

Original article

# In vitro study of natural plant products against oral bacteria

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## Abstract

**Objective:** To analyze the effect of selected plant product against several bacterial which commonly causes oral infection. It was hope that in future, this product will become the remedy for treatment of oral infection and with the hope that it can substitute antibiotics. **Methods:** A total of 5 species of oral bacteria from American Type Culture Collection (ATCC) were employed in this study (*S. mutans*, *S. aureus*, *P. aeruginosa*, *S. sobrinus* and *L. salivarius*). Three types of natural plants crude extracts were used (garlic, curry leaves and cloves). Bactericidal and bacteriostatic effects of these herbs were tested. **Results:** It was shown garlic had antibacterial effects on all bacteria. The Minimal Inhibitory Concentration (MIC in g/mL) of garlic towards *S. aureus*, *P. aeruginosa*, *S. mutans*, *S. sobrinus* and *L. salivarius* were 0.3, 1.8, 1.2, 0.5 and 1.8, respectively. There was significant difference among the MIC of garlic on tested bacteria. It was more potent toward *S. aureus*. The curry leaf solution on the other hand, did not show any zone of inhibition in all bacteria plates but adversely showed enhanced growth of those bacteria. Clove had shown its antibacterial effects on *S. aureus* and *P. aeruginosa*. The clove was more potent toward *S. aureus* with the MIC of 0.45 g/mL. *P. aeruginosa* was more sensitive to clove compared to garlic. For *S. aureus*, it was more sensitive to garlic compared to clove. **Conclusion:** The antibacterial activity of garlic and clove crude extracts shown in our study further confirm these natural plants' potential usage in therapeutic use for oral diseases or infections. This could be the platform for the interested party to do research and development on it and to produce oral health products which are more affordable for lower economic income groups and with fewer side effects as seen in synthetic drug.

**Keywords:** Garlic; Clove; Curry leaves; MIC; Antibacterial effect

## INTRODUCTION

Plants have been the medicine for many cultures in the world since the ancient times. For instance, the Egyptians, Babylonians, Greek, Chinese and Romans used garlic for healing purposes<sup>[1]</sup>. Pasteur also noted garlic's antibacterial activity in 1858, and garlic was used as antiseptic to prevent gangrene

during World War I and World War II. While 25 % to 50 % of current pharmaceuticals are derived from plants, none are used as antimicrobials<sup>[2,3]</sup>. Natural products account for more than 40 % of all pharmaceuticals on the market today. A large number of plants are used to combat different diseases<sup>[4,5]</sup> and possess antimicrobial activity<sup>[6-9]</sup>.

Dentists are using it nowadays to relieve gum and tooth pain and it may be useful as a topical antiseptic in mouthwash. Curry leaves are also being observed as helpful in reducing halitosis after the subjects held the curry leaves in their mouths for 5-7 mins<sup>[10]</sup>.

Several spices particularly garlic, ajowain, black

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pepper, clove, ginger, cumin and caraway are used extensively in the Indian diet and in Indian medicine. Garlic with its antibacterial properties is widely used for a number of infectious diseases. There are quite a number of studies being conducted previously to investigate the antimicrobial activity of the garlic extract, and these studies had shown the antibacterial, antifungal and antiproteolytic activity of garlic<sup>[11]</sup>. The active compound in garlic that responsible for these antimicrobial activities is known as allicin. Allicin is one of the sulfur containing compounds in garlic, and it is formed when alliin, a sulfur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed or chewed<sup>[10]</sup>. Eugenol, an active principle of clove is used as an antiseptic and possesses local anaesthetic activity; it is therefore used for toothache<sup>[12]</sup>. Mahanine is a carbazole alkaloid and is a major constituent of the edible parts of the Thai vegetable *Micromelum minutum*<sup>[13]</sup>. The compound has also been reported to be present in the curry leaf plant *Murraya koenigii* and in some related species. Mahanine has been shown to exhibit a wide range of pharmacological effects, antimutagenicity against heterocyclic amines, antimicrobial activity against Gram positive bacteria and an anti-inflammatory effect<sup>[13-15]</sup>. The aim of this study was to analyze the effect of selected plant product against several bacterial which commonly causing oral infection. It was hope that in future, this product will become the remedy for treatment of oral infection and with the hope that it can substitute antibiotics.

## MATERIALS AND METHODS

A total of 5 species of oral bacteria from American Type Culture Collection (ATCC) were employed in this study. These were *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus sobrinus* and *Lactobacillus salivarius*. Three types of natural plants crude extracts were used. Those plant were garlic (*Allium sativum*), curry leaves (*Murraya koenigii* Spreng.) and cloves.

Seventy (70) gram of fresh garlic, clove, and curry leaves were blended in 35 mL of distilled H<sub>2</sub>O. Then the material were centrifuged, ultrafiltered through a 10 kDa cut-off membrane and sterilized by filtration (0.45 μm). By subtracting the weight of

the insoluble material from the weight of the original products the final concentration in the solution was determined as 57.1 % (W/V). the product were stored at -20°C.

Oral bacteria from ATCC medium was inoculated and grown in nutrient broth for 24 hours at 37°C. The oral bacteria from nutrient broth was then be inoculated on Mueller Hinton agar plate. Five wells were made on that agar plate by using borer. Different concentrations of natural plant crude extracts were poured in the wells from the stock solution. The agar plates were incubated for 24 hours at 37°C and presence of zone of inhibition were observed around each well after incubation.

Those plates were further incubated overnight at 37°C and zone of inhibition were noted after 24 hours to confirm the bactericidal effects of the natural plant crude extracts.

Control for each oral bacterium was performed with sterile saline in same volume.

## Minimal Inhibitory Concentration (MIC) determination by agar well diffusion method

The most diluted concentration but still showing zone of inhibition after 24 hours incubation was selected for further dilution into another 4 concentration. These secondary series of diluted concentrations were poured into new inoculated plates and incubated at 37 °C overnight. After the second incubation, the well with the most diluted concentration but still showing zone of inhibition was considered as MIC.

## RESULTS

The bactericidal effect of garlic against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus salivarius* had shown zone of inhibition that was 0.5 g/mL. But in case of clove it was bactericidal only against *Staphylococcus aureus* and *Pseudomonas aeruginosa* that is 0.5 g/mL.

The minimal inhibitory concentration of garlic, towards *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus salivarius* had shown its antibacterial effects on all bacteria. The curry leaf solution on the other hand, did not show any zone of inhibition in all bacteria plates but adversely showed enhanced

growth of those bacteria. Triplication of the experiment did show the same result. There was significant difference among the MIC of garlic on tested bacteria ( $P < 0.05$ ). The garlic also was more potent toward *Staphylococcus aureus* with the MIC of 0.30 g/mL.

There was significant difference among the MIC of clove on bacteria ( $P < 0.05$ ). The clove extract had antibacterial effect only on *Staphylococcus aerues* and *Pseudomonas aeruginosa*. The clove was more potent toward *Staphylococcus aureus* with the MIC of 0.45 g/mL.

From the result, *Pseudomonas aeruginosa* was more sensitive to clove with MIC of 0.9 g/mL compared to garlic 1.8 g/mL of MIC. For *Staphylococcus aerues*, it was more sensitive to garlic with MIC of 0.3 g/mL compared to clove with MIC of 0.45 g/mL. For *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus salivarius*, these bacteria only response to garlic paste with MIC of 1.2 g/mL, 0.5 g/mL, and 1.8 g/mL, respectively.

## DISCUSSION

Ethno-pharmacologists, botanists, microbiologists, and natural-products chemists are combing the Earth for phytochemicals and "leads" which could be developed for treatment of infectious diseases. Our study has found that garlic aqueous crude extract show both bacteriostatic and bactericidal activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus sobrinus*, *Lactobacillus salivarius*. Antibacterial activity of garlic against *Staphylococcus aureus* and *Streptococcus mutans* in our study is in coherence with other studies conducted previously<sup>[11-17]</sup>. Antibacterial activity of garlic against *Pseudomonas aeruginosa*, *Streptococcus sobrinus*, and *Lactobacillus salivarius* shown in our study, according our knowledge, is being studied and proved for the first time. Varied concentration of garlic crude extracts in achieving bacteriostatic and bactericidal effects in these oral bacteria demonstrate different degree of sensitivity. This might be due to the ability of allicin to penetrate cell membranes, the features of the bacterial cell envelope may have penetration influence on periplasmic and cytoplasmic enzymes<sup>[18]</sup>. Although the oral bacteria in our study were all Gram positive bacteria (except *Pseudomonas aeruginosa*), but each species

has its own thickness of peptidoglycan layer in its Gram-positive cell envelope<sup>[16]</sup>. The oral bacteria with thicker peptidoglycan layer might have impaired access of the allicin to certain extent to the cell membrane and thus cause less sensitivity effect of garlic on *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Lactobacillus salivarius* as shown in our study.

Cloves crude extract also showed bacteriostatic and bactericidal activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in our study. This result is consistent with other studies which stated that eugenol as the active compound with antimicrobial activity<sup>[17,19-21]</sup>. Eugenol is a cyclic terpene compound contained in cloves. The antimicrobial action of cloves is due to the impairment of a variety of enzyme systems involving in the production of energy or synthesis of structural components in bacteria cells<sup>[22]</sup>. Cyclic terpene compounds like eugenol was reported to cause dissipation of proton motive force and loss of membrane integrity<sup>[23]</sup>. Thus, eugenol, a constituent of clove, has been used for analgesic, local anesthetic, anti-inflammatory and antibacterial effects. It is used in the form of a paste of mixture as dental restorative material. Our study had further confirmed its antibacterial activity even in crude extract form.

Antibacterial activity of garlic and clove crude extracts against *Pseudomonas aeruginosa* demonstrated in our study indicates the possible usage of these natural plants extracts against infections caused by *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa*, being one of the common agents in causing nosocomial infection in hospital, might be able to be curbed through the usage of these natural plant extracts as compared to antibiotic. As mentioned earlier, it is 1 000 fold less easy for bacteria to develop resistance towards garlic extract compared to antibiotic<sup>[24]</sup>. Thus, this augurs well for the prognosis of the patients with Pseudomonal infection. However, further studies about the exact active constituents that cause this antibacterial activity in these natural plants are needed.

Curry leaves crude extract, on the other hand, had failed to show bacteriostatic or bactericidal activity against any of the 5 oral bacteria being studied. In the contrary, it actually enhanced the growth of these 5 oral bacteria by the appearance of denser growth around the wells in the agar plates. Although

it is postulated the antibacterial activity of curry leaves is due to its terpenes compound, nevertheless this compound might have been denatured or lost during preparation of the crude extract. The enhancing effects on growth of oral bacteria could be due to its glucose or sucrose content, thus causes denser growth of colonies of oral bacteria around the wells of agar plates. Hence, further extensive studies on antibacterial activity of curry leaves are needed to confirm its antibacterial activity against oral bacteria.

In conclusion, our study managed to demonstrate the preliminary evidence of the bacteriostatic and bactericidal effects of garlic and clove crude extracts against oral bacterial species. Whereas the curry leaves crude extract in our study was showing the contradictory results with previous studies. Instead of antibacterial effects, it promotes the growth of the oral bacteria to certain extent. The antibacterial activity of garlic and clove crude extracts shown in our study further confirm these natural plants' potential usage in therapeutic use for oral diseases or infections. This could be the platform for the interested party to do research and development on it and to produce oral health products which are more affordable for lower economic income groups and with less side effects as seen in synthetic drug.

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