Research article

*In vitro antibacterial efficacy of Ananas comosus and Vitis vinifera on cariogenic microorganism*

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ABSTRACT

**Introduction and Aim:** Human health concerns are converging, necessitating a concerted effort to find preventive and therapeutic approaches. Fruits and vegetables are the primary sources of essential nutrients and have various bioactive compounds suitable for treating oral diseases in children. In the Indian sub-continent, the most consumed fruit juices are Pineapple - *Ananas comosus* and Grape - *Vitis vinifera* because they are easily accessible, cost-effective, and have increased beneficial effects on oral microorganisms. This present study aimed to assess the antibacterial efficacy of fruit juice extracts of *A. comosus* and *V. vinifera* on two major cariogenic bacteria, namely *Streptococcus mutans* and *Lactobacillus acidophilus*.

**Materials and Methods:** The ethanolic extracts of *A. comosus* and *V. vinifera* were prepared, and their antibacterial effects were evaluated by agar well diffusion method against *S. mutans* and *L. acidophilus*.

**Results:** The overall mean difference of zone of inhibition of *A. comosus* and *V. vinifera* juice extracts was assessed using One way ANOVA. *V. vinifera* (P-value of <0.01) showed increased antibacterial efficacy against *S. mutans* and *L. acidophilus* when compared to *A. comosus*. Further, *V. vinifera* juice extract showed a significant zone of inhibition against *S. mutans* when compared to *L. acidophilus*. (P = 0.001*)

**Conclusion:** The ethanolic extract of *V. vinifera* juice extract had better antibacterial efficacy against both microorganisms when compared to *A. comosus*.

**Keywords:** Pineapple; Grape; *Streptococcus mutans*; *Lactobacillus acidophilus*; Ethanolic extract.

**INTRODUCTION**

Dental caries is a multifactorial infectious disease influenced by host response, diet, and nutrition. Despite the addition of fluoride, dental caries continues to be the most frequent infectious disease affecting people, particularly children. Missed school days often occur due to the negative impact on children's learning abilities (1-3). Although many oral pathogens are present in the mouth, *S. mutans* and *L. acidophilus* are critical players in developing dental caries (4). Antimicrobial agents such as mouthwashes play a crucial role in preventing dental caries, especially in areas of the mouth that are difficult to access with mechanical plaque control methods like brushing and flossing. Therefore, antimicrobial agents like mouthwash can be essential to daily oral hygiene practices (5). Several antimicrobial agents have been studied for their effectiveness against oral microorganisms, including Chlorhexidine, Triclosan, Cetyl pyridinium chloride, and Fluoride-based solutions. Among these agents, Chlorhexidine is the chemical plaque control agent of choice due to its broad-spectrum antimicrobial activity and substantivity to bond with both soft and hard tissues in the oral cavity. However, its long-term usage has been linked to adverse consequences, including brown discoloration of the teeth and restorative materials, brown tongue dorsum, taste disturbance, oral mucosal ulceration, unilateral/ bilateral parotid enlargement, and accelerated supra gingival calculus formation (6). While these methods effectively reduce the growth of oral bacteria, they might not be enough to thoroughly remove plaque from all areas of the oral cavity.

Herbs are a popular alternative to synthetic or chemical antioxidant and antibacterial substances (7). In recent decades, children's beverage consumption habits have shifted away from healthier options, such as milk and water, towards sugar-sweetened beverages. The consumption of 100% fruit juice has risen significantly to overcome the above trend. The increase in fruit juice consumption might be attributed to several factors, including the convenience of juice as a snack, the perception that it is a healthy beverage option, its lower cost, and efforts that advertise juice as a substitute for whole fruits and vegetables (8).

*A. comosus* (pineapple) is one of the significant tropical fruits. The active substance in *A. comosus* is Bromelain, a protein-digesting enzyme that can accelerate a hydrolysis reaction of protein. Bromelain has been widely used in the fields of the manufacture of food products, chemicals, and pharmaceuticals. The bromelain enzyme targets the cell wall polypeptides so that the formation of cell walls is hindered, which in turn causes the bacterial cell to lyse due to increased osmotic pressure (9).
V. vinifera (grapes) has been implicated worldwide in the health research literature for several phytonutrients, especially in its skin and seed. They are rich in flavonoids and polyphenolic compounds like monomeric catechin and oligomeric proanthocyanidins (10). These flavonoids interact with bacterial proteins and cell wall structures, which might cause damage to cytoplasmic membranes, decrease membrane fluidity, and suppress nucleic acid synthesis, cell wall formation, or energy metabolism (11).

The antibacterial effectiveness of several fresh fruits, including apples, pomegranates, and watermelon, has been thoroughly explained in the available literature by numerous in vitro and in vivo investigations. Studies on A. comosus and V. vinifera are scarce, and the only components of these fruits that have been evaluated against cariogenic bacteria are their peels, pulp, and seeds. Since the fresh whole fruit juice extracts without added sugars are simple to prepare and easy to consume by children, the present in vitro investigation was conducted to evaluate and compare the antibacterial efficacy of A. comosus and V. vinifera, against the most common caries-causing oral pathogens, S. mutans and L. acidophilus.

MATERIALS AND METHODS

Ethical guidelines

The present study was conducted per the standards and protocols of Meenakshi Academy of Higher Education (MAHER) University. The approval of the Ethical Committee was obtained from the Institutional Review Board at MAHER University. The working protocol of the study was conducted using the facility and support of the Microbiology laboratory, Royal Bio Research Centre, Chennai.

Plant material

Fresh indigenous fruits of young A. comosus (Kew-King variety) and V. vinifera (Paneer grapes- Muscat Hamburg variety), cultivated in Tamil Nadu, were procured from a local organic farm in Chennai. Before further processing, the fruits were thoroughly washed with running tap water and subjected to surface sterilization using 70% Ethanol. The fruits were then rinsed with sterile distilled water. For A. comosus, the fruit was peeled and cut into small pieces, while for V. vinifera, the berries were carefully separated from the stem and pedicel. Subsequently, individual sterile conical flasks were filled with ten grams of the prepared cut/separated fruits.

Preparation of ethanolic fruit extract

Preparing the ethanolic fruit extract involved adding 100 ml of Ethanol to conical flasks. These flasks were left undisturbed in the laboratory at room temperature for 72 hours. Then, the ethanolic fruit juice extracts were individually filtered using sterile filter paper. The filtrates were placed in a mantel heater at 35°C for 10 minutes to remove the Ethanol. After the Ethanol evaporation, the filtrates were weighed, and the final yield was determined based on their weight.

Using the agar well diffusion method, a sample size of 34 zones was determined for each test organism per fruit juice extract to estimate the mean zone of inhibition. The sample size was calculated to achieve a statistical power of 90%.

Evaluation of antibacterial efficacy

The microorganisms, S. mutans, and L. acidophilus, were isolated from the saliva of patients with dental caries to mimic the exact oral environment, and stock cultures were made. Active cultures for the present experiment were prepared by transferring a loop of cells from the stock cultures to test tubes of nutrient broth for bacteria incubated for 24h at 37°C.

A total of 28 petri dishes were utilized, divided into four groups comprising seven plates each. One group had plates containing A. comosus fruit juice extract, while another contained V. vinifera fruit juice extract. Both groups were subjected to independent testing against S. mutans and L. acidophilus. Then, the inoculums were spread on the respective plates, and five wells per plate, each measuring 1 cm in diameter, were created for pouring the fruit juice extracts. Then, 100 µl of A. comosus and V. vinifera fruit juice extract was added to the wells of the corresponding plates using a micropipette. The petri dishes were then incubated at room temperature (37°C) for 24 hours. Following the incubation period, the zone of inhibition around each well was measured in millimetres, using digital Vernier calipers, at its maximum possible diameter.

Determination of the minimal inhibitory concentration (MIC)

This experiment tested the effects of different concentrations of ethanolic extracts of A. comosus and V. vinifera on the growth of S. mutans and L. acidophilus to determine the MIC value. The test compounds were prepared at 1000, 500, 250, 125, and 62.5 µg/ml concentrations. A serial dilution method was employed to determine the MIC value, as mentioned by Jayaraman et al. (12), for which five ml of Muller Hinton broth was taken in 20 tubes (five tubes/group). Then, 100 µl of the S.mutans or L.acidophilus cultures was added to each tube. Additionally, 100 µl of the fruit extracts at different concentrations (1000, 500, 250, 125, and 62.5 µg/ml) were added to the respective tubes. The tubes were then incubated for 24 hours at 37°C to allow bacterial growth. A calorimeter determined the microbial growth at 600/620 nm wavelength. The Optical Density (OD) values were measured to assess the inhibitory effect of the fruit extracts on the growth of S. mutans and L. acidophilus as detected by the absence of visual turbidity. The MIC concentration was confirmed by inoculating Muller Hilton agar culture plates with serial
dilutions of both extracts in a laminar airflow environment. After 24 hours of incubation at room temperature, the zone of inhibition was measured to calculate the MIC. The obtained OD and zone of inhibition values showed that the MIC values for the fruit juice extract of A. comosus were 250µg/ml against S. mutans and 125µg/ml against L. acidophilus. The MIC value for the fruit juice extract of V. vinifera against both test organisms was 250 µg/ml. **Antibacterial efficacy**

Table 1 shows that the mean difference in the zone of inhibition was more for the fruit juice extract of A. comosus against S. mutans when compared to L. acidophilus (2.28±5.8); however, the difference was not statistically significant(p=0.339).

Table 2 demonstrates a highly significant result (p=0.001*) indicating a notable difference in the mean zone of inhibition for the fruit juice extract of V. vinifera when tested against both S. mutans and L. acidophilus.

Table 3 reveals results comparing the overall mean difference in the zone of inhibition between the four groups. The mean value of V. vinifera (6.571) shows a larger zone of inhibition, which indicates that V. vinifera shows a statistically significant difference (P <0.001*) when compared to A. comosus.

**DISCUSSION**

In the present study, A. comosus and V. vinifera showed significant antibacterial activity against S. mutans and L. acidophilus. One of the key strengths of the present study is the utilization of fresh fruit juice extracts, which have been shown to have several beneficial properties in pediatric dentistry. Fresh fruit juices offer many advantages due to their rich content of phytonutrients, which are bioactive substances naturally occurring in plants and can scavenge free radicals (13). Another advantage is that the high polyphenol content in fruit juices causes a high antioxidant activity (11). As demonstrated in the present study, the ethanolic preparation of fruit juices offered the significant advantage of minimizing contamination risks associated with handling natural products. The present study is the first in vitro experimental research which compared A. comosus and V. vinifera ethanolic fruit juice extracts against S. mutans and L. acidophilus. The results of the present study that established the antibacterial effect of the

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**Table 1:** Comparison of the mean difference of zone of inhibition of A. comosus against S. mutans and L. acidophilus.

<table>
<thead>
<tr>
<th>Mean Difference</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% CI</th>
<th>t-value</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.285</td>
<td>5.822</td>
<td>2.200</td>
<td>-7.670</td>
<td>3.099</td>
<td>6</td>
<td>0.339</td>
</tr>
</tbody>
</table>

*Significant P value (P<0.05).

**Table 2:** Comparison of the mean difference of zone of inhibition of V. vinifera against S. mutans and L. acidophilus.

<table>
<thead>
<tr>
<th>Mean Difference</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% CI</th>
<th>t-value</th>
<th>Degree of freedom</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.571</td>
<td>2.636</td>
<td>0.996</td>
<td>-9.010</td>
<td>14.132</td>
<td>6</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

**Table 3:** Comparison of the mean difference of zone of inhibition of A. comosus and V. vinifera against S. mutans and L. acidophilus.

<table>
<thead>
<tr>
<th>Fruit extracts</th>
<th>Test organisms</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean square</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>A. comosus</td>
<td>S. mutans</td>
<td>245</td>
<td>3</td>
<td>81.67</td>
</tr>
<tr>
<td></td>
<td>L. acidophilus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. vinifera</td>
<td>S. mutans</td>
<td>L. acidophilus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant P value (P<0.05).
extracts highlight their use as potential antimicrobial agents in preparing mouthwashes for young children.

Vargas et al., studied the effect of fresh fruit juices on dental caries in children. They concluded that there was no association between 100% fruit juice consumption and dental caries among young children (7). An in vivo study concluded that drinking juices was associated with less early childhood caries and severe early childhood caries among preschoolers (14). A review article on the role of plant extracts in inhibiting the growth of oral pathogens concluded that plant extracts reduced the development of biofilms and dental plaque, influenced the adhesion of bacteria to surfaces, and reduced the symptoms of oral disease (15). The children with SECC had a statistically higher count of salivary levels of S. mutans and Lactobacillus than caries-free children (16). Hence the above two microorganisms were tested in the present study.

The diffusion technique used to assess the antibacterial activity can be done on paper or in a well, is frequently used to evaluate natural extracts with antibacterial activity and has the benefit of having highly reproducible results. Due to the drawbacks of the paper method, the agar well diffusion method was preferred in the present study; since the extract comes into direct contact with the agar, it is regarded to be a more effective method to determine the antibacterial activity of any material of interest (17).

MIC values obtained for A. comosus and V. vinifera in the present study were in contrast with the findings from the previous in vitro studies. A previous study concluded that S. mutans was sensitive at a concentration of 2 mg/ml to the Bromelain enzyme found in the pineapple core (18). In an in vitro study that assessed the MIC values of the grape seed extract against S. mutans, the MIC value was determined to be 2 mg/mL (19). However, it must be considered that the variance in MIC values could be attributed to examining specific parts of the fruits, as the essential components attributing to antimicrobial activity could vary based on the part of the fruit used. The overall antioxidant activity, pulp firmness, total soluble solids, and Bromelain activity increase in the pineapple fruit during the fifth month of harvesting after anthesis, whereas the ascorbic acid content decreases with age (20,21). Thus, in the present study, young fruits of A. comosus were used.

In a previous in vitro study where the antibacterial activity of pineapple weevil extract was studied, the results revealed that the weevil extract displayed a significant bactericidal effect on the growth of S. mutans (22). Goudarzi et al., concluded in their in vitro study that the pineapple peel showed significant antibacterial activity against S. mutans (23). The available literature highlights that chewing the pineapple fruit and stem has been observed to prevent a drop in plaque pH after eating snacks. The above practice is essential in preventing the initiation and progression of dental caries (24). Previous studies show that the Bromelain enzyme isolated from the core of pineapple showed the largest clear zone of inhibition against S. mutans (25,26). Hence in the present study, the core and flesh of A. comosus was the preferred choice to test its antibacterial activity.

When used as a mouthwash in children, the grape seed extract displays antimicrobial activity against S. mutans compared to a potent antiseptic such as Chlorhexidine (27). The biological activity of S. mutans was inhibited by the phenolic compounds found in grapes at various concentrations of 500 and 250 µg/ml (28). Among the two fruit juice extracts in the present study, V. vinifera had increased antibacterial efficacy against S. mutans, as per the studies mentioned earlier.

**Limitations**

Within the limitations of the current in vitro experimental study, the antibacterial efficacy of A. comosus and V. vinifera against S. mutans and L. acidophilus was confirmed. The main drawback of the present study was that the antibacterial effect of these fruit juice extracts was not tested against conventional antimicrobial drugs. Therefore, additional studies need to be performed comparing the antibacterial efficacy of fruit juices with gold-standard antibiotics utilizing various concentrations of fruit juice extracts. Also, its shelf life, salivary pH and plaque pH concentrations must be investigated. Conducting thorough investigations to validate the potential benefits of fruit juice extracts as a mouthwash is crucial. The impact of the extracts on oral microbiota in vivo, particularly their ability to combat biofilm formation, must be studied.

Moreover, comprehensive testing on various oral microorganisms is necessary to establish the spectrum of antibacterial properties exhibited by these extracts. Further clinical studies with extended follow-up periods are required. By conducting the extended studies, robust evidence regarding the efficacy and safety of the interventions could be obtained, thereby ensuring that the results are reliable and applicable to the pediatric population.

**CONCLUSION**

The present study demonstrated the potential of A. comosus and V. vinifera extracts as alternatives to conventional mouthwashes, which might pose risks to young children. With the prevention of dental caries and antibiotic drug resistance in children becoming increasingly challenging for pediatric dentists, using herbal mouthwashes offers a promising approach to alleviate the burden. The results indicated that V. vinifera exhibited more potent antibacterial activity against common caries-causing pathogens in children, namely S. mutans and L. acidophilus than A. comosus.
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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES


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