

ANTIBACTERIAL POTENTIAL OF ACMELLA OLERACEA AGAINST BACTERIA CAUSING DENTAL CARIES-AN IN-VITRO STUDY

Dental Science

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ABSTRACT

Aim: This study intended to evaluate the antibacterial potential of *Acmella oleracea* against dental caries causing microorganisms, namely *Streptococcus mutans* and *Lactobacillus acidophilus*.

Materials and methods: *Acmella oleracea* leaves were shade dried and ground well. Acetone and methanol extracts prepared were screened for in vitro antimicrobial activity and zones of inhibition determined using agar well diffusion method.

Results: Of all the different extracts tested, acetone extract of *Acmella Oleracea* at higher concentrations were found to be more potent against *Streptococcus mutans*, with a zone of inhibition of 14 mm compared to amoxicillin control (16 mm). Acetone extract of *Acmella oleracea*, had a zone of inhibition of 10 mm against *Lactobacillus acidophilus*.

Conclusion: Acetone extract of *Acmella Oleracea* was found to possess good antibacterial property against *Streptococcus mutans* and *Lactobacillus acidophilus*.

KEYWORDS

Acmella Oleracea, Antimicrobial, Dental caries

INTRODUCTION

Dental caries is a major public health problem globally and is the most widespread non communicable disease (NCD).¹ The Global Burden of Disease Study 2016 estimated that oral diseases affected half of the world's population (3.58 billion people) with dental caries (tooth decay) in permanent teeth being the most prevalent condition assessed. Dental treatment is costly, averaging 5% of total health expenditure and 20% of out-of-pocket health expenditure in most high-income countries. The oral health care demands are beyond the capacities of the health care systems in most low-and middle-income countries (LMICs). Oral health inequalities exist among and between different population groups around the world and through the entire life course. Social determinants have a strong impact on oral health.²

Although the oral cavities of most mammalian species contain billions of microbial cells, most scientists have concluded that *Streptococcus mutans* is the bacterium that is responsible for dental caries, or decay.³ Under cariogenic conditions, *S.mutans* play the critical role required for the colonization of lactobacilli in the oral cavity (i.e. creating a retentive niche) which in turn contribute to the progression of lesions, acting as secondary invaders.

Different antibiotics can prevent bacterial infection, but the surging problem of antibiotic resistance poses a challenge and urges renewed efforts to seek alternatives. One of the possible strategies towards this objective is rational localization of bioactive phytochemicals.⁴ Use of medicinal plants contributed as an alternative for antibiotic effects such as hypersensitivity reaction, supra infections and teeth stainings.⁵ The World Health Organization has estimated that about 80 % of the population in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant-based.⁶ *Acmella Oleracea*, also called the toothache plant has been used to treat toothache and throat and gum infections from a long time and is a known folk remedy. In India, spilanthos has long been integrated into ayurvedic medicine under its Sanskrit name *Sarahattika* and is used to support the immune system, improve digestion, treat headaches, relieve toothaches and alleviate nausea.⁷

The main constituents of the plant, namely, *spilanthol* and *acemellonate* are sometimes used to reduce the pain and can induce saliva secretion.⁸ Xerostomia or dry mouth can also increase the chance of developing dental decay, demineralization of teeth, tooth sensitivity, and/ or oral infections.⁹ *A.oleracea*, with its action as a sialogogue help alleviate

xerostomia and prove beneficial for overall oral health. It has also been projected that it has multiple pharmacological actions as antipyretic, anti-inflammatory, antifungal, diuretic, vasorelaxant, antimalarial, etc and minor side effects.

With the growing concern of antibiotic resistance and as it leads to higher medical costs, there exists an unprecedented urgency in finding alternatives, hence the present study was planned to evaluate the antibacterial activity of *Acmella oleracea* against the most common cariogenic microorganisms, namely *Streptococcus mutans* and *Lactobacillus acidophilus*. There is a dearth of literature on *Acmella oleracea* calibrating its effect as a safe and novel alternative to antibiotics currently in use, which reinforce the need for the present study.

MATERIALS AND METHODS

Fresh leaves of *Acmella oleracea* were collected and properly washed with distilled water. Afterwards, it was shade dried and grounded into a powdered form using a sterile mortar and pestle. Soxhlet extraction method was employed to prepare acetone and methanol extracts. Bacterial isolates obtained from laboratory were uniformly spread using a sterile swab on Mueller-Hinton agar plates. Four agar plates were prepared, each with four wells of 6mm diameter made with a sterile cork borer. Out of the four, two agar plates were of *Streptococcus mutans* and two of *Lactobacillus acidophilus*. Acetone and methanol extracts in increasing concentrations (25µl, 50µl, 100µl) were introduced into the three wells on the two *S.mutans* swabbed agar plates, and fourth well had Amoxicillin, a standard drug as a positive control. Similarly, extracts and positive control were introduced into wells of lactobacillus plates. For the extracts to diffuse into the agar medium, plates were allowed to stand at room temperature for one hour, after which the plates were incubated at 37°C for 18 hours. After incubation, plates were observed for results and zones of inhibition measured.

RESULTS

- Acetone extract of *Acmella Oleracea* at 100µl effectively inhibited growth of *Streptococcus mutans* (14mm) and it was comparable to that of positive control, amoxicillin (16mm) as in fig 1.
- Methanol extract of *A.oleracea* at 100µl inhibited growth of *S.mutans* (10mm) compared to amoxicillin (12mm). Fig 2.
- Inhibition zone measurements for acetone extracts of *A.oleracea*

- at 100 μ l against *Lactobacillus acidophilus* was found to be 10mm and for methanol extracts at 100 μ l, it was 5mm.
- Extracts showed concentration-dependant activity against both the bacterial strains and inhibition zone increased with increase in the concentration.
 - *A.oleracea* exhibits lower antibacterial activity when it comes to *L.acidophilus* compared to *S.mutans*.
 - At the same time, acetone extract was found to be more effective compared to methanol extract against both bacteria.

Fig 1. Zone of inhibition values for different concentrations of A.Oleracea acetone extracts and control on S.mutans and L.acidophilus.

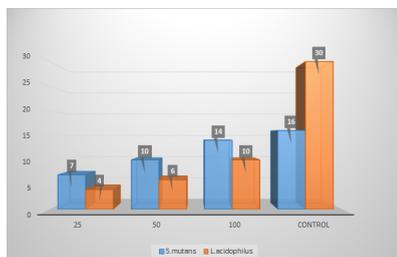
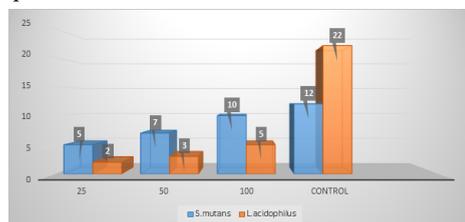


Fig 2. Zone of inhibition values for different concentrations of A.Oleracea methanol extracts and control on S.mutans and L.acidophilus.



DISCUSSION

Dental health is integral to general wellbeing and relates to the quality of life that extends beyond the functions of the craniofacial complex.¹⁰ Among all the diseases affecting oral health, dental caries is responsible for a high rate of morbidity among the population and are the main cause of tooth loss among the population.

Antibiotics commonly used to treat oral infections like penicillin, cephalosporin, erythromycin, tetracycline and derivatives and metronidazole seem to develop bacterial resistance over long term use. The herbal medicine has an edge over conventional antibiotic treatment that suffer the limitation of low benefit to high risk as compared to herbal treatment that possess high benefit to low-risk ratio.¹¹ Over the past decades, pharmaceutical companies have been interested in investigating plants as sources for new phytotherapeutic agents with proven efficacy, safety and quality.¹²

As an increased acquired resistance to conventional antibiotics is observed, it is rational to attempt combination therapy of standard antibiotics with plant extracts that possess bioenhancing activity to attain bactericidal synergism. Use of such combination therapy against resistant bacteria may lead to newer options for the treatment of infectious diseases. Combination therapy can be used for expansion of the antimicrobial spectrum, prevention of the emergence of resistant mutants, minimizing the toxicity. Bio-enhancers may act by (1) increasing drug absorption (2) modulating biotransformation of drugs in the liver or intestines (3) modulating active transport (4) decreasing elimination (5) immune modulatory activity.¹³

Spilanthes spp. are popular, over-the-counter remedies; they are sold over the internet under various names and are widely used in traditional medicine in various cultures. *Spilanthes* spp. are used for more than 60 types of disorders. They are predominantly used as extracts in personal care products, traditional medicines, and the pharmaceutical and culinary areas.¹⁴ *Acmella oleracea* also known by *Spilanthes acmella* and *Spilanthes oleraceae* has been traditionally used for treatment of toothache. Its antibacterial activity by itself is enhanced by its action as a sialogogue.

In the present study, acetone extract of *A.oleracea* showed

antimicrobial activity against *S.mutans* exhibiting maximum zone of inhibition of 14mm at 100 μ l concentration. Better results were found in a study conducted by T.Thompson *et al.* using acetone extract, wherein the zone of inhibition was found to be 20mm.¹⁵ The difference observed could be attributed to variations in the parts of the plant material used and the quality of plant sample, differences in the microbiological techniques used and difference in solvent-sample ratio.

In the present study, methanol extract of *A.oleracea* showed antimicrobial activity against *S.mutans* with maximum zone of inhibition of 10mm at 100 μ l and this antimicrobial activity was comparable to that of the positive control amoxicillin(12mm). Similar result was obtained by a study conducted by T.Thompson *et al.* where zone of inhibition of 13 mm was seen, supporting the findings of our present study. In contrast to the present study, much better results were obtained in a study conducted by Onoriode O *et al.* with maximum zone of inhibition for methanol extract of *A.Oleracea* at 29 mm.¹⁶ In the same study, against *L.acidophilus*, the methanol extracts showed an average zone of inhibition of 24mm. In a study conducted by Jency *et al.* methanol extracts of *A.oleracea* had shown a max zone of inhibition of 14.2 mm against *L.acidophilus*.¹⁷ Meanwhile in the present study, methanol extract of *A.Oleracea* showed a minimal zone of inhibition of just 5mm at 100 μ l concentration. The difference observed may be attributed to the method employed for extraction of solvent (In soxhlet extraction there is a chance of thermolabile compounds getting damaged) and in variation in polarity of the two solvents used as well as the incubation time.

CONCLUSION

Acmella Oleracea has antibacterial activity against predominant cariogenic bacteria namely *S.mutans* and *L.acidophilus*. Difference in susceptibility of two test bacteria towards extracts could be due to nature of the antibacterial agents present and mode of action on the bacteria. As with any another *in vitro* study, results should be interpreted cautiously because same antibacterial activity of *A.oleracea* extracts against the tested microorganisms cannot be expected if the microorganisms are present as a part of dental biofilm.

Hence, further *in vitro* studies in biofilm models followed by properly designed randomized controlled trial should be carried out to substantiate the antibacterial potential of *A.oleracea* extracts against *S.mutans* and *L.acidophilus*.

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