ANTIBACTERIAL ACTIVITY OF ACTINIDIA DELICIOSA (KIWI FRUIT) EXTRACT AGAINST PERIODONTOPATHOGENIC BACTERIA: AN IN-VITRO STUDY

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ABSTRACT

Introduction: Kiwi fruit belongs to the Actinidiaceae family which is mainly cultured in the tropics and subtropics such as New Zealand, France, and Japan. Kiwi fruits have been eaten stewed, in jams and jelly juice. It contains a high medicinal value. Objective: To determine the antibacterial potential of pure Actinidia deliciosa (Kiwi Fruit) extract against periodontopathogenic bacteria; Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn) and Aggregatibacter actinomycetemcomitans (Aa). Materials and Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to assess the antibacterial efficacy of Actinidia deliciosa extract against periodontopathogenic bacteria by serial dilution method and colony-forming units respectively. Results: The MIC value of Actinidia deliciosa extract was achieved in the range between 12.5-100µg/ml for Pg, Pi, Fn, and Aa, suggesting that periodontal pathogens tested were susceptible to Actinidia deliciosa extract. MBC value of Actinidia deliciosa extract revealed no growth of colony forming units at the highest tested concentration (100 µg/ml) for all tested periodontopathogenic bacteria. Conclusion: Actinidia deliciosa extract exhibited potent antibacterial activity and further investigations are recommended to evaluate the in-vivo effect in different formulations (gel, mouthwashes, etc.) for the management of periodontal disease.
KEYWORDS: Actinidia, minimum bactericidal concentration, minimum inhibitory concentration, periodontal pathogens, Vitamin C.

INTRODUCTION
Periodontitis is an inflammatory disease associated with multifarious microbiota dwelled in the subgingival area that includes gram-negative, anaerobic bacteria and some motile species, destroying the tooth-supporting tissues.[1,2] Among the pathogens, Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi), and Aggregatibacter actinomycetemcomitans (Aa) are strongly insinuated in the etiology of chronic periodontitis.[3,4]

Conventional treatment of chronic periodontitis includes mechanical therapy that can only remove the local factors but does not eliminate bacteria residing in periodontal tissue which can be eliminated either by administration of systemic antibiotics or locally delivered antibiotics into the periodontal pocket.[5] However, long-term administration of antibiotics can lead to antibiotic resistance against anaerobic bacteria species, allergies, and toxicity problems.[6]

Thus, there is a need for the development of new strategies for the management of periodontal disease, to partially or completely exclude the use of antibiotics. Consequently, this has led to the shift from an allopathic mode of therapy to naturopathic therapy, thereby minimizing the adverse effects of systemic administration, and making it more site-specific by using a local drug delivery system.[7]

The most important tenets of naturopathy are that the etiology of the disease is eliminated so that the body tends to use its inherent abilities to heal itself. Fruits are one such naturally available source, which has rich amounts of bioactive compounds, such as flavonoids, phenolic compounds, vitamins and enzymes which have various properties like scavenger and angiogenic effects which further initiate a disease-free environment.[8]

Actinidia deliciosa commonly known as kiwi fruit is an excellent supplementation of vitamin C. It is also rich in antioxidants such as lutein, oxycarotenoid, omega-3 fatty acid and alphalinolenic acid, thereby induces robust anti-inflammatory property. In addition, it has valuable medicinal properties like anti-bacterial, anti-asthmatic, anti-platelet, anti-nociceptive, anti-
tumor, anti-hypertensive, etc. These numerous pharmacological properties of the kiwi fruit can be attributed to the presence of various bioactive compounds present in it.\cite{9} (Table 1)

To the best of our knowledge, till date no study has been conducted to know the antibacterial potential of *Actinidia delicosa* on the most common periodontal pathogens. Hence, the present study aimed to find out the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of pure *Actinidia delicosa* extract that in the future can be safely and efficiently administered as controlled drug delivery system against specific periodontopathogenic bacteria.

### Table 1: Chemical composition of *Actinidia delicosa*.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td>Anti-bacterial, anti-inflammatory, antioxidant</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Anti-bacterial, anti-oxidant, anti-cancer, anti-inflammation, analgesic and comparative immune-modulation</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Normal cell function, growth and development, immune function</td>
</tr>
<tr>
<td>Minerals</td>
<td>Electrical neutrality, nerve transmission maintains acid-base balance immune system health</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>Anti-inflammatory, anti-viral, anti-cancer and anti-oxidant</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Anti-inflammatory, antibacterial, anti-viral, anti-listerial,anti-cancer and anti oxidant</td>
</tr>
<tr>
<td>Tannins</td>
<td>Antiviral, antibacterial and antitumour activities</td>
</tr>
</tbody>
</table>

### MATERIALS AND METHODS

*Actinidia delicosa* (Kiwi fruit) extract  
About 100% pure *Actinidia delicosa* extract in powder form was obtained from Navchetana Kendra health care private limited, New Delhi.

**Bacterial strains**  
The tests were performed using the American type cell culture collection strains, Manassas, VA, USA. The bacterial strains used were Pg, ATCC 33277, Pi ATCC 25611, Fn, ATCC 25586, and Aa, ATCC 29523.
Minimum inhibitory concentrations (MIC)

MIC is the most minimal concentration of antimicrobial agent that will inhibit the obvious growth of bacteria after overnight incubation. A stock solution containing Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi), and Aggregatibacter actinomycetemcomitans (Aa) was prepared by addition of 100 μg Actinidia deliciosa extract to 1 ml thioglycollate (TG) broth medium. According to the standard protocols given by Schwalbe et al, nine dilutions of the Actinidia deliciosa extract were prepared with the TG broth medium. 10 μl of each stock culture were added to the test tube containing antimicrobial agents. The tubes were sealed airtightly and incubated at 37°C for 24 hours in an anaerobic jar and observed for turbidity. The minimum concentration of the Actinidia deliciosa extract in the tube which does not show any turbidity is considered as the MIC of the drug. The procedure was repeated five times to minimize errors.

Minimum bactericidal concentration

After the MIC procedure, dilution tubes that were showing sensitivity to the Actinidia deliciosa extract at lower concentrations were incubated in an anaerobic jar for ≥48 hours into the respective culture medium to check the growth of microorganisms then colonies were counted.

RESULTS

The results revealed that Actinidia deliciosa extract restricted the growth of all the four tested bacteria at the highest tested concentration (100 μg/ml). Minimum inhibitory concentration value of Actinidia deliciosa extract against Pi, Pg, Aa, and Fn were 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 100 μg/ml respectively (Table 2 and Figure 1). Minimum bactericidal concentration value of Actinidia deliciosa extract against Pi, Pg, Aa, and Fn were 12.5 μg/ml, 25 μg/ml, 50 μg/ml and 100 μg/ml respectively (Table 3 and Figure 2). However, MBC values showed there was no colony forming units at 100 μg/ml concentration suggesting that all tested periodontopathogenic bacteria were sensitive at the highest tested concentration.
Figure 1: Minimum inhibitory concentration procedure performed using serial dilution method.

Figure 2: Minimum bactericidal concentration; a) *Prevotella intermedia* (Pi), b) *Aggregatibacter actinomycetemcomitans* (Aa), c) *Porphyromonas gingivalis* (Pg), and d) *Fusobacterium nucleatum* (Fn)
Table 2: MIC values of Actinidia deliciosa extract.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>100 μg/ml</th>
<th>50  μg/ml</th>
<th>25  μg/ml</th>
<th>12.5 μg/ml</th>
<th>6.25 μg/ml</th>
<th>3.12 μg/ml</th>
<th>1.6  μg/ml</th>
<th>0.8  μg/ml</th>
<th>0.4  μg/ml</th>
<th>0.2  μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Pg</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Aa</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Fn</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
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Table 3: MBC values of Actinidia deliciosa extract.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>100 μg/ml</th>
<th>50  μg/ml</th>
<th>25  μg/ml</th>
<th>12.5 μg/ml</th>
<th>6.25 μg/ml</th>
<th>3.12 μg/ml</th>
<th>1.6  μg/ml</th>
<th>0.8  μg/ml</th>
<th>0.4  μg/ml</th>
<th>0.2  μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>68</td>
<td>79</td>
<td>98</td>
<td>102</td>
<td>112</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>Pg</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>48</td>
<td>52</td>
<td>69</td>
<td>85</td>
<td>92</td>
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<tr>
<td>Aa</td>
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<td>Fn</td>
<td>NG</td>
<td>32</td>
<td>45</td>
<td>54</td>
<td>85</td>
<td>99</td>
<td>126</td>
<td>138</td>
<td>206</td>
<td>215</td>
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</table>

DISCUSSION

Naturopathy is a field of alternative medicine which beliefs the ideology of vitalism and folk medicine. Essential nutrients that cannot be synthesized by the human body must be consumed in the diet. Fruits are one such naturally available source that is rich in nutrients, minerals, and vitamins to our body.

Actinidia deliciosa (Kiwi fruit) is one such nutrient-dense fruit, containing a noteworthy medicinal value that includes an array of nutrients, antioxidants, phytonutrients and enzymes that act to provide functional, metabolic benefits and homeostasis in the body.[8]

However, there are limited studies regarding the antibacterial properties of Actinidia deliciosa. Sudha et al. assessed the antimicrobial potency of Emblica officinalis, Hylocereus undatus, Vitis vinifera, Syzygium cumini and Actinidia deliciosa against K. pneumonia, S. aureus, P. aeruginosa, E. coli, and C. albicans and concluded that Actinidia deliciosa showed the maximum antimicrobial activity against K. pneumonia followed by S. aureus, P. aeruginosa, E. coli, and C. albicans.[11] Mishra et al. conducted a study which assayed the antimicrobial, antioxidant and chemopreventive potential extracts of Carambola, Guava, Kiwi, Papaya and Strawberry against E.coli and it was inferred that kiwi fruit demonstrated the best results, among all the selected fruits.[12]

In the present study, the antibacterial potency of Actinidia deliciosa extract was evaluated against four specific periodontopathogenic bacteria; Porphyromonas gingivalis (Pg), Fusobacterium nucleatum (Fn), Prevotella intermedia (Pi) and Aggregatibacter
Actinomycetemcomitans (Aa). The range of MIC values of pure Actinidia deliciosa extract were 12.5-100µg/ml, with the highest value noted against Fn and the lowest value noted against Pi. The MBC values of Actinidia deliciosa extract indicated that there was no growth of colony-forming units at the highest tested concentration (100 µg/ml) against the tested periodontopathogenic bacteria. The number of colony-forming units was inversely proportional to the concentration of extract. Thus, suggesting that the pure Actinidia deliciosa extract has predictable bactericidal activity against periodontopathogenic bacteria. (Table 2 and 3)

Antibacterial activity of kiwi fruit can be attributed due to the presence of manifold of bioactive compounds such as Phenolic acids, Coumarins, Anthocyanins, Organic acids, and Tannins. Specifically, Phenolic compounds and Coumarins have the capacity to inactivate the bacterial cellular enzymes which are responsible for penetration of the bacterial substance into the host cell. Tannins, also a key component of kiwi fruit exhibit antimicrobial activity by inhibition of extracellular microbial enzymes thus leading to deprivation of the substrates required for microbial growth. Tannins directly act on microbial metabolism by inhibiting oxidative phosphorylation.

Biochemical studies revealed that kiwi fruit contains Vitamin C (ascorbic acid) which increases the hydroxylation of proline, which is required for collagen biosynthesis. Vitamin C also scavenges free radicals and other reactive oxygen species, which actively diminishes inflammation, thus acting as a potent antioxidant.

From this in-vitro study, it can be inferred that Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Fusobacterium nucleatum (Fn) and Aggregatibacter actinomycetemcomitans (Aa) were sensitive to pure Actinidia deliciosa extract. Further investigations are recommended to perform an in-vivo clinical trial to determine the beneficial effect of pure Actinidia deliciosa extract.

CONCLUSION
Embracing the paradigm shift towards naturopathy, in the present in-vitro study it can be concluded that Actinidia deliciosa exhibited potent antimicrobial activity against the four tested periodontal pathogens. Further studies are suggested to assess and compare the in-vivo efficacy of Actinidia deliciosa extract with other traditionally approved antimicrobials for the management of polymicrobial periodontal disease and also to evaluate the in-vivo effect of
Actinidia deliciosa extract in different formulations (gel, films, chips, mouthwashes, patches, etc.) with variable concentrations.

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Conflicts of interest
There are no conflicts of interest.

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