Evaluation of antibacterial activity of Achyranthes aspera extract against Streptococcus mutans: An in vitro study

Abstract

Dental caries and periodontal diseases have historically been considered the most important global oral health burdens. Many chemicals and synthetic drugs have marked the side effects. Hence, there has been a paradigm shift from the use of modern drugs to the age-old herbs. Achyranthes aspera is one such important plant with various established pharmaceutical properties. The aim of this study was to assess the antibacterial activity of the A. aspera extract against Streptococcus mutans. Aqueous extract of A. aspera was prepared. Different concentrations of the root and stem extracts of A. aspera were transferred to the agar plates, which had been streaked with the bacterium S. mutans. The plates were incubated aerobically at 37°C for 24 h, and the zones of inhibition were measured using cup plate method. A. aspera extract showed statistically significant zones of inhibition. A. aspera showed marked antibacterial activity against S. mutans.

Key words: Achyranthes aspera, antibacterial activity, apamarga, chlorhexidine, Streptococcus mutans

INTRODUCTION

Dental caries and periodontal diseases have historically been considered the most important global oral health burdens.⁴ Many chemicals and synthetic drugs have proven to be effective in the prevention of these diseases, but they also have marked side effects. In recent times, there has been a marked shift toward herbal cures because of the pronounced cumulative and irreversible reactions of modern drugs.⁴,⁵

Achyranthes aspera Linn. is a species of plant in the Amaranthaceae family, commonly known as Apamarga or Chirchita in Hindi. It is a popular medicinal plant which has occupied a pivotal position in folk medicine throughout the tropical Asian and African countries. It is distributed throughout the tropical world and it is commonly found as a weed throughout India.

This herb is reported to have various pharmacological actions such as anti-inflammatory,⁴ analgesic, and antipyretic activities.⁵ It is used to treat many problems such as piles, digestive disorders, fever, cough, dysentery, psoriasis, paralysis, spleen enlargement, control of fertility, and postpartum bleeding.⁶ While some people also use the roots of this plant as tooth brush, it still has a long way to go to be established as a recognized entity in the prevention and treatment of oral diseases.

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How to cite this article: Yadav R, Rai R, Yadav A, Pahuja M, Solanki S, Yadav H. Evaluation of antibacterial activity of Achyranthes aspera extract against Streptococcus mutans: An in vitro study. J Adv Pharm Technol Res 2016;7:149-52.
Various studies report that the aqueous solution of *A. aspera* has shown antibacterial activity against *Staphylococcus aureus*, *Streptococcus heamolyticus*, and *Bacillus typhosus*, while alcoholic and aqueous extract of its leaves possess antibacterial activity against *S. aureus* and *Escherichia coli*.\(^7\)

Very few studies have been conducted on the antibacterial effect of *A. aspera* extracts against oral pathogens. Therefore, the aim of this study is to evaluate the *in vitro* antibacterial activity of *A. aspera* against *Streptococcus mutans*.

**MATERIALS AND METHODS**

*About the plant and microorganism*

The *A. aspera* plant was collected from the outskirts of Gurgaon, Haryana, and was authenticated by an expert, National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi. Pure strains of *S. mutans* (Microbiological Type Culture Collection [MTCC] 890) were obtained from MTCC, Institute of Microbial Technology, Chandigarh.

*Preparation of Achyranthes aspera extract*

Fresh stems and roots of *A. aspera* were collected and dried under shade and then in an oven at a temperature <50°C. The stems and roots were ground into a fine powder. Then, 100 g of powdered stems and roots were boiled in 2000 ml of distilled water separately in two flasks for 2 h. One thousand and three hundred milliliters of filtrate was obtained from the stems and roots, which was then filtered using a filter paper. It was reduced to obtain a solid residue of 6 g by heating it at 60°C. The solid residue obtained was then stored at a low temperature. Dimethyl sulphoxide was used to dissolve the solid residue made from the stems and roots to make different concentrations. One gram of solid residue was mixed in 10 ml of dimethyl sulphoxide to make 10% concentration and it was kept as a stock solution. From this stock solution, further different concentrations were made.

*Culture media*

Brain heart infusion agar was used as a medium to grow the lyophilized bacteria. The bacteria were streaked to the agar slants in test tubes and were kept in an incubator at 37°C for 48 h. Cup plate method was used to determine the zones of inhibition.\(^8\)\(^-\)\(^11\) In this method, the circular wells that can incorporate different volumes of the test agent were cut in the agar plates using a template. The extract of stems and roots with different concentrations (0.5%, 1%, 1.5%, 2%, 2.5%, 5%, 7.5%, and 10%) and at different volumes was transferred to the agar plates.

About 0.2% chlorhexidine at 10 µl was used as control and it was also transferred to agar plates which were incubated aerobically at 37°C for 24 h. Antibacterial activity was interpreted from the size of the diameter of zones of inhibition measured in millimeter using a measuring guide in all the agar plates. Figures 1-3 show the zones of inhibition formed by the stem and root extracts of *A. aspera* and chlorhexidine, respectively.
Statistical analysis
Statistical package for social sciences version 17 (SPSS Version 17, Chicago, IL, USA) for windows was used for statistical analysis. Comparison of mean values between the stem and root extract was done by using Student’s t-test.

RESULTS

The lowest concentration of the extract, which inhibited the growth of the tested microorganisms, was found to be 2.5% for both the stem and root extracts. The minimum zone of inhibition of 14 mm was seen at 2.5% concentration and 100 µl volume of root extract. While the maximum zone of inhibition of 21 mm was seen at 10% concentration and 250 µl volume. Similarly, the minimum zone of inhibition of 12 mm was seen at 2.5% concentration and 100 µl volume of stem extract while the maximum zone of inhibition of 16 mm was seen at 10% concentration and 250 µl volume [Table 1].

The mean value of zone of inhibition of the stem and root extracts was 13 and 15.2 at the lowest volume and 14.7 and 18.5, respectively, at the highest volume [Table 2]. While for chlorhexidine at 2% concentration, the zone of inhibition obtained was 19 mm at 10 µl volume.

DISCUSSION

Herbal medications have been around for ages. Our ancestors had been known to use plant life to treat and alleviate illnesses that affect the human body. Medicinal plants are today being once again preferred because of the various reasons such as their easy availability, negligible side effects, low cost of treatment, and their effectiveness. The present study proved that A. aspera possesses a significant antibacterial activity against S. mutans, which is the causative organism playing a major role in the pathogenesis of dental caries.

In the present study, chlorhexidine gluconate which is considered a gold standard for antibacterial agents against oral pathogens was taken as control. At 0.2% concentration, it showed a zone of inhibition of 19 mm at 10 µl volume. While, for A. aspera, the maximum zone of inhibition was found to be 16 mm at 10% concentration and 250 µl volume and 21 mm at 10% concentration at 250 µl volume for stem and root extracts, respectively. Though the plant extract was found to be effective at a higher concentration and volume than chlorhexidine gluconate, it showed a marked antibacterial activity and should be considered to replace the synthetic drugs and chemicals because of their irreversible side effects. A study conducted by Giri et al.[12] showed 28 ± 1.4 zone of inhibition at 50% concentration of A. aspera extract. Similar studies were conducted by Kumar et al.[13] and Manjula et al.,[14] in which the extracts of A. aspera were reported to have a marked antibacterial activity against various pathogenic strains.

The cup plate method was used in this study which depends on the diffusion of the test agent to an extent that growth of the added bacteria is prevented completely in a zone around the circular wells containing the solution of the test agent. This method has been found to give precise results as also reported by the studies of Vahabi et al.,[5] Prabhat et al.,[9] Phatak et al.,[9] and Agarwal et al.[11]

The antibacterial activity of A. aspera can be attributed to the alkanoids and tannins. Tannin is a phenolic compound which is soluble in water and it could be one of the components responsible for the antibacterial activity.[4]

Extracts of the leaves and callus of this plant in various solvents have been reported to show antimicrobial activity.[15] Prabhat et al.[9] reported that methanolic extracts possess antimicrobial activity while Khan et al.[16] reported that the ethanol and chloroform extracts of the seeds of A. aspera show mild–to-moderate antibiotic activity against Bacillus subtilis, E. coli, and Pseudomonas aeruginosa. Jebashree et al.[17] demonstrated the anticiarigenic activity of A. aspera by using ethyl acetate extracts of A. aspera, which showed high antibacterial activity against S. mutans than other solvent extracts. However, in the present study, aqueous extract of A. aspera was used, which is most easy and safe to obtain and showed a marked antibacterial activity.

Any antimicrobial agent is considered effective, given the size of inhibition zone produced by it measures 2 mm or more.[18] In the present study, the minimum zone of inhibition obtained were 12 mm and 14 mm for stem and root extracts of A. aspera, respectively. It has proved to have potent antibacterial property.

Table 1: Zone of inhibition for stem and root extracts

| Concentrations (%) | Volumes (µl) | Stem (mm) | Root (mm) | Stem (mm) | Root (mm) | Stem (mm) | Root (mm) | Stem (mm) | Root (mm) |
|--------------------|-------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
|                    | 100         | 150       | 200       | 250       |
| 2.5                | 12          | 14        | 12        | 16        | 13        | 16        | 14        | 17        |
| 5                  | 13          | 15        | 13        | 16        | 14        | 17        | 14        | 18        |
| 7.5                | 13          | 16        | 14        | 17        | 15        | 17        | 15        | 18        |
| 10                 | 14          | 16        | 15        | 16        | 16        | 20        | 16        | 21        |
Table 2: Mean values of stem and root extracts at different volumes and concentrations

| Extracts | n | Mean±SD | t  | P          |
|----------|---|---------|----|------------|
| 100 µl   |   |         |    |            |
| Root     | 4 | 15.25±0.96 | 3.576 | 0.012*     |
| Stem     | 4 | 13±0.82   |     |            |
| 150 µl   |   |         |    |            |
| Root     | 4 | 16.25±0.5 | 3.973 | 0.007**    |
| Stem     | 4 | 13.5±1.29 |     |            |
| 200 µl   |   |         |    |            |
| Root     | 4 | 17.5±1.73 | 2.777 | 0.032*     |
| Stem     | 4 | 14.5±1.29 |     |            |
| 250 µl   |   |         |    |            |
| Root     | 4 | 18.5±1.73 | 3.79 | 0.009**    |
| Stem     | 4 | 14.75±0.96|     |            |

*Significant, **Highly significant. SD: Standard deviation

CONCLUSION

Antibacterial agents against oral microorganisms, especially those contributing to sub- and supra-gingival biofilm formation, play an important role in the prevention of dental caries and periodontal disease. Since some chemical synthetic drugs including chlorhexidine can cause brown staining of the teeth, tongue, transient impairment of taste perception toxic effects on connective tissues, dryness and soreness of oral cavity, allergic reactions in patients, and oral desquamation in children, herbs with medicinal properties can serve as a useful and effective source of treatment for various dental diseases. Hence, they can be utilized along with conventional medicine that can assure us of greater health in the future. Further studies can be conducted in future to assess the safety levels of such herbs.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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