

Original Article

Determination of Antibacterial Activity of Anacyclus Pyrethrum Extract against Some of the Oral Bacteria: An In Vitro Study

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KEY WORDS

Anacyclus Pyrethrum;
Antibacterial effect;
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ABSTRACT

Statement of Problem: There are more than 500 different bacterial species in the oral cavity which can cause tooth decay and periodontal diseases. Anacyclus Pyrethrum has been used to manage dental and periodontal diseases in traditional Iranian medicine.

Purpose: The purpose of this study was to determine the antibacterial activity of Anacyclus Pyrethrum against some of the oral bacteria, such as Staphylococcus aureus, Streptococcus mutans, Streptococcus sanguis and Pseudomonas aeruginosa.

Materials and Method: At first, The antibacterial effect of serial concentrations (1/10 to 1/100 mg/ml) of methanolic extract of Anacyclus Pyrethrum root were tested by using well assay method on Staphylococcus aureus, Streptococcus mutans, Streptococcus sanguis and Pseudomonas aeruginosa. In the second examination, 150-1000 mg/ml concentrations were tested and the agar dilution method, recommended by the Clinical and Laboratory Standards Institute Standards, was used. Then, the lowest concentrations of the extract which inhibited visible growth of organisms on the media plate; Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), were determined.

Results: The inhibition zone was only seen in the 1.10 mg/ml concentration. The diameters were 15 and 12 mm in Staphylococcus aureus and Streptococcus sanguis agar media plate, respectively. In the second examination, the greatest inhibition zones were 27 mm for Staphylococcus aureus and 21 mm for Streptococcus sanguis in 1000 mg/ml. There was not any inhibition zone for Streptococcus mutans and Pseudomonas aeruginosa in the concentrations. The MBC was achieved as 800 mg/ml for Staphylococcus aureus and Streptococcus sanguis. Streptococcus mutans and Pseudomonas aeruginosa grew in all the concentrations.

Conclusion: The antibacterial effect of Anacyclus Pyrethrum extract against Staphylococcus aureus and Streptococcus sanguis was not significant. Anacyclus Pyrethrum had no antibacterial effect against either Streptococcus mutans or Pseudomonas aeruginosa.

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Introduction

Anacyclus Pyrethrum (pellitory, Spanish chamomile) from Asteraceae family and Anacyclus genus is a native plant of India and Arabic countries and its root has therapeutic effects [1].

The root of Anacyclus Pyrethrum is fusiform with a hard and compact structure. It is 5 to 10 cm in length and dark, grayish brown, in color. It has a strange taste and causes saliva to flow [2].

Pyrethrine is the active substance of Anacyclus

Pyrethrum. The other compounds of this plant are resinous and include peltelin, tannin, gum, potassium sulfate and carbonate, potassium chloride, calcium phosphate, and carbonate [3].

It has been reported that the root of *Anacyclus Pyrethrum* is a strong sialagogue. It has neurogenic effects on head, face and tongue. Toothache relief is another medicinal effect of the root of this plant [1-2].

Anacyclus Pyrethrum has been used to provide dental pain relief in traditional Iranian medicine for many years. Preventing from tooth decay progression and treating gingival diseases have been the other medical uses of pellitory in Iranian medicine [4].

More than 500 different bacterial species exist in the oral cavity. The first bacterial colonies of the oral cavity, before tooth eruption, include *Streptococcus salivarius*, *Streptococcus mitis*, *Veillonella* spp, *Neisseria* spp, and *Staphylococcus* spp. *Lactobacillus* spp, *Fusobacterium* spp, and *Prevotella* spp colonies appear after tooth eruption. *Streptococcus mutans* colonies emerge after the first year of life and *Streptococcus sanguinis* colonies emerge after tooth eruption [5]. It has been stated that *Pseudomonas aeruginosa* is one of the subgingival plaque bacteria in women of child-bearing age who are involved with periodontitis [6].

It has been reported that *Pseudomonas aeruginosa* involve in necrotizing oral lesions with different clinical aspects from necrotizing periodontal disease [7].

With regard to periodontal diseases, *Streptococcus sanguinis* and *Staphylococcus aureus* lead to gingivitis and peri implantitis, respectively. *Streptococcus mutans* is one of the major causes of tooth decay [5].

A search through traditional Iranian medicine reveals that *Anacyclus Pyrethrum* has been used for the management of dental and periodontal diseases. This study was an attempt to find an answer to the following question: Which of the effects of *Anacyclus Pyrethrum* made Hakims (traditional physicians) use *Anacyclus Pyrethrum* in treating the oral cavity diseases; the antibacterial effects or some other ones? The aim of this study was to determine the antibacterial activity of *Anacyclus Pyrethrum* against some of the oral bacteria. To the authors' best of knowledge, this was the first study on antibacterial activity of *Anacyclus Pyrethrum* against *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sanguis*, and

Pseudomonas aeruginosa.

Materials and Method

The antibacterial effect of of methanolic extract of *Anacyclus Pyrethrum* root was tested in vitro. In order to conduct the study, the roots of *Anacyclus Pyrethrum* were purchased from traditional Shirazian pharmacies (Atari). Dried roots, 200g, were stored in a round bottom flask (No. 72) and refluxed with 800 ml of 95% methyl alcohol (Merck, Germany). After 24 hours, Watman paper (No.1), 150µm in diameter, was used for liquor filtration. The filtered solution was evaporated (GFL, 1009, Germany) at 70°C and for one week. The roots of *Anacyclus Pyrethrum* were collected and stored in sterile foils at 4°C. The bacteria were obtained from the bacterial stock, Department of Microbiology, School of Medicine, Shahed University. *Anacyclus Pyrethrum* extract was screened against *Staphylococcus aureus* (ATCC25923), *Streptococcus mutans* (ATCC1601), *Streptococcus sanguis* (ATCC1-449), and *Pseudomonas aeruginosa* (ATCC25923). Muller-Hinton broth plates (Liofilchem Company, Italy) were sterilized and inoculated through using a sterile swab dipped into culture in columns adjusted to 1.5×10^8 bacterial/ml (0.5 Mc Farland turbidity standards). The well diffusion method was used. Wells, which were 6mm in diameter, were punched on the agar media and *Anacyclus Pyrethrum* extract was placed on the media surface. Plates were kept at room temperature for 30 minutes and then they were incubated at 37°C under aerobic conditions. After an incubation time of 24 hours, the zones of inhibition were measured through using an Antibiotic Zone Reader. Based on the recommended dilution by the Clinical and Laboratory Standards Institute (CLSI), different serial concentrations, from 1/10 mg/ml, 1/20 mg/ml, 1/30 mg/ml, 1/40 mg/ml, 1/50 to 1/100 mg/ml were used. To obtain final confirmation, the 150-1000 mg/ml concentrations were tested in the second examination [8].

The MIC (Minimum Inhibitory Concentration) was identified as the lowest concentration of the extract which inhibited visible growth of the organism on the agar plates. MBC (Minimum Bactericidal Concentration) was detected from MIC range. Under completely sterile conditions, one ml. of incubated

Table 1 The inhibition zone diameter of metanolic extract of Anacyclus Pyrethrum from 1/10 mg/ml to 1/100 mg/ml concentrations

Bacteria	Concentrations					
	1/100 mg/ml	1/50 mg/ml	1/40 mg/ml	1/30 mg/ml	1/20 mg/ml	1/10 mg/ml
Staphylococcus aureus	0	0	0	0	0	15mm
Streptococcus mutans	0	0	0	0	0	0
Streptococcus sanguis	0	0	0	0	0	12mm
Pseudomonas aeruginosa	0	0	0	0	0	0

solution with determined MIC were cultured and saved at 37°C for 24 hours. The first concentration which had no bacterial growth was considered as MBC.

Results

The results of this study revealed that Anacyclus Pyrethrum stimulated some antibacterial activity against Staphylococcus aureus and Streptococcus sanguis in the 1/10 mg/ml concentration and the mean diameters of inhibition zone were 15 mm and 12 mm, respectively. There was not any inhibition zone for Streptococcus mutans and Pseudomonas aeruginosa. In the second examination, too, Anacyclus Pyrethrum extract stimulated some antibacterial activity against Staphylococcus aureus and Streptococcus sanguis in the 150-1000 mg/ml concentrations. There was not any inhibition zone either for the Streptococcus mutans or for the Pseudomonas aeruginosa media. The diameter of the inhibition zone for different concentrations has been displayed in Tables 1 and 2.

The Minimum Bactericidal Concentration of Anacyclus Pyrethrum extract was in 800 mg/ml for Staphylococcus aureus and Streptococcus sanguis. Streptococcus mutans and Pseudomonas aeruginosa had growth in all the concentrations. The Minimum Bactericidal Concentration of Anacyclus Pyrethrum extract has been summarized in Table 3.

Discussion

The results of this study indicated that Anacyclus Pyrethrum extract produced little antibacterial effect

against Staphylococcus aureus and Streptococcus sanguis. Anacyclus Pyrethrum had no antibacterial effect against Streptococcus mutans and Pseudomonas aeruginosa. To the researchers' knowledge, this study has been the first on the antibacterial activity of Anacyclus Pyrethrum extract against the above name mentioned bacteria.

Anacyclus Pyrethrum (Akarkara in Persian lingo) has been used for the management of toothache and periodontal diseases. The patient might use it as a mouth wash or might chew the herb. It has been stated that Anacyclus Pyrethrum was used for easier tooth extraction. The conventional technique was to apply a mixture of Akarkara and vinegar to the root apex [4].

It has been reported that Anacyclus Pyrethrum is involved in immunostimulating and immunomodulatory activities [9-10].

Pahuja et al. investigated the preventive effects of Anacyclus Pyrethrum against seizure induced oxidative stress and its role in normalizing the intensity of cholinesterase activity of seizures [11].

The inhibitory cyclooxygenase activity and antioxidant effects are other reported achievements of Anacyclus pyrethrum [12-13].

Considering the medical uses of Anacyclus Pyrethrum in traditional Iranian medicine, the question was: Does Anacyclus Pyrethrum has any antibacterial Anacyclus Pyrethrum has been used for the management of dental and periodontal diseases in traditional Iranian medicine for many years. In spite of the researchers' attempts, they could not find any research on antibacterial activity of Anacyclus Pyrethrum.

Table 2 The inhibition zone diameter of metanolic extract of Anacyclus Pyrethrum from 150 mg/ml to 1000 mg/ml concentrations

Bacteria	Concentrations					
	1000mg/ml	800 mg/ml	600 mg/ml	400 mg/ml	300 mg/ml	150 mg/ml
Staphylococcus aureus	27mm	25mm	23mm	20mm	18mm	23mm
Streptococcus mutans	0	0	0	0	0	0
Streptococcus sanguis	21mm	18mm	16mm	16mm	15mm	17mm
Pseudomonas aeruginosa	0	0	0	0	0	0

Table 3 The minimum bactericidal concentrations of *Anacyclus Pyrethrum* extract

Bacteria	Concentrations				
	1000 mg/ml	800 mg/ml	600 mg/ml	400 mg/ml	300 mg/ml
<i>Staphylococcus aureus</i>	-	-	+	+	+
<i>Streptococcus mutans</i>	+	+	+	+	+
<i>Streptococcus sanguis</i>	-	-	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+

However, there was a research on the larvicidal activity of *Anacyclus Pyrethrum*.

Pandey et al. claimed that the Akarkara (the Persian name for *Anacyclus Pyrethrum*) stimulated strong larvicidal activity against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say) [14].

The findings of this study showed that *Anacyclus Pyrethrum* extract had little antibacterial effect against *Staphylococcus aureus* and *Streptococcus sanguis* and it had no antibacterial effect against *Streptococcus mutans* and *Pseudomonas aeruginosa*. Based on this finding, it seems that the antibacterial effect of *Anacyclus Pyrethrum* extract was not the main reason for its uses in dental and periodontal disease management. The antioxidant and anaesthetic effects of *Anacyclus Pyrethrum* may be the appropriate answers to the above question [2-12]. To examine these effects, the root of *Anacyclus Pyrethrum* has to be analyzed chemically. This study was based on some standard species of bacteria. To obtain more information, further investigations on mouth derived microorganisms are required.

Conclusion

Anacyclus Pyrethrum extract produced little antibacterial effect against *Staphylococcus aureus* and *Streptococcus sanguis*. The plant did not have any antibacterial effect against *Streptococcus mutans* and *Pseudomonas aeruginosa*.

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Conflict of Interest

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