antioxidant properties. This article is mainly focused to determine the antibacterial effect of ginger and garlic on subgingival plaque of periodontitis patients. **Materials and Methods:** This study is conducted with the ethanolic extract of garlic. The extract is used undiluted. Sterile antibiotic disks were impregnated with 15 μ L of the extract. Standard strain of coagulase-negative *Staphylococcus* and *Lactobacillus* was grown on surface agar culture. They were suspended separately in normal saline with turbidity matching 0.5 McFarland Standard. These standard strains were coated on nutrient agar on Petri dish. Then, the disk containing the garlic extract was placed on it. Then, the plates were incubated at 37°C overnight. After incubation, the plates were examined for zone of inhibition. **Results:** After 24 h, on examination there was no zone of inhibition on the agar plates for both the organisms-lactobacillus and coagulase-negative *Staphylococcus*. **Conclusion:** All the isolates used, in our study, were not susceptible to the garlic extracts.

KEY WORDS: Antimicrobial, Garlic, Microbes, Oral, *Staphylococcus*

INTRODUCTION

Herbal medicine is an increasingly common form of alternative therapy throughout the world. Consequently, herbal medicines are finding their more and more usefulness in the arena of dentistry and their armamentarium. Herbal extracts are effective because they interact with specific chemical receptors within the body. Herbal medicines have less side effect in comparison with traditional medicines, but side effects do occur and are safer to use than conventional medications.^[1]

Herbal products can vary in their potency. Therefore, care must be taken in their selection. The biggest challenge and problem are the lack of information about the effect of herbs on oral tissues, mechanism of action, and side effects. Herbal medicines have two special characteristics that differentiate them from chemical drugs that are use of crude herbs and

prolonged usage. Experience has shown that there are real benefits in the long-term use of whole medicinal plants and their extracts, since the constituents in them work in conjunction with each other.^[2] Several popular conventional drugs on the market are from various herbs. Natural products have been used for thousands of years in folk medicine and they are believed to be the new source of antimicrobial agents.

Earlier they were limited to as an important ingredient of tooth pastes, mouthwashes, and as pain reliever, but, nowadays, they are increasingly being used in all possible treatments in dentistry such as root canals, surgeries, periodontal therapies, and anti-plaque agents to name a few. Herbs have been used for centuries to prevent and control dental disease. Medicinal plants are known for their considerable antibacterial activity against various microorganisms including bacteria's responsible for dental caries. Phytochemicals for the prevention, treatment, and maintenance of the periodontal diseases are identified. They can be tannins, terpenoids, flavonoids, alkaloids, polyphenols, etc. Antimicrobial activities of these have

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been found to be particularly useful for periodontal diseases.^[3]

Historically, garlic was used in China to the lower blood pressure, in Egypt to increase physical strength, in Europe to prevent the plague, and in India as a home remedy for various minor ailments such as flu and cough. Garlic has been used not only for flavoring food but also because it contains a sulfur-rich derivative of cysteine known to have medicinal benefits.

Garlic contains several different organosulfur compounds (OSCs) in addition to amino acids, vitamins, and micronutrients. Its anticarcinogenic actions may be explained by particular OSCs. Diallyl sulfide, for example, which is responsible in part for its strong taste and odor, has been shown to selectively inhibit as well as induce certain P-450 enzymes.^[4]

Garlic extract is effective against *Streptococcus mutans* when tested both *in vitro* and *in vivo* conditions. As *S. mutans* play an important role in the dental caries development, and, in this study, garlic extract has been shown to be effective against *S. mutans*, garlic extract mouth rinse might be used as an effective remedy in the prevention of dental caries.^[5]

Allicin is measured as the most therapeutic constituent of garlic. Similar study conducted using broth dilution method exposed that planktonic growth of the cariogenic, Gram-positive species *S. mutans*, *Streptococcus sobrinus*, and *Actinomyces oris* was repressed by various allicin concentrations. Planktonic growth of the tested Gram-negative perio-pathogenic species *Aggregatibacter actinomycetemcomitans*, and the *Fusobacterium nucleatum* was also inhibited by allicin. *Porphyromonas gingivalis* is an anaerobic, Gram-negative pathogen and the bacteria most associated with chronic periodontitis have established lower levels of sensitivity to allicin in comparison to the other oral pathogens.^[6] It was also found in a study that antimicrobial properties were present in garlic *in vitro* against streptococci and also has anticariogenic properties against oral microorganism.^[7]

Garlic has a variety of properties such as diaphoretic, expectorant, antispasmodic, antiseptic, bacteriostatic, antiviral, antihelminthic, and hypotensive effects; it is commonly used to treat chronic bronchitis, recurrent upper respiratory tract infections, and influenza.^[8]

The main active component of garlic is allicin. It is antibacterial and has immune regulatory functions. Allicin destroys cell wall and cell membrane of root canal bacteria.^[9] This is used as an irrigant alternative to NaOCl. Garlic extract inhibits the growth of oral pathogens such as *S. mutans* and *P. gingivalis* and hence used for the management of dental infections such as periodontitis.^[10] Despite of its antibacterial

function, the garlic extract also increases the biofilm formation by *S. mutans* to orthodontic wire, through the upregulation of glucosyltransferase expression. Garlic extract also plays a key role in increased microbial attachment to orthodontic wires.^[11]

Garlic (*Allium sativum*) and onion (*Allium cepa*) herbs were evaluated for their action against different organisms such as Gram-positive organisms and Gram-negative organisms and fungi in their aqueous form. A significant growth inhibition is shown by most of the organisms. The activity of the garlic extract on the mouth flora of volunteers was investigated. A mouth wash containing 10% garlic in quarter ringer solution produced a drastic reduction in the number of bacteria in the oral cavity.^[12]

When crushed, *Allium sativum* yields allicin, which is an antibiotic^[13] and antifungal compound (phytoncide). It has been also claimed that it can be used as a home remedy to help speed recovery from sore throat or other minor ailments because of its antibiotic properties. Sulfur components such as the ajoene, diallyl sulfide, dithiin, S-allylcysteine, and enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products, are present in garlic. A non-sulfur compound – phytoalexin (allixin) was also present along with an antioxidant, antimicrobial effects,^[14] promoting effects, inhibition of aflatoxin B2 DNA binding,^[15] and also neurotrophic effects.

Gram-negative and Gram-positive bacteria, such as *Escherichia coli*, Salmonella, *Staphylococcus*, and *Streptococcus* species are resistant to garlic. Many of the bacteria do not help in developing resistance to allicin, despite being resistant to antibiotics. The related study has formulated the potential for garlic to act as a meat preservative. The study also showed that the garlic could limit the growth of all the bacteria tested.^[16] It has also been observed that allicin is capable of killing methicillin-resistant *Staphylococcus aureus* (MRSA). In a study conducted by Culter and Wilson, it was found that a more stable extract of allicin inhibits all strains of MRSA tested at a concentration of 256 µg/mL.^[17] Garlic was also shown to be effective in combating *Helicobacter pylori*, which has been linked to stomach cancer. Using 5 mg/mL of an aqueous garlic extract, researchers were able to inhibit the growth of 90% of their *H. pylori* populations.^[18] These studies have shown that garlic possesses some antimicrobial properties.

The main objective of the present investigation was to determine the inhibitory activity of the garlic extract on *Lactobacillus* and coagulase-negative *Staphylococcus* using the disk diffusion method.

In comparison with tetracycline, pure garlic extract shows more efficient antimicrobial activity against cecal bacteria.^[19] Garlic extract also exhibits antifungal and

antiviral activity.^[20,21] For instance, garlic is reported to an effective fungicidal agent against *Candida albicans*, a fungus that is usually present in the oral cavity.^[22] Garlic is also suggested to act synergistically with antibiotics.^[23]

Dental caries and periodontal diseases are the most common chronic diseases in the world which is prevalent in most of the countries.^[24] The acidic oral environment is well tolerated by *S. mutans* and *Lactobacillus acidophilus*. These bacteria are strongly stimulated by sucrose and are known as the main organisms responsible for human tooth decay. *S. mutans* mainly initiate tooth decay while *L. acidophilus* makes it progress to dental cavity-like lesion.^[24] Agents such as *S. mutans* and facultative anaerobic Gram-positive bacteria are usually found in the human oral cavity. These bacteria are the most common pathogens isolated from human dental plaque and play an important role in dental caries by converting sucrose into lactic acid and predisposing teeth to decay.^[25]

A major public health problem throughout the world is periodontal disease and is recognized as the most common cause of tooth loss in adults. Periodontal disease is a general term used to describe the several pathological conditions that affect the supporting structures/tissues of teeth. Periodontal treatment aims to cure the inflamed tissue and reduce the number of pathogenic bacteria and also eliminate the unhealthy pockets. Mechanical therapy, chemotherapy and systemic administration of antibiotics are some of the clinical methods being utilized currently. Conventional therapy also includes scaling – removal of the calculus and the plaque, curettage clearing the inflamed soft-tissue, and root planning – removal of necrotic tissues on the root surface. Periodontal diseases are usually associated with bacterial infections; therefore, antibacterial treatment seems to be an appropriate method of improving the condition of the inflamed tissues. One of the major problems associated with conventional treatment of systemic administration of antibiotics is the distribution of drug throughout the body, which is not required and it can also give rise to toxicity problems. One method of reducing the distribution of therapeutic agents in the body is through the use of local drug delivery system. Mouth rinses, irrigating solutions, and sustained release devices are some of the local delivery systems.^[26]

MATERIALS AND METHODS

Preparation of Extract

Ethanollic extract of garlic in the undiluted form was used.

Inoculum Preparation

Standard strain of coagulase-negative *Staphylococcus* and *Lactobacillus* was grown on surface agar culture.

They were suspended separately in normal saline with turbidity matching 0.5 McFarland Standard.

These standard strains were coated on nutrient agar on Petri dish.

Antimicrobial Assay

Antimicrobial activity of the garlic extract was determined by disk diffusion method. *Lactobacillus* and coagulase-negative *Staphylococcus* were grown on the agar plates. These standard strains were coated on nutrient agar on Petri dish. Sterile antibiotic disks were impregnated with 15 and 20 μ L of the extract. Then, the disk containing the garlic extract was placed on it. Then, the plates were incubated at 37°C overnight. After incubation, the plates were examined for the zone of inhibition.

RESULTS

The main aim of the study was to evaluate the antimicrobial activity of garlic on the oral microbes using the disk diffusion method. The agar plates were examined for the zone of inhibition after 24 h. On examination there was no zone of inhibition on the agar plates for both the organisms-*Lactobacillus* and Coagulase-negative *Staphylococcus*.

Figure 1 shows the antimicrobial effect of garlic on *Staphylococcus* and *Lactobacillus* by disk diffusion method.

DISCUSSION

This study was to determine the antimicrobial effect of garlic extract on the oral microbes- *Staphylococcus* and *Lactobacillus*.

In our study, there was no effect of garlic extract on coagulase-negative *staphylococcus* and *lactobacillus*. In another study conducted by Houshmand *et al.*, different concentrations of the garlic extract were used against the organisms; the concentration of 100% of garlic extract was significantly less effective than other concentrations against *Lactobacillus* spp.^[27] Garlic extract inhibits the growth of oral pathogens such as *S. mutans* and

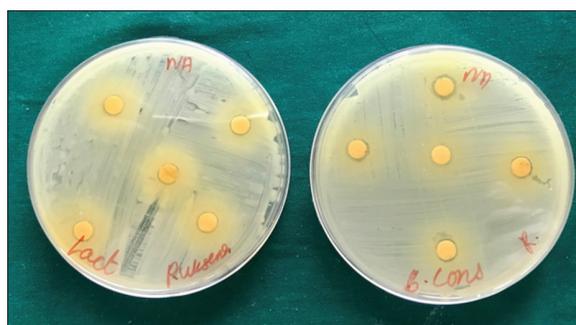


Figure 1: Antimicrobial effect of garlic on *Staphylococcus* and *Lactobacillus* by disk diffusion method

P. gingivalis, hence, used for the management of dental infections such as periodontitis.^[10]

An essential therapeutic aid for alleviating the ailments of human kind has been satisfied by the medicinal plants. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Nowadays, there is a current belief that natural herbal drugs are comparatively safe and more dependable than the costly synthetic drugs, many of which have adverse side effects.^[28]

Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*. *Mycobacterium tuberculosis* which is an acid fast bacteria is sensitive to garlic^[29]. Garlic extracts are also effective against *H. pylori*, the cause of gastric ulcers^[30]. Garlic extracts can also prevent the formation of *Staphylococcus* enterotoxins A, B, and C1 and also thermonuclease^[31]. On the other hand, it seems that garlic is not effective against toxin formation of *Clostridium botulinum*^[32].

In a study conducted by Houshmand *et al.*, the antibacterial effects of different concentrations of garlic extracts on various strains of bacteria were similar to each other. However, the concentration of 100% of garlic extract was significantly less effective than other concentrations against *Pseudomonas aeruginosa* and *Lactobacillus* spp. Furthermore, according to their results the extract is somewhat more effective against *Lactobacillus* spp. than against the other studied bacteria.^[27]

In a study, Elnima *et al.* have reported the significant effect of 10% garlic solution in decreasing levels of oral microorganisms,^[12] Groppo *et al.*^[7] have observed a remarkable reduction of mutans streptococci after gargling with a 2.5% garlic mouthwash solution. Another study, reporting a reduction in levels of *S. mutans* after garlic mouthwash, mentioned that a 3% concentration was the minimum concentration at which a zone of inhibition was observed.^[5] Multidrug-resistant and non-multidrug-resistant strains of *S. mutans* have also been reported to be sensitive to garlic extract.^[33] In addition, combination of garlic with lime is reported to have a noticeable antibacterial effect against isolates from carious teeth.^[34]

Garlic extract has inhibitory effect against MRSA.^[17] It is very effective against a range of oral Gram-negative species while being less active against oral Gram-positive species.^[35]

Antibacterial effect of garlic extract against bacteria other than *S. mutans* and *L. acidophilus* has been

reported in previous studies. Li *et al.* reported in 2007 that the garlic extract is effective against not only *S. mutans* and *L. acidophilus* but also against *Actinomyces*, *Wolinella*, *Peptococcus*, *Prevotella*, and *Fusobacterium*.^[36] In a study conducted by Groppo *et al.* in 2002 he reported that both garlic and chlorhexidine have antimicrobial activity against *S. mutans*, but have no impact on the other oral microorganisms.^[7] In addition, Groppo *et al.* also stated in 2007 that 2.5% of garlic mouthwash solution has *in vivo* antimicrobial activity and is competent to inhibit the activity of *S. mutans* and other oral microorganisms.^[37]

Experimental studies have shown that the chemopreventive activity of *Allium* vegetables is related to their content of OSCs. Moreover, how these compounds achieve chemoprevention is not fully understood, several modes of action have been proposed.^[37-40] These include: (1) Effect on drug metabolizing enzymes (i.e., induction of Phase II detoxification enzymes, including glutathione transferases, quinine reductase, epoxide hydrolase, and glucuronosyltransferase that inactivate toxic substances and facilitate their excretion); (2) antioxidant activity (garlic preparations exhibit radical scavenging activity and decrease lipid peroxidation, which is relevant in the light of the observation that tumor promotion may involve oxygen radicals); (3) tumor growth inhibition that has been documented in several carcinoma cell lines, including prostate carcinoma cells; (4) induction of apoptosis, which coincides with an increase in the percentage of cells blocked in the G2/M phase of the cell cycle (possibly through a depression in p34cdc2 kinase); and (5) effective stimulation of the immune response (OSCs) stimulates proliferation of lymphocytes and macrophage phagocytosis, induce the infiltration of macrophages and lymphocytes in transplanted tumors, induce splenic hypertrophy, stimulate release of interleukin-2, tumor necrosis factor- α and interferon- γ , enhance natural killer cell, killer cell, and lymphokine-activated killer cell activity.^[41]

CONCLUSION

All the isolates used, in our study, were not susceptible to the garlic extracts. In both the concentrations of the garlic extract, there was no zone of inhibition found on the agar plates. All the isolates were resistant to garlic indicating that it is not an efficient microbial agent. Further researches can be done for evaluating the antimicrobial effect of garlic using higher concentrations of the extract. Clinical studies are necessary to evaluate the efficacy of garlic extract.

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