

Antibacterial efficacy of epicatechin and rutin from *Acacia catechu* leaf extract against *Enterococcus faecalis* and *Streptococcus mutans* - An *in vitro* study

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ABSTRACT

To evaluate *in vitro* antibacterial effect of epicatechin and rutin from *Acacia catechu* leaf against *Streptococcus mutans*, *Enterococcus faecalis*. Chlorhexidine is used as a positive control. ATCC strains were obtained from HI media (Mumbai). Using agar well diffusion method, susceptibility tests were performed overnight incubation at 37°C. All tests were performed in duplicate. Minimum inhibitory concentration and minimum bactericidal concentration were also performed by broth dilution method. Epicatechin showed significant antibacterial efficacy against the oral microbes tested whereas rutin showed moderate efficacy against the oral microbes tested. Indian herbal extracts are used since ancient times to treat various disease ailments as these natural products exert broad-spectrum actions. *E. faecalis*, *S. mutans* is a normal inhabitant of the oral cavity. *E. faecalis* commonly causes Root canal failure. The prevalence of *S. mutans* is increased in oral rinse samples from patients receiving dental treatment. Hence, the finding suggests that epicatechin from ethanolic leaf extract of *A. catechu* showed good activity against *S. mutans* when compared to rutin.

Keywords: Antibacterial, broth dilution, acacia catechu leaf, epicatechin, rutin

Introduction

Oral microbial flora is dominated by gram positive micro organisms and hence dental plaque which is formed on the tooth surface contains gram positive cocci and bacilli.^[1] Since decades Natural products have been investigated as promising agents to prevent oral diseases like Dental caries, Dental plaque, periodontal disease.^[2]

Acacia catechu Willd. (Mimosaceae) is commonly called as khair or black cutch. It is known as khadira in Sanskrit and Karungali in Tamil.^[3] People in Kerala drink the boiled juice for digestive disorders. In India.^[4] Epicatechin is an odorless white powder. Epicatechin is a flavonol belonging to the group of flavonoids. Epicatechin is a strong antioxidant, has insulin mimic action and improves heart health. It is used in management of diabetes mellitus.^[5-7] Epicatechin 3 gallate is the most predominant catechins which is used to treat dental caries

and periodontal infections.^[8] Rutin is a bioflavonoid. Pure rutin is yellow or yellow-green coloured needle-shaped crystal. Rutin is a flavonol glycoside comprised of the quercetin and the disaccharide rutinose. The richest source is buckwheat. Rutin is also found in citrus fruits, noni, black tea, apple peel. During digestion much of the rutin is metabolized to its aglycone, quercetin.^[9] It has antioxidant, anti-inflammatory, anticancer and antiobesity activity.^[10-12] Hence, in this study, we investigated the antibacterial activity of Epicatechin and Rutin from *Acacia catechu* leaf extract against oral bacteria.

Materials and Methods

Drugs

Epicatechin and rutin were purchased from Sigma-Aldrich, India.

Test microorganism

Streptococcus mutans - ATCC 25175 *Enterococcus faecalis* - ATCC 29212.

Minimum inhibitory concentration (MIC)^[13-15]

To 100 µL of sterile broth, 100 µL of the sample was added and mixed to attain a concentration of 10 mg/mL. This was serially diluted to obtain the dilutions of 50 mg/mL, 25 mg/mL, 15 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and finally 1 mg/mL. Each concentration was inoculated with 0.01 mL of 24 h bacterial cell

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suspension and incubated at 37°C for 24 h. Chlorhexidine is used as a positive control. The presence of cloudiness or turbidity of the broth indicates positive growth. The concentration which inhibits the bacterial growth is considered as the MIC.

Result

The result depicted in Table 1 shows that epicatechin shows no growth when tested against *S. mutans* and *E. faecalis*, positive control chlorhexidine also showed no growth. Rutin showed no growth at higher concentration than Epicatechin against *S. mutans* and *E. faecalis*.

Discussion

Dental caries/dental plaque is one of the major infectious diseases of the oral cavity World Wide. Dental plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest threat of disease.^[16,17]

Table 1: MIC of epicatechin and rutin from *Acacia catechu* leaf extract

Standard	<i>S. mutans</i> (mg/ml)	<i>E. faecalis</i> (mg/ml)
Epicatechin	25	25
Rutin	50	25
Control	10	10

MIC: Minimum inhibitory concentration, *S. mutans*: *Streptococcus mutans*, *E. faecalis*: *Enterococcus faecalis*, *A. catechu*: *Acacia catechu*

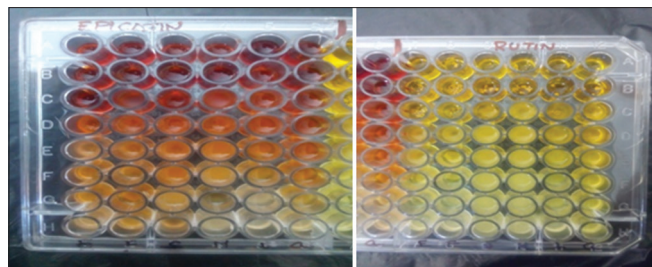


Figure 1: Broth dilution assay of *Acacia catechu* leaf extract

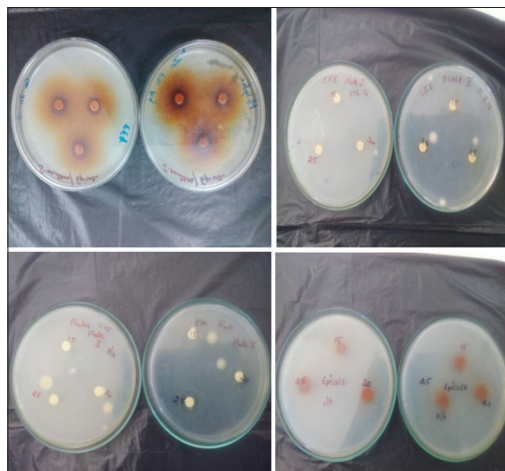


Figure 2: Agar well diffusion assay of *Acacia catechu* leaf extract

In this study, epicatechin shows no growth at 25 mg/ml tested against *S. mutans* and *E. faecalis*, chlorhexidine as positive control showed no growth in 10 mg/ml. Rutin showed no growth at 50 mg/ml against *S. mutans* and at 25 mg/ml against *E. faecalis*. When compared to chlorhexidine the efficacy was less, but epicatechin and rutin showed antibacterial activity against *S. mutans* and *E. faecalis* with a moderate action (Figures 1 and 2).

Current research indicate that the polyphenols, being secondary metabolites, are present in rich amount in various plants.^[18-20]

Therapeutic properties *A. catechu* willd and other herbal extracts contains many active constituents in it like Catechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, phloroglucin, protocatechuic acid, poriferasterol, poriferasterol acylglucosides, lupenone, kaempferol, dihydrokaemferol, Quercetin, Taxifolin etc.^[21]

Conclusion

In conclusion, it is suggested that the significant antibacterial efficacy of epicatechin and rutin found in this study could be attributed due to the presence of polyphenols. However, further clinical trials are required to prove the anticariogenic efficacy.

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