

# Antibacterial Activity of *Cissus quadrangularis* (Veldt Grape) Against *Porphyromonas gingivalis*, a Keystone Pathogen in Periodontal Disease: An In Vitro Study

Received 06/27/2024  
Review began 07/13/2024  
Review ended 07/29/2024  
Published 08/07/2024

© Copyright 2024

Nair et al. This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 4.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

DOI: 10.7759/cureus.66377

Aathira R. Nair <sup>1</sup>, Prem Blaisie Rajula <sup>1</sup>, Ravishankar PL <sup>1</sup>, Sai Sri Soury Geddani <sup>1</sup>, Subhashini B <sup>1</sup>, Lochini S <sup>1</sup>

1. Periodontology, SRM Kattankulathur Dental College and Hospital, Chennai, IND

**Corresponding author:** Prem Blaisie Rajula, premlam@srmist.edu.in

## Abstract

**Background:** *Porphyromonas gingivalis*, a keystone pathogen and one of the primary pathogens responsible for periodontitis, leads to a chronic inflammatory condition that destroys the periodontal tissues and ultimately results in tooth loss. While conventional non-surgical therapy combined with antibiotics and local drug delivery systems are commonly used to treat periodontitis, certain medicinal herbs have also demonstrated efficacy in its prevention. *Cissus quadrangularis* L. (CQ), a perennial plant from the Vitaceae family, is widely recognized and used as a medicinal herb in many tropical countries, predominantly in India, Sri Lanka, Thailand, Java, West Africa, and the Philippines.

**Aim:** The aim of the study was to determine the antibacterial activity of CQ against the periodontal keystone pathogen *P. gingivalis*.

**Method:** Aqueous and ethanolic extracts of CQ were prepared using a Soxhlet extractor. The antibacterial effectiveness of these extracts against the periodontal pathogen *P. gingivalis* was evaluated at different concentrations, and the minimal inhibitory concentration (MIC) was determined using broth microdilution.

**Results:** The ethanolic extract of CQ mixed with 10% dimethyl sulfoxide (DMSO) showed higher inhibition compared to the aqueous extract of CQ against *P. gingivalis*.

**Conclusion:** Our study revealed the potent inhibitory effects of CQ against *P. gingivalis*. Both aqueous and ethanolic extracts displayed MIC values of 500 µg/mL. Notably, the ethanolic extract of CQ, dissolved in 10% DMSO, demonstrated superior efficacy with a lower IC50 value of 194.36 µg/mL. These findings indicate promising potential for CQ in the management of periodontal disease.

**Categories:** Dentistry, Infectious Disease, Therapeutics

**Keywords:** porphyromonas gingivalis, minimum inhibitory concentration, ethanolic extract, cissus quadrangularis, antibacterial activity

## Introduction

The keystone pathogen *Porphyromonas gingivalis* is prominently implicated in periodontitis, a chronic inflammatory condition that results in tooth loss and the damage of gingiva, alveolar bone, and periodontal ligaments [1]. Among the myriad bacterial species inhabiting the oral cavity, this specific gram-negative rod bacterium is non-motile and requires anaerobic conditions to survive. After 3-5 days of incubation, it forms black-pigmented colonies on supplemented blood agar plates [2,3]. By interacting with commensal *Streptococci*, *P. gingivalis* causes secondary infections in periodontal tissues [4]. Its capacity to elude host immune responses and disseminate via cell-to-cell interactions or hematogenous routes sustains persistent irritation in the peripheral regions [5].

Presently, periodontitis management involves systemic and localized antibiotic therapy, sometimes coupled with surgical interventions for addressing deep-pocket irritations [6]. Anaerobic bacteria, especially *P. gingivalis*, are the target of antibiotics. A range of antibiotics such as metronidazole, amoxicillin, amoxicillin/clavulanic acid, clindamycin, tetracycline, and fluoroquinolones are utilized alongside mechanical debridement [7,8].

Failure to perform debridement can lead to antibiotics being unable to penetrate biofilm, resulting in significantly higher minimal inhibitory concentrations (MIC) [9]. Moreover, *P. gingivalis* strains exhibit considerable resistance rates, reaching up to 21.56% for metronidazole, 25.49% for amoxicillin, and 23.52% for clindamycin [10]. Notable resistance is also observed for penicillin, erythromycin, azithromycin, and tetracycline [11].

### How to cite this article

Nair A R, Blaisie Rajula P, PL R, et al. (August 07, 2024) Antibacterial Activity of *Cissus quadrangularis* (Veldt Grape) Against *Porphyromonas gingivalis*, a Keystone Pathogen in Periodontal Disease: An In Vitro Study. Cureus 16(8): e66377. DOI 10.7759/cureus.66377

As antibiotic resistance escalates, the imperative for new antimicrobials intensifies, yet the rate of new antibiotic discoveries has declined [12]. Medicinal plants offer another avenue for combating *P. gingivalis* infections. Not only do plant species represent a diverse reservoir of antibacterial compounds [13], but ethnobotanical knowledge can guide the selection of plant extracts for discovering novel antibacterials [14]. Extensive ethnobotanical documentation exists regarding plant usage for oral health, yet further research is assured to establish the pharmacological efficacy of these ethnomedicinal practices against dental pathogens.

*Cissus quadrangularis* (CQ), also known as Veldt Grape or Devil's Backbone, belongs to the Vitaceae family. It is recognized by various names, including Asthisamharaka, Hadjod, Pirandai, Mangara Valli, and *Vitis quadrangularis* [15-19]. Utilized in Ayurvedic and Siddha medicine traditions, it offers a range of therapeutic benefits, including antimicrobial, antiulcer, antioxidative, anti-osteoporotic, gastroprotective, cholinergic, antihyperglycemic, and cardiovascular properties [19]. This cactus-like plant is characterized by jointed climbers with simple tendrils and leaves that are either simple or lobed, cordate, serrate, or dentate. Its small, greenish-white, bisexual, and tetramerous flowers complement its versatile medicinal properties (Figure 1). This plant possesses the capability to mend bones, earning it the epithet "bone setter" [20].

It was discovered to exhibit anti-osteoporotic, antioxidant, antimicrobial, anticonvulsant, sedative, neuropharmacological, central nervous system (CNS) activity, anthelmintic, antidiabetic, antistress, anticancerous potential, and antinociceptive properties [18,21].

Previous research has revealed varying degrees of antibacterial efficacy against different microorganisms [16], yet scant literature has examined its antibacterial activity specifically against *P. gingivalis*. Therefore, this study aimed to assess the antibacterial potential of CQ against this significant periodontal pathogen.

## Materials And Methods

### Collection of sample

Commercially available CQ powder (Bixa Botanicals, Dhar, India), also known as Hadjod stem powder, was used.

### Preparation of ethanolic extract of CQ (Soxhlet extraction method)

Ethanolic extract of CQ was prepared using a Soxhlet extractor (Figure 1). Among conventional methods, Soxhlet was the most powerful method and had the most extraction yields [22]. The solvent (250 mL ethanol) was filled in the percolator. Finely ground powder of CQ (25 g) was packed using Whatman filter paper and placed inside the thimble. The solvent (ethanol) was heated by adjusting the temperature of the mantle to 50°C to reflux. The solvent vapor that travels up the distillation arm was cooled by the water that circulates in the condenser. The active compounds of CQ were extracted in ethanol. The cycle was repeated thrice to enable the complete extraction of the bioactive compounds present in CQ. After extraction, the solvent was evaporated to yield the extracted compounds (Figure 2). The ethanolic extract of CQ was dissolved in 10% dimethyl sulfoxide (DMSO) at a concentration of 25 mg/mL. Similarly, the aqueous extract was also obtained using the Soxhlet extraction method.



**FIGURE 1: Extract preparation using a Soxhlet apparatus**



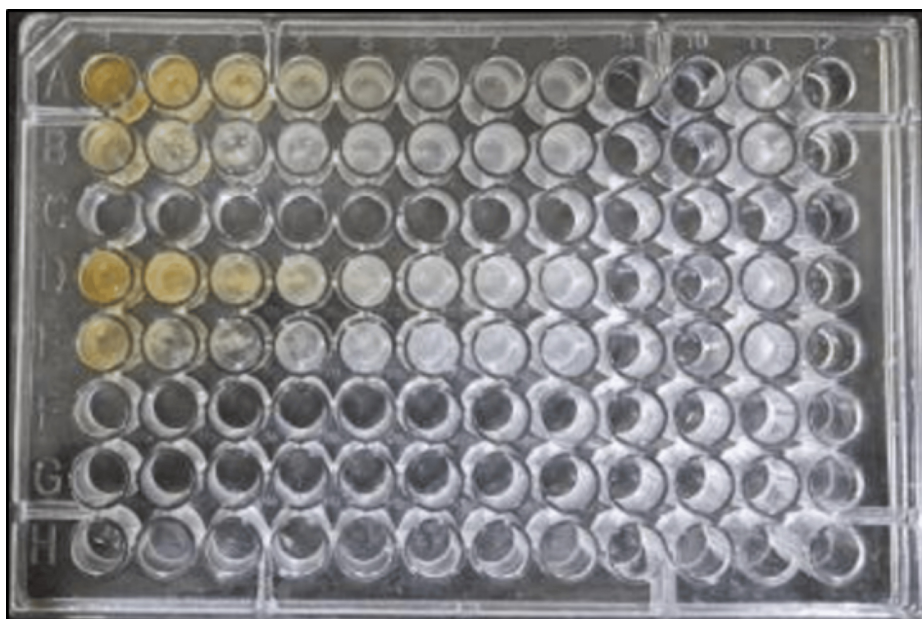
**FIGURE 2: Aqueous and ethanolic extract of *Cissus quadrangularis***

### Preparation of bacterial suspension

From the maintained frozen stock cultures of *P. gingivalis* (ATCC No-33277), a small quantity of cells was recovered and subcultured. Brain heart infusion (BHI) broth was used as the culture medium to support the growth of bacteria. This culture was transferred into tubes containing 2 mL of the BHI medium to obtain a culture suspension of *P. gingivalis*. The selected test bacterial strain was adjusted to 0.5 McFarland turbidity standards.

### MIC by broth dilution method

The MIC was calculated using the broth microdilution method with a 96-well polystyrene flat bottom plate. From columns 2 to 8, 50  $\mu\text{L}$  of Mueller Hinton broth (MHB) was added to the microtiter plate. Then, 100  $\mu\text{L}$  of the sample was added to the first well and serially diluted to the eighth well (1000  $\mu\text{g}/\text{mL}$  to 7.8  $\mu\text{g}/\text{mL}$ ) (rows A and D: aqueous extract of CQ; rows B and E: ethanolic extract of CQ dissolved in 10% DMSO). The 10th and 11th wells served as sterility and negative controls. Then, 50  $\mu\text{L}$  of the *P. gingivalis* (ATCC® 33277) suspensions (0.5 McFarland) was added to all columns except the sterility column (Figure 3). The 96-well plate was read at 600 nm after being incubated for 24 hours at 37 °C. MIC was determined by comparing the growth rate of the tested bacterial strain with the negative controls. The lowest extract concentration that inhibited bacterial growth was noted as MIC.



**FIGURE 3: Microdilution. Rows A and D: aqueous extract of CQ. Rows B and E: ethanolic extract of CQ dissolved in 10% DMSO.**

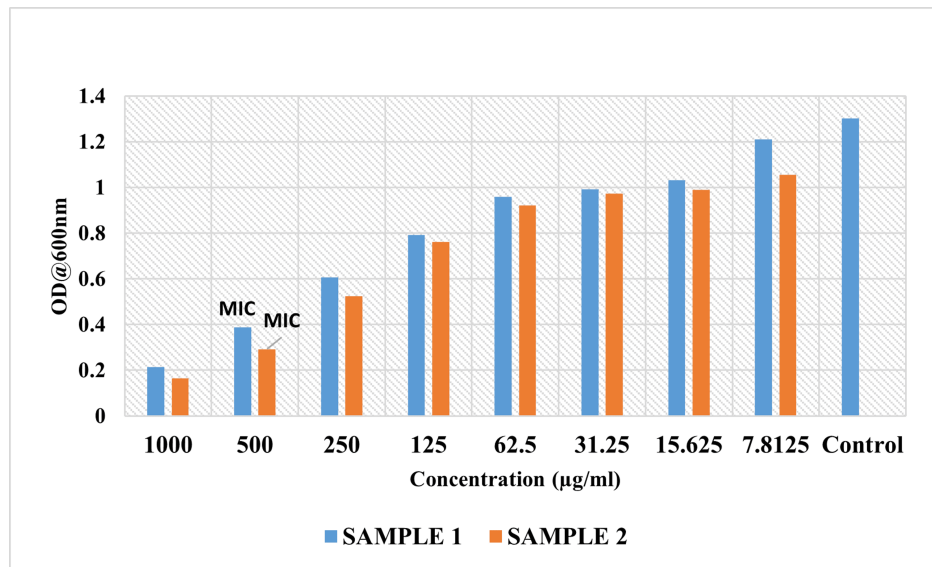
CQ: *Cissus quadrangularis* L., DMSO: dimethyl sulfoxide.

## Results

The MIC data reveal intriguing insights into the inhibitory potential of Sample 1 (an aqueous extract of CQ) and Sample 2 (an ethanolic extract of CQ dissolved in 10% DMSO) at varying concentrations. The concentrations tested ranged from 1000  $\mu\text{g/mL}$  to 7.8125  $\mu\text{g/mL}$ . The MIC, representing the minimum concentration at which microbiological development is prevented, is a critical parameter in assessing the effectiveness of antimicrobial agents. The MIC of Samples 1 and 2 was determined to be 500  $\mu\text{g/mL}$ , with inhibitory percentages of 70.19% and 77.57%, respectively (Table 1 and Figure 5). As per standard protocol, the IC<sub>50</sub> of Samples 1 and 2 was determined to be 240.46  $\mu\text{g/mL}$  and 194.36  $\mu\text{g/mL}$ , respectively. The inhibitory effect of the ethanolic extract of CQ dissolved in 10% DMSO was better than that of the aqueous extract of CQ. The broth microdilution method illustrated in Figure 3 provides a visual representation of the antimicrobial activity of the samples, highlighting their ability to inhibit bacterial growth at varying concentrations. Furthermore, Figure 4 depicts the activity of the samples compared to the control in terms of optical density (600 nm), reaffirming their effectiveness in suppressing bacterial proliferation.

Concentration ( $\mu\text{g/mL}$ )	Sample 1	Sample 1 - IC <sub>50</sub>	Sample 2	Sample 2 - IC <sub>50</sub>
1000	83.56	240.46 $\mu\text{g/mL}$	87.33	194.36 $\mu\text{g/mL}$
500	70.19		77.57	
250	53.37		59.75	
125	39.09		41.47	
62.5	26.34		29.26	
31.25	23.89		25.34	
15.63	20.74		24.04	
7.81	7.07		18.89	

**TABLE 1: Inhibitory Percentage and IC<sub>50</sub>**

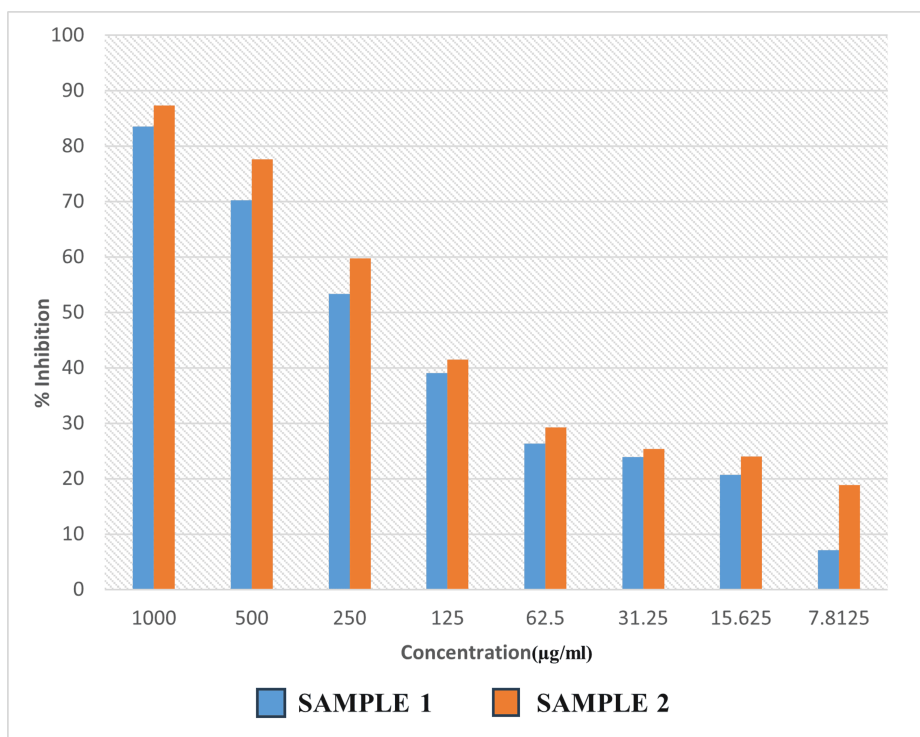


**FIGURE 4: Minimum inhibitory concentration of aqueous and ethanolic extract of *Cissus quadrangularis***

Sample 1: Aqueous extract of *Cissus quadrangularis*.

Sample 2: Ethanolic extract of *Cissus quadrangularis* dissolved in 10% DMSO.

MIC: minimum inhibitory concentration, DMSO: dimethyl sulfoxide, OD: optical density.



**FIGURE 5: Percentage inhibition of aqueous and ethanolic extract of *Cissus quadrangularis***

Sample 1: Aqueous extract of *Cissus quadrangularis*.

Sample 2: Ethanolic extract of *Cissus quadrangularis* dissolved in 10% DMSO.

DMSO: dimethyl sulfoxide.

## Discussion

Gingivitis and periodontitis are prevalent periodontal disorders that impact a substantial proportion of the worldwide population. These chronic inflammatory diseases are predominantly caused by an excess of certain anaerobic bacterial species in dental plaque. This is usually treated with mechanical methods (scaling and root planing, or SRP), but these methods are not always successful because of pathogens that can invade tissue. Systemic and local antibiotics may be beneficial when used in conjunction with mechanical treatment. However, widespread antibiotic use can lead to antibiotic resistance. As a result, research over the past ten years has concentrated on medicinal plants with anti-inflammatory and antibacterial properties to treat these ailments.

The foundations of traditional medicinal systems have historically been plants [23]. Plant extracts, in particular, have emerged as promising natural antibacterial agents with diverse therapeutic applications for combating pathogenic bacteria. CQ, for instance, contains a plethora of beneficial compounds such as carotene, phytosterol, terpenoids,  $\beta$ -sitosterol,  $\delta$ -amyrin,  $\delta$ -amyrone, calcium, flavonoids like quercetin, vitamin C, and stilbene derivatives such as resveratrol, piceatannol, pallidol, perthenocissin, and phytosterols [15].

The aqueous and ethanolic extracts of CQ were selected for the current investigation in order to assess their antibacterial activity against *P. gingivalis*, the keystone periodontal pathogen. The results of our study shed light on the inhibitory potential of two different samples of CQ aqueous and ethanolic extracts against *P. gingivalis*, a prominent periodontal pathogen. The MIC data reveal the inhibitory potential of Sample 1 (an aqueous extract of CQ) and Sample 2 (an ethanolic extract of CQ dissolved in 10% DMSO) at varying concentrations. The concentrations tested ranged from 1000  $\mu\text{g/mL}$  to 7.8125  $\mu\text{g/mL}$ . The MIC of Samples 1 and 2 was determined to be 500  $\mu\text{g/mL}$ . Additionally, the inhibitory percentages of Sample 1 and Sample 2 were found to be 70.19% and 77.57%, respectively.

The determination of the half-maximal inhibitory concentration (IC<sub>50</sub>) further elucidated the potency of the samples, with Sample 2 exhibiting a lower IC<sub>50</sub> value (194.36  $\mu\text{g/mL}$ ) compared to Sample 1 (240.46  $\mu\text{g/mL}$ ). These findings indicate that the ethanolic extract of CQ formulated with 10% DMSO possessed enhanced

antibacterial activity against *P. gingivalis* compared to the aqueous extract (Sample 1). The results were consistent with the findings of Nadhini et al., who reported that the ethanolic extract of CQ exhibited the highest level of antibacterial activity against clinical pathogens [24]. They stated that the ethanolic extract was most frequently used and showed a reasonable antibacterial effect.

Multiple clinical investigations have indicated that administering CQ within a range of 1.2-10 g/day for durations spanning 4-12 weeks led to an acceleration in bone fracture healing, without encountering severe adverse events (AEs). These clinical observations find reinforcement in various in vivo and in vitro studies focusing on bone-related outcomes. Such collective evidence underscores the potential of CQ as an alternative approach to mitigating bone loss [25,26].

The traditional medicinal practice of Ayurveda has long utilized CQ to address a spectrum of diseases and ailments [27]. Its recognized osteogenic properties prompted an exploration into its potential application for periodontitis. Our findings confirm the antibacterial properties of CQ, shedding light on its potential therapeutic role in combating periodontal infections. The efficacy of both samples in preventing the growth of *P. gingivalis* is of significant clinical relevance, as it suggests their potential utility in the prevention, treatment, and management of periodontal diseases.

The strength of our study lies in its novel focus on the antibacterial activity of CQ against *P. gingivalis*, a key pathogen in periodontal disease. The demonstrated efficacy of CQ, particularly the ethanolic extract, suggests its potential as an adjunctive therapy for periodontal diseases. Furthermore, incorporating CQ into local drug delivery systems, such as mouth rinses and gels, could enhance its therapeutic application in dental practice.

Despite the promising results, the study is subject to certain limitations. While the extracts effectively targeted *P. gingivalis*, their efficacy against other periodontal pathogens remains unknown due to the exclusive focus on this single bacterium. Furthermore, the controlled laboratory environment (in vitro) necessitates clinical trials to validate their effectiveness for further clinical applications.

## Conclusions

In conclusion, our findings highlight the significant antibacterial properties of CQ aqueous and ethanolic extracts against *Porphyromonas gingivalis*. The demonstrated efficacy of CQ extracts in inhibiting *P. gingivalis* opens new avenues for the development of innovative periodontal treatment strategies. To fully harness the potential of CQ extracts, further research is necessary to delve into the underlying mechanisms of their antibacterial action. Understanding these mechanisms will provide valuable insights into how these extracts can effectively combat *P. gingivalis*.

## Additional Information

### Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

**Concept and design:** Prem Blaisie Rajula, Aathira R. Nair, Ravishankar PL, Sai Sri Soury Geddamm, Subhashini B, Lochini S

**Acquisition, analysis, or interpretation of data:** Prem Blaisie Rajula, Aathira R. Nair, Ravishankar PL, Sai Sri Soury Geddamm, Subhashini B, Lochini S

**Drafting of the manuscript:** Prem Blaisie Rajula, Aathira R. Nair, Ravishankar PL, Sai Sri Soury Geddamm, Subhashini B, Lochini S

**Critical review of the manuscript for important intellectual content:** Prem Blaisie Rajula, Aathira R. Nair, Ravishankar PL, Sai Sri Soury Geddamm, Subhashini B, Lochini S

**Supervision:** Prem Blaisie Rajula, Ravishankar PL

### Disclosures

**Human subjects:** All authors have confirmed that this study did not involve human participants or tissue.

**Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue.

**Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.



## Acknowledgements

The authors are very grateful to Dr. Kesavaram Padmavathy, Professor, Department of Microbiology, Sree Balaji Dental College and Hospital, Chennai, India, and Prashanth KK, Director, SimBioen Labs and Scientific Private Limited, Chennai, India, for providing the necessary facilities to carry out this study. They would also like to acknowledge the use of ChatGPT (Open AI, Open AI Inc, San Francisco, California, USA) in improving the grammar, punctuation, and overall clarity of the scientific paper.

## References

1. Singhrao SK, Harding A, Poole S, Kesavalu L, Crean S: Porphyromonas gingivalis periodontal infection and its putative links with Alzheimer's disease. *Mediators Inflamm.* 2015, 2015:137357. [10.1155/2015/137357](https://doi.org/10.1155/2015/137357)
2. Mysak J, Podzimek S, Sommerova P, et al.: Porphyromonas gingivalis: major periodontopathic pathogen overview. *J Immunol Res.* 2014, 2014:476068. [10.1155/2014/476068](https://doi.org/10.1155/2014/476068)
3. Conrads G, Klomp T, Deng D, Wenzler JS, Braun A, Abdelbary MM: The antimicrobial susceptibility of Porphyromonas gingivalis: genetic repertoire, global phenotype, and review of the literature. *Antibiotics (Basel).* 2021, 10:10.3390/antibiotics10121438
4. Sakanaka A, Takeuchi H, Kuboniwa M, Amano A: Dual lifestyle of Porphyromonas gingivalis in biofilm and gingival cells. *Microb Pathog.* 2016, 94:42-7. [10.1016/j.micpath.2015.10.003](https://doi.org/10.1016/j.micpath.2015.10.003)
5. Belstrøm D, Holmstrup P, Damgaard C, Borch TS, Skjødt MO, Bendtzen K, Nielsen CH: The atherogenic bacterium Porphyromonas gingivalis evades circulating phagocytes by adhering to erythrocytes. *Infect Immun.* 2011, 79:1559-65. [10.1128/IAI.01036-10](https://doi.org/10.1128/IAI.01036-10)
6. Jepsen K, Jepsen S: Antibiotics/antimicrobials: systemic and local administration in the therapy of mild to moderately advanced periodontitis. *Periodontol 2000.* 2016, 71:82-112. [10.1111/prd.12121](https://doi.org/10.1111/prd.12121)
7. Ardila CM, Granada MI, Guzmán IC: Antibiotic resistance of subgingival species in chronic periodontitis patients. *J Periodontol Res.* 2010, 45:557-63. [10.1111/j.1600-0765.2010.01274.x](https://doi.org/10.1111/j.1600-0765.2010.01274.x)
8. Beikler T, Prior K, Ehmke B, Flemmig TF: Specific antibiotics in the treatment of periodontitis--a proposed strategy. *J Periodontol.* 2004, 75:169-75. [10.1902/jop.2004.75.1.169](https://doi.org/10.1902/jop.2004.75.1.169)
9. Larsen T: Susceptibility of Porphyromonas gingivalis in biofilms to amoxicillin, doxycycline and metronidazole. *Oral Microbiol Immunol.* 2002, 17:267-71. [10.1034/j.1399-302x.2002.170501.x](https://doi.org/10.1034/j.1399-302x.2002.170501.x)
10. Ardila CM, López MA, Guzmán IC: High resistance against clindamycin, metronidazole and amoxicillin in Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans isolates of periodontal disease. *Med Oral Patol Oral Cir Bucal.* 2010, 1:947-51. [10.4317/medoral.15.e947](https://doi.org/10.4317/medoral.15.e947)
11. van Winkelhoff AJ, Herrera Gonzales D, Winkel EG, Dellemeijn-Kippuw N, Vandenbroucke-Grauls CM, Sanz M: Antimicrobial resistance in the subgingival microflora in patients with adult periodontitis. A comparison between The Netherlands and Spain. *J Clin Periodontol.* 2000, 27:79-86. [10.1034/j.1600-051x.2000.027002079.x](https://doi.org/10.1034/j.1600-051x.2000.027002079.x)
12. Brown ED, Wright GD: Antibacterial drug discovery in the resistance era. *Nature.* 2016, 529:336-43. [10.1058/nature17042](https://doi.org/10.1058/nature17042)
13. Silva LN, Zimmer KR, Macedo AJ, Trentin DS: Plant natural products targeting bacterial virulence factors. *Chem Rev.* 2016, 116:9162-236. [10.1021/acs.chemrev.6b00184](https://doi.org/10.1021/acs.chemrev.6b00184)
14. Gyllenhaal C, Kadushin MR, Southavong B, et al.: Ethnobotanical approach versus random approach in the search for new bioactive compounds: support of a hypothesis. *Pharm Biol.* 2012, 50:30-41. [10.3109/13880209.2011.634424](https://doi.org/10.3109/13880209.2011.634424)
15. Mosaib MG, Maruf MAA, Islam R, et al.: Antibacterial activity of Cissus quadrangularis stem extract on the pathogenic and industrial waste watered bacteria. *Eur J Med Health Sci.* 2020, 2:28-38. [10.34104/ejmhs.020.28038](https://doi.org/10.34104/ejmhs.020.28038)
16. Kashikar ND, George I: Antibacterial activity of Cissus quadrangularis Linn. *Indian J Pharm Sci.* 2006, 68:245-7. [10.4103/0250-474X.25727](https://doi.org/10.4103/0250-474X.25727)
17. Chidambara Murthy KN, Vanitha A, Mahadeva Swamy M, Ravishankar GA: Antioxidant and antimicrobial activity of Cissus quadrangularis L. *J Med Food.* 2003, 6:99-105. [10.1089/10966200332223495](https://doi.org/10.1089/10966200332223495)
18. Anwar PZ, Sezhian UG, Narasingam A: Bioactive compound analysis and bioactivities of endophytic bacteria from Cissus quadrangularis. *Int J Pharm Sci Res.* 2020, 11:5553-60. [10.15040/IJPSR.0975-8252.11\(11\).5553-60](https://doi.org/10.15040/IJPSR.0975-8252.11(11).5553-60)
19. Katheeja R, Manish S, Ilangoar IGK, Selvaraj J, Vasugi S: Analyzing the antihyperglycemic effect of Cissus quadrangularis and Bacopa monnieri on 3T3-L1 cell lines. *Cureus.* 2024, 16:e52661. [10.7759/cureus.52661](https://doi.org/10.7759/cureus.52661)
20. Tiwari M, Gupta PS, Sharma N: A preliminary study on in vitro antioxidant and in vivo anti-inflammatory activity of Cissus quadrangularis Linn. *Res J Pharm Technol.* 2021, 14:2619-24. [10.52711/0974-360X.2021.00461](https://doi.org/10.52711/0974-360X.2021.00461)
21. Kapadia S, Kanase V: Evaluation of antidepressant activity of ethanolic extract of Cissus quadrangularis on swiss albino mice. *Res J Pharm Technol.* 2021, 14:4197-202. [10.21205/rs.3.rs-4340058/v1](https://doi.org/10.21205/rs.3.rs-4340058/v1)
22. Haido MH, Matti AH, Taher SM: Optimization of extraction conditions of bioactive compounds from Kurdistan species Urtica dioica. *Cureus.* 2024, 16:e61146. [10.7759/cureus.61146](https://doi.org/10.7759/cureus.61146)
23. Singh N, Singh V, Singh RK, Pant AB, Pal US, Malkunje LR, Mehta G: Osteogenic potential of cissus quadrangularis assessed with osteopontin expression. *Natl J Maxillofac Surg.* 2013, 4:52-6. [10.4103/0975-5950.117884](https://doi.org/10.4103/0975-5950.117884)
24. Nandhini K, Tharani J, Sangeetha D: Screening of antibacterial activity of Cissus quadrangularis and Boerhavia diffusa against clinical pathogens. *Int J Sci Res.* 2022, 11:668-72. [10.37398/JSR.2020.640113](https://doi.org/10.37398/JSR.2020.640113)
25. Brahmshatriya HR, Shah KA, Ananthkumar GB, Brahmshatriya MH: Clinical evaluation of Cissus quadrangularis as osteogenic agent in maxillofacial fracture: A pilot study. *Ayu.* 2015, 36:169-75. [10.4103/0974-8520.175542](https://doi.org/10.4103/0974-8520.175542)
26. Benjawan S, Nimitphong H, Tragulpiankit P, Musigavong O, Prathantururug S, Pathomwichaiwat T: The effect of Cissus quadrangularis L. on delaying bone loss in postmenopausal women with osteopenia: a randomized placebo-controlled trial. *Phytomedicine.* 2022, 101:154115. [10.1016/j.phymed.2022.154115](https://doi.org/10.1016/j.phymed.2022.154115)

27. Pillay D, Emery VC, Mutimer D, et al.: Guidelines for laboratory monitoring of treatment of persistent virus infections. *J Clin Virol.* 2002, 25:73-92. [10.1016/s1386-6532\(02\)00018-5](https://doi.org/10.1016/s1386-6532(02)00018-5)