Original Article

Antibacterial Activity of *Acalypha Indica* Extract on *Porphyromonas Gingivalis*: An *In vitro* Study

Prarthana Manoharan, Lalithambigai Gunasekaran, Girish R. Shavi, Shankar Shanmuagam, John J. Smith
Department of Public Health Dentistry, Vivekanandha Dental College for Women, Namakkal, Tamil Nadu, India

Abstract

Introduction: *Acalypha indica* plant is traditionally used to treat dysentery, diarrhea, malnutrition, and malaria. This plant extract reported a wide antibacterial spectrum against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis,* and *Pseudomonas aeruginosa.* **Objective:** This study aimed to assess the antimicrobial efficacy of *A. indica* plant extract at the concentrations of 10–200 mg/ml against *Porphyromonas gingivalis*. *A. indica* plant metabolites were extracted by the maceration technique using methanol. **Materials and Methods:** The literature revealed the analysis of different phytochemicals such as alkaloids, flavonoids, tannins, saponins, and phenols. The antibacterial activity was analyzed using minimum inhibitory concentration (MIC) and minimum bactericidal concentration. **Results:** The n-hexane extract of *A. indica* has the best antibacterial potency at 200 mg/ml against *P. gingivalis* with an initial zone of inhibition at 100 mg/ml 12 mm, which is compared to the gold-standard chlorhexidine and MIC value of 150 mg/ml. **Conclusion:** Among major periodontal pathogens, *P. gingivalis* appears to be one of the prime etiological agents in the pathogenesis and progression of the inflammatory events of periodontal disease. This infinitesimal herb *A. indica* found throughout India is readily available and can be utilized as an effective antimicrobial agent against *P. gingivalis* and periodontal diseases.

Keywords: *Acalypha indica*, alternative medicine, antibacterial, periodontal diseases, *Porphyromonas gingivalis*

Introduction

Oral disease is one of the public health problems highly correlated to bacterial etiology. The most common preventable disease throughout one's lifetime, i.e., periodontal disease and dental caries, has been attributed to tooth loss and pain. It is often not self-limiting without a proper care. It is a form of chronic disease found in the majority of the population.[1]

Periodontal disease is predominantly a bacterial disease. Evidence reveals the burden of periodontal disease as 20%–50% across the globe. However, in developing countries like India, the prevalence of periodontal disease was found to be 51%. Periodontal disease is attributed to one of the major causes of tooth loss, masticatory compromise, esthetics, self-confidence, and quality of life.

Among major periodontal pathogens, *Porphyromonas gingivalis* appears to be one of the prime etiological agents in the pathogenesis and progression of the inflammatory events of periodontal disease. The literature revealed the implication of various pathogenic periodontal bacteria and their toxic products in circulatory problems.[2] Subgingival plaque samples from patients with chronic periodontitis have been attributed to contain 85.75% of the periodontopathic bacterium.[3]

Before modern medicines began to take their shape, we were highly dependent on natural resources for various ailments, known as conventional medicine. Civilization and the modern era have led way for more modern medicines, paving way for more antimicrobial resistance and reemerging diseases.[4] Before COVID-19, the world faced an emergent threat of antimicrobial resistance. The literature revealed that among the COVID-19-infected patients, only 4% had true bacterial coinfection. The overuse of antibiotics in the current situation paving way for a 10% increase in antimicrobial resistance.[5]

Address for correspondence: Dr. Prarthana Manoharan, Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode, Namakkal - 637 205, Tamil Nadu, India.
E-mail: lolitapreethi@gmail.com

Received: 17-05-2022 **Revised:** 10-08-2022 **Accepted:** 30-12-2022 **Published:** 31-03-2023

Access this article online

Quick Response Code:

Website: www.jiaphd.org

DOI: 10.4103/jiaphd.jiaphd_105_22

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Manoharan P, Gunasekaran L, Shavi GR, Shanmuagam S, Smith JJ. Antibacterial activity of *Acalypha indica* extract on *Porphyromonas gingivalis*: An *in vitro* study. J Indian Assoc Public Health Dent 2023;21:39-43.

COVID-19, the most deadly pandemic in the recent memory, was caused by the SARS-CoV-2 virus. According to the literature, a combination therapy of herbal medicine and western medicine significantly contributed to reduce a wide range of symptoms from fever to decreased syndrome score.[6]

The nature has nurtured us with various medicinal plants available around our settlements, spreading alongside roads, and households. One among those nurtured medicines is *Acalypha indica*, a small annual herb plant growing naturally in wet, temperate, and tropical areas along the equator continents of Asia. Africa, Europe, South and North America, and Australia.[7]

Conventionally, *A. indica* plant is used to treat dysentery, diarrhea, malnutrition, and malaria. These beneficial activities of the plant are allied to various chemical constituents and essential oil present in it. This plant extract reported a wide antibacterial spectrum against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis*, and *Pseudomonas aeruginosa*.[8] The literature reveals that the n-hexane extracts of *A. indica* have the best antibacterial potency at 50 mg/ml against *Streptococcus mutans*.[9]

A. indica, an infinitesimal herb, is readily available and can be utilized as an effective antimicrobial agent against *P. gingivalis*.[10] Various studies have shown the effective antimicrobial efficacy of *A. indica* against various oral pathogens; however, none of the authors explored its efficacy against periodontal pathogens. Hence, the present study aimed to evaluate the antibacterial efficacy of *A. indica* extract against *P. gingivalis*. The objective focused on calculating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *A. indica* against *P. gingivalis* by calculating the colony-forming units (CFU/ml).

Materials and Methods

After getting ethical clearance from the Institutional Ethical Committee of Vivekanandha Dental College for Women, *A. indica* plant was procured from the local nursery and dried under sunlight for 8 days, before grinding. Hazali *et al*. (2016) studied the subchronic toxicity effect of *A. indica* on the Sprague-Dawley rats based on the hematological and biochemical parameters and observed no sign of toxicity and mortality in all groups throughout the study. There was no significant difference ($P > 0.05$) in body weight gain and food and water intake between the control and treatment groups.[11-13] Procurement of pure form of *Porphyromonas gingivalis* bacterial strains was from the Dextrose Laboratory, Bengaluru. This *in vitro* study has been conducted at the Dextrose Technologies Private Limited, Bengaluru Urban, Karnataka.

Extraction of *Acalypha indica*

Using the maceration technique,[14] the extracts of *A. indica* plant was extracted and powdered. Sample powder was stored in an air-tight bag at room temperature and used for extraction. Fifty grams of the plant sample was mixed with 375 ml of solvent (n-hexane) in a 1000 ml volumetric flask for 48 h incubation. A vacuum rotary evaporator has been used to remove the solvent from the sample. Dimethyl sulfoxide has been added to the extracted samples for further analysis as a transport medium.

Phytochemical profiling

Samples were analyzed using thin-layer chromatography (TLC)[15] on a 20 cm × 10 cm silica gel aluminum plate. Two microliters of each extract were deposited and developed in a glass twin-trough chamber with hexane:ethyl acetate:formic acid, 7:10:0.1 (v/v/v), as a mobile phase. We dried the plates with forced air, and then visualized them under ultraviolet 254, 366 nm. Retardation factor (RF) values were recorded after devitalization with vanillin-sulfuric acid and anisaldehyde spray reagent [Figure 1].

Microbiology methods

P. gingivalis ATTC 33277 has been procured from the Dextrose Technologies Pvt. Ltd laboratory, Bengaluru, for culture preparation. Samples were stored in Tryptone Soya Agar (TSA) broth at 37°C incubation until further use.

We conducted three trials of antibacterial activity to minimize the procedural errors.

As positive control, triple antibiotic paste which is a standard antibacterial agent (metronidazole, ornidazole, ciprofloxacin) and as negative control distilled water was utilized against the bacterium.

Zone of inhibition test

The well-diffusion technique has been employed to evaluate the antibacterial activity. Autoclaved agar plates were dried and punctured 6 mm on its surface. The agar plates were seeded with 100 µl of the inoculums and spread evenly over the plate with a sterile glass spreader. Each sample (from 10 mg) was added to separate wells in the culture plates and incubated at 30°C for 24 h. After 24 h of incubation, the diameter of the zone of inhibition was measured to the nearest millimeter (mm) using a vernier caliper [Figure 2].

Minimum inhibitory concentration

Microbial susceptibility was tested by a spectrophotometric method using the dye 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT). Microtitration plates were prepared and incubated for 24 h at 35°C, with concentrations of 1 mg/ml − 225 mg/ml. Further, the author dispensed a 25 µl concentration of MTT dye to each agar plate. The operated executed spectrometric optical density (OD) measurement, after 30 min of incubation at room temperature and gentle agitation at 540 nm.

Minimum bactericidal concentration

The TSA plate is added with 100 µl sample and incubated at 37°C for 24 h. After incubation, the total viable colonies were counted and noted.

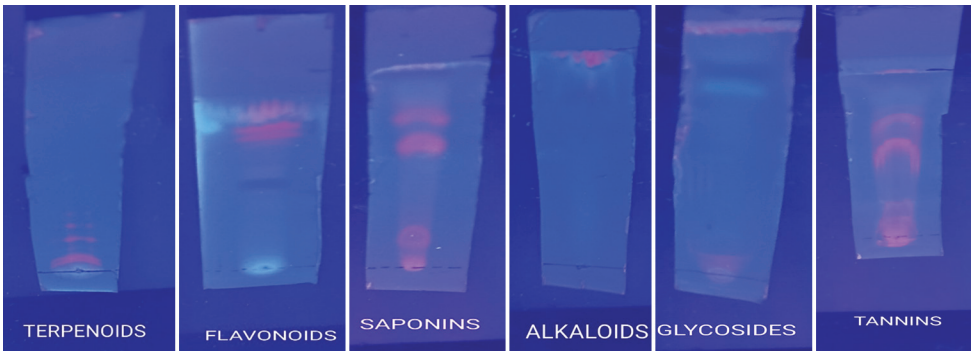


Figure 1: Thin-layer chromatographic analysis – polyphenols separated



Figure 2: Zone of inhibition

Results

In the present study, n-hexane solvent-extracted *A. indica* is utilized to evaluate its efficacy against *P. gingivalis.* The mobile phase thin-layer chromatographic analysis is carried out in a glass twin-trough chamber. Further, the RF values are recorded from TLC.

Initially, Table 1 depicts the TLC RF values.

The TLC results show the presence of six major phytochemicals, namely, alkaloids, flavonoids, tannin, terpenoid, saponin, and glycoside. RF values of each phytochemical, which means the ratio of the distance moved by the solute to the distance moved by the solvent, show glycoside with high RF value and alkaloids with low RF value. These infer that glycosides are polar and alkaloids are comparatively less polar. Later, the antibacterial activity of the extract is established by employing the zone of inhibition.

The results of the zone of inhibition are depicted in Table 2.

The effective zone of inhibition measured at 100 mg/ml concentration of *A. indica* extract was 12 mm and highly comparable with the gold-standard chlorhexidine gluconate 2 mg/ml which is 12 mm. Lower concentrations of the extract showed no effect against *P. gingivalis* [Table 2 and Figure 2].

Table 1: Thin-layer chromatography retardation factor values

Photochemical tested	RF value Sample-*Acalypha indica*
Alkaloid	0.86
Flavonoid	0.88
Tannin	0.87
Terpenoid	0.87
Saponin	0.88
Glycoside	0.89

RF: Retardation factor

Table 2: Zone of inhibition

Acalypha indica extract concentration (mg/mL)	Zone of inhibition (mm)
1	Resistant
5	Resistant
10	Resistant
20	Resistant
40	Resistant
60	Resistant
80	Resistant
100	12
125	13

Table 3 shows the MIC and minimum bactericidal activity (MBC) OD values of *A. indica* extract against *P. gingivalis* and their bacterial activity. Graph 1 shows the decreasing activity of bacteria with increasing *A. indi0ca* extract concentration. Positive control of 2 mg/ml and *A. indica* extract of 150 mg/ml showed 0 bacterial count.

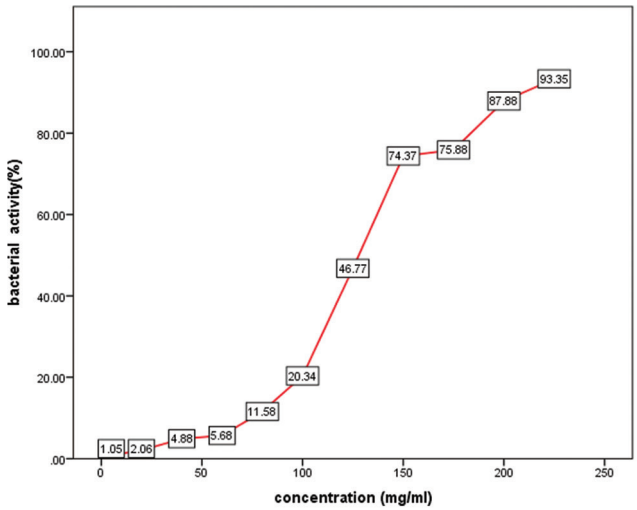
Discussion

Increased side effects, tolerance of modern medicines, and easy availability of low-cost traditional medicines have urged the modern era back to traditional medicines. The indigenous medicinal practice had been in existence for an extended period before our modern medicinal era. It has multidimensional chemical structures making them more diverse and efficient.[1] Even now, some parts of Asia and Africa rely 80% on traditional medicine for their primary health care.

Table 3: Minimum inhibitory concentration and minimum bactericidal activity optical density values of *Acalypha indica* extract against *Porphyromonas gingivalis*

Concentration (mg/mL)	MIC OD at 540 (nm)	MBC (CFU/mL)	Bacterial activity (%)
Negative control	1.986	1.2×10^{6}	
1	1.967	TNTC	0.95
5	1.965	TNTC	1.05
10	1.961	TNTC	1.25
20	1.945	TNTC	2.06
40	1.889	TNTC	4.88
60	1.873	TNTC	5.68
80	1.756	1.1×10^{3}	11.58
100	1.582	0.8×10^{3}	20.34
125	1.057	0.6×10^{3}	46.77
150	0.509	0	74.37
175	0.479	0	75.88
200	0.243	0	87.88
225	0.132	0	93.35
Positive control (triple antibiotic)	0.126	0	

TNTC: Too numerous to count, CFU: Colony forming unit, MBC: Minimum bactericidal concentration, MIC: Minimum inhibitory concentration, OD: Optical density



Graph 1: Anti - bacterial activity with increase in concentration of Acalypha indica extract

Our descendants are found to be using plant and their products for various treatments of ailments. The present study proved that *A. indica* plant extract possesses significant antibacterial activity against *P. gingivalis*, a major periodontal pathogen.

The present study utilizes a standard antibacterial agent, triple antibiotic paste (metronidazole, ornidazole, and ciprofloxacin) against oral pathogens, as a positive control. Further, distilled water is utilized as a negative control. Astonishingly, *A. indica* extract showed a zone of inhibition of 12 mm at 100 mg/ml concentration which is comparable with the 0.2% concentration of triple antibiotic paste.

The literature reveals numerous data on *A. indica* against various oral pathogens such as *S. aureus, Candida Albicans,* and many others.[8] The present study is supposed to be the first to demonstrate the antibacterial activity of *A. indica* extract against *P. gingivalis*. The chloroform extract of stem bark of *A. indica* evaluated by Ishak *et al*. in 2013 showed a 10 mm zone of inhibition at 200 mg/ml concentration against *S. aureus* bacteria and *C. albicans* fungi.[16]

Sekar *et al*., in 2012, concluded that the ethanol extract was more efficient than the acetone extract of the above-mentioned plant against various pathogens, which include *S. aureus, Streptococcus pyogenes,* and *P. aeruginosa*.[17]

MIC is the lowest concentration of the chemical, which can prevent the visible growth of the bacteria, and MBC is a complementary test for MIC, which depicts the minimum concentration of chemicals causing bacterial death. Batubara *et al*. showed MIC of 500 mg/ml in the n-hexane extract of *A. indica* against *Streptococcus* species. Further investigation under Govindarajan *et al*. depicted 625 mg/ml of MIC against staphylococcus and *Streptococcus* species.[17] This is the first study to depict the MIC and MBC of *A. indica* extract against *P. gingivalis*, with a MIC of 150 mg/ml.

Evidence put forward 10 million worldwide deaths could result from antibiotic resistance, making it deadlier than cancer in 2050.[6]

Conventional medicine is increased in the recent times partly due to the long unsustainable economic situation in our country and partly due to the high level of adversities related to modern medicines. Traditional medicines with comparatively cost-effective, more easily accessible, and lesser side effects can serve as an effective source, for treating ailments. Further studies on the toxicological effects of the plant extract are indispensable. The oral cavity is a plethora of microorganisms, necessitating the exploration of various other pathogens.

Conclusion

Conventional medicinal practices based on the natural plants have been recognized by the World Health Organization as reliable medicinal sources for therapeutic activities. Numerous phytochemicals have been evident in the Indian variety of *A. indica*, which is proven to be an effective antimicrobial against oral pathogens. The zone of inhibition of *A. indica* extract is comparable with the gold-standard chlorhexidine which is 12 mm. The antibacterial activity of *A. indica* extract is effective at 150 mg/ml against the major periodontal pathogen, *P. gingivalis*.

Acknowledgment

We would like to thank Mr. Aravind Ganessin, Managing Director, Dextrose Technologies Pvt Ltd., for allowing us carry out the present study at their laboratory and helping us complete the research project. We would like to thank one and all who helped us in this research project.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References
1. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986;50:353-80.
2. Cimpoiu C. Analysis of some natural antioxidants by thin-layer chromatography and high performance thin-layer chromatography. J Liq Chromatogr Relat Technol 2006;29:1125-42.
3. National Institutes of Health (US); Biological Sciences Curriculum Study. NIH Curriculum Supplement Series. Understanding Emerging and Re-Emerging Infectious Diseases. Bethesda (MD): National Institutes of Health (US); 2007.
4. Govindarajan M, Jebanesan A, Pushpanathan T, Samidurai K. Studies on effect of *Acalypha indica* L. (*Euphorbiaceae*) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 2008;103:691-5.
5. Sekar D, Kolanjinathan K, Saranraj P, Gajendiran K. Screening of *Phyllanthus amarus*, *Acalypha indica* and *Datura metel* for its antimicrobial activity against selected pathogens. Int J Pharm Biol Arch 2012;3:1231-6.
6. Ishak FD, So'ad SZ, Jauhari AH, Mashud NN, Hassan NM. *In vitro* study of antimicrobial activity of *Acalypha indica* Linn. Extract. Open Conf Proc J 2013;4 Suppl 2:57-60.
7. Nasr IH, Seppälä M. Scully's Medical Problems in Dentistry. India: Elsevier; 2015.
8. Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G, Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chem 2016;194:135-42.
9. Hazali N, Nazri NN, Ibrahim M, Masri M. Subchronic toxicity of *Malaysian Acalypha indica*: Biochemistry and haematology analysis of rat. J Teknologi 2016;78(5-5).
10. Zahidin NS, Saidin S, Zulkifli RM, Muhamad II, Ya'akob H, Nur H. A review of Acalypha indica L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential. J Ethnopharmacol 2017;207:146-173.
11. How YK. *Porphyromonas gingivalis*: An overview of periodontopathic pathogen below the gum line. Front Microbiol 2016;7:53.
12. Sivaranjani D, Saranraj P, Manigandan M, Amala K. Antimicrobial activity of *Plectranthus amboinicus* solvent extracts against human pathogenic bacteria and fungi. J Drug Deliv Ther 2019;9:36-9.
13. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global prevalence of periodontal disease and lack of its surveillance. ScientificWorldJournal 2020;2020:2146160.
14. Ang L, Song E, Lee HW, Lee MS. Herbal medicine for the treatment of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis of randomized controlled trials. J Clin Med 2020;9:1583.
15. Arshad M, Mahmood SF, Khan M, Hasan R. Covid-19, misinformation, and antimicrobial resistance. BMJ 2020;371:m4501.
16. Subramaniam G, Girish M. Antibiotic resistance – A cause for reemergence of infections. Indian J Pediatr 2020;87:937-44.
17. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants 2015;4:196.