# CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY OF ACACIA NILOTICA AND THYMUS VULGARIS BASED MOUTHWASH COMPARED WITH CHLORHEXIDINE COMMERCIAL MOUTHWASH AGAINST ORAL MICROBES.

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**Abstract**

**Background:** Caries is one of the most commonly affecting diseases of the oral cavity. There are many methods of removing caries but prevention is always better than cure. Mouthwash is a well studied chemotherapeutic agent. Though many mouthwashes are available in the market, the quest for a good and effective mouthwash is still there. Acacia nilotica and thymus vulgaris have been used in the present study as mouthwash.
**Materials and methods:** Mouthwashes have been prepared using Acacia nilotica and Thymus vulgaris and the anti microbial activity and cytotoxicity of the mouthwashes have been evaluated against various oral microbes and compared with commercially available chlorhexidine mouthwash.
**Results:** Acacia and Thyme have both shown good antimicrobial and less cytotoxic activity. Synergistic activity is shown by both the mouthwashes.
**Conclusion:** Acacia nilotica and thymus vulgaris can be used as effective mouthwash.

**Keywords:** Mouthwash, Acacia, Thyme, antimicrobial activity.

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## 1. Introduction

Oral cavity is affected by many diseases. Of all these, dental caries is the most commonly seen one.(1) The main etiological agents of dental caries are Streptococcus mutans and Lactobacillus species.They can easily colonize the tooth surface, cause production of acid and cause demineralization of tooth tissues.(2)
As it is known, different methods have been followed for caries removal.(3) But, prevention is always better than curing a disease. Caries prevention program should be primarily aimed to reduce cariogenic bacteria.

A mouth rinse is a chemotherapeutic agent used as an effective home care remedy to enhance oral hygiene and prevent dental caries by targeting the cariogenic bacteria. A variety of synthetic mouthwash is available in the market which claims to reduce the cariogenic bacteria by preventing plaque accumulation on tooth surfaces. Chlorhexidine is the most commonly used synthetic mouthwash.(4)

The usage of antimicrobial herbal products in dentistry has been well documented in prevention of dental caries.(5) In spite of various commercially available anticaries agents, the search for an effective herbal antimicrobial mouthwash still continues. Medicinal plants are an important source of raw materials for manufacturing many drugs.
In our study we have made use of Acacia nilotica and Thymus vulgaris as key ingredients to prepare mouthwash.

Acacia nilotica is commonly called babul tree or gum arabic tree.It is known for its anti-microbial, anti-plasmodial and antioxidant activity and used for treatment of human immunodeficiency virus, hepatitis C virus and cancer. (6)It is useful for treatment of venereal diseases, nausea, burns and wounds, stomachache and diarrhea.Different parts of the tree are used for different purposes.(7) We have made use of the bark of the tree for preparation of mouthwash.

Thymus vulgaris is commonly called thyme or garden thyme. It belongs to the family Laminacae and is native to Europe and Mediterranean region.It is known to have anti-inflammatory, antimicrobial, and antioxidant effects that may be useful in treating everything from intestinal infections to skin conditions. (8)We have used thyme leaves for preparation of mouthwash.
The aim of the present study is to compare the cytotoxicity and antimicrobial activities of Acacia nilotica, Thymus vulgaris and Acacia,Thymus mixed mouthwash with that of commercially available chlorhexidine mouthwash.

## 2. Materials and Methods

**Preparation of Acacia nilotica, Thymus vulgaris mouthwash:**
Bark powder of Acacia nilotica and dry leaves of Thymus vulgaris were separately taken into two different beakers. To each of the beakers, 50 ml of distilled water is added and stirred till the constituents are mixed and no lumps are seen. The mixture was heated till the volume was reduced to 5 ml. 1ml of herbal extract concentrate was taken in a separate 10 ml falcon tube. To each of the tubes, 0.3gram sucrose was added as sweetening agent, 0.001gram sodium benzoate was added which acts as preservative, 0.01 gram sodium lauryl sulfate was added which served as detergent. Finally 9ml of distilled water was added to the solution to make 10ml of mouthwash.
Similarly mixed mouthwash was prepared by mixing both Acacia nilotica and Thymus vulgaris mouth washes.

Figure1: Bark of A. nilotica

Figure2: A. nilotica solution

Figure3: A nilotica concentrate

Figure4: Leaves of T vulgaris

Figure 5: T vulgaris solution

Figure6: T vulgaris concentrate

**Anti microbial activity of Acacia nilotica and Thymus vulgaris mouthwashes**

Antibacterial activity of the prepared mouthwash was assessed against the strains of oral microbes Streptococcus mutans,Lactobacillus, Enterococcus faecalis and Candida albicans. Muller Hilton Agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and was let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The mouthwash solutions of different concentrations were loaded onto plates and were incubated for 24 hours at 37 ° C. After the incubation time, the zones of inhibition were measured. Control used was commercially available mouthwash.

**Brine Shrimp Lethality Assay(Cytotoxicity Test)**

**Saltwater preparation:**

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water.

6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL, 10µL, 20µL, 40µL, 80µL).Nauplii is the early larval stage of shrimps.This organism is one of the important test organisms for toxicity test because of its characteristics such as easy culturing (hatching from eggs gives organisms of similar age, genotype and physiological condition), short life cycle, and resistance to manipulation, wide geographic distribution, simplicity and cost-effectiveness of performed tests.

Then Acacia nilotica, Thymus vulgaris mouth washes were added separately to different wells according to the concentration level. Control used was commercially available mouthwash. The plates were incubated for 24 hours.

After 24 hours, the ELISA plates were observed and noted for the number of live nauplii's presence and calculated by using the following formula,

number of dead nauplii/number of dead nauplii+number of live nauplii×100

## 3. Results:

**Antimicrobial Activity of Acacia nilotica mouthwash:**

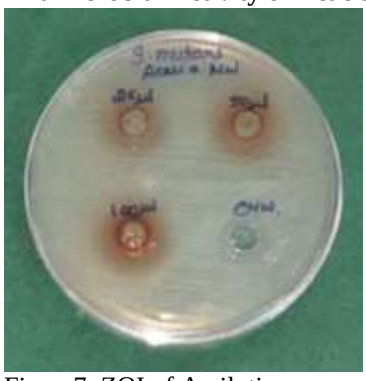

Figure7: ZOI of A nilotica against S mutans.

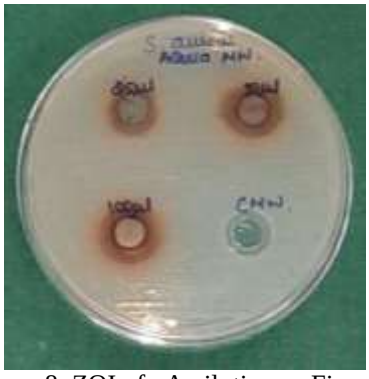

Figure 8: ZOI of A nilotica against S aureus

Figure 9: ZOI of A nilotica against C albicans

**Antimicrobial Activity of Thymus vulgaris mouthwash:**

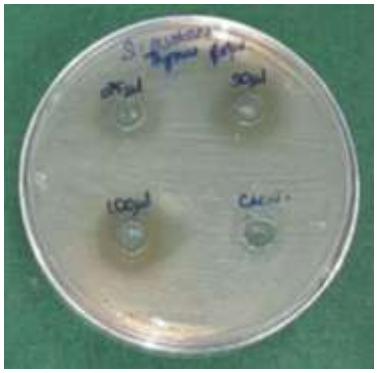

Figure 10: ZOI of T vulgaris against S mutans.

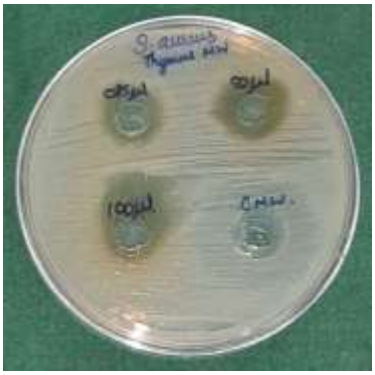

Figure 11: ZOI of T vulgaris against S aureus.

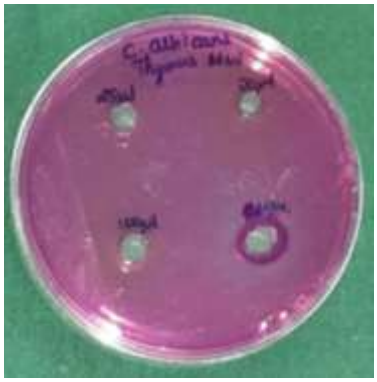

Figure 12: ZOI of T vulgaris against C albicans.

**Antimicrobial Activity of A nilotica & T vulgaris mixed mouthwash:**

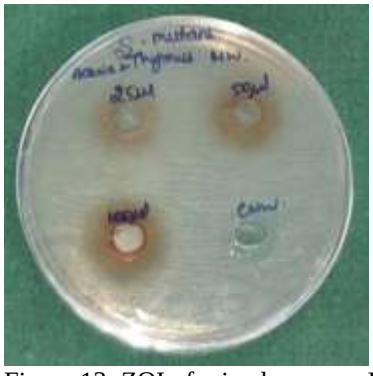

Figure 13: ZOI of mixed Mouthwash against S mutans.

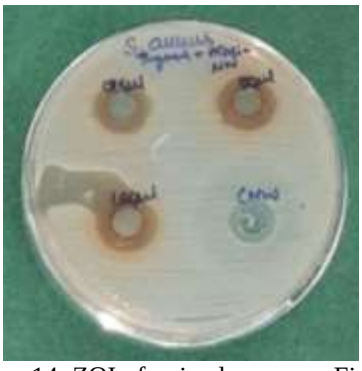

Figure 14: ZOI of mixed Mouthwash against S aureus.

Figure 15: ZOI mixed mouthwash against C albicans.

**Cytotoxicity Activity of Acacia nilotica:**

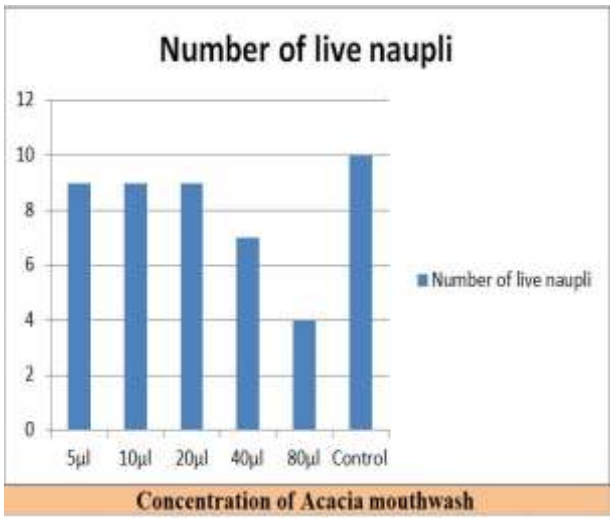


Table 1: Table showing live and dead nauplii after incubation for A nilotica mouthwash

| Concentration | Number of live nauplii | Number of dead nauplii |
|---|---|---|
| 5µL | 9 | 1 |
| 10µL | 9 | 1 |
| 20µL | 9 | 1 |
| 40µL | 7 | 3 |
| 80µL | 4 | 6 |
| Control | 10 | 0 |

**Cytotoxicity Activity of Thymus vulgaris:**

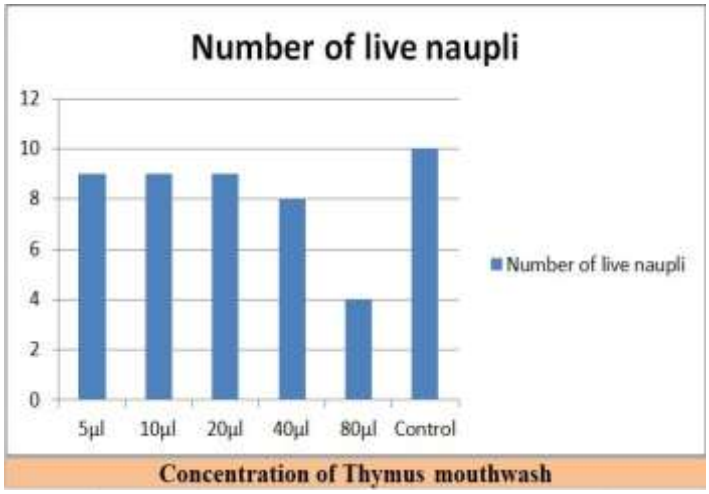


Table 1: Table showing live and dead nauplii after incubation for T vulgaris mouthwash

| Concentration | Number of live nauplii | Number of dead nauplii |
|---|---|---|
| 5µL | 9 | 1 |

| | | |
|---|---|---|
| 10µL | 9 | 1 |
| 20µL | 9 | 1 |
| 40µL | 8 | 2 |
| 80µL | 4 | 6 |
| Control | 10 | 0 |

**Cytotoxicity Activity of Acacia nilotica & Thymus vulgaris mixed mouthwash:**

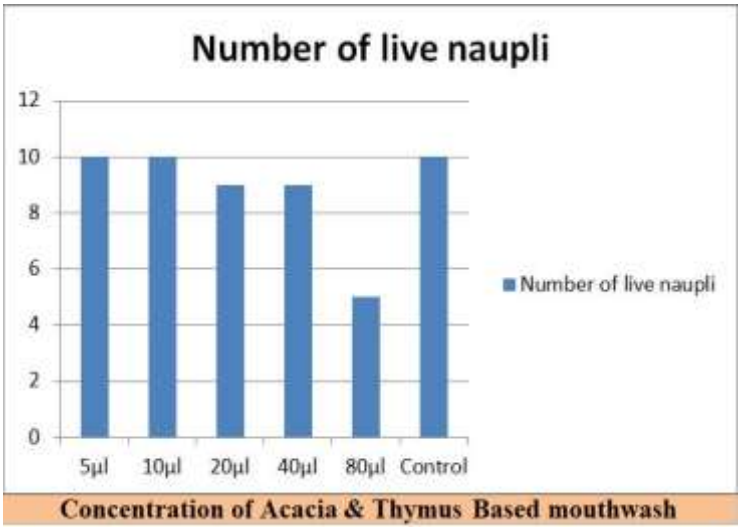


Table 3: Table showing live and dead nauplii after incubation for A nilotica & T vulgaris mixed mouthwash

| Concentration | Number of live nauplii | Number of dead nauplii |
|---|---|---|
| 5µL | 10 | 0 |
| 10µL | 10 | 0 |
| 20µL | 9 | 1 |
| 40µL | 9 | 1 |
| 80µL | 5 | 5 |
| Control | 10 | 0 |

## 4. Discussion

Dental caries affects a vast majority of the world population. The caries causing microbes can colonize in the oral environment and cause demineralization of tissues. There are many methods by which caries can be removed and restored but always prevention is better than cure. Mouthwashes are one of the most popular home remedies to prevent the accumulation of food debris thereby preventing dental caries and can also be used to maintain oral hygiene.(9)

Commercially available mouthwashes include listerine, chlorhexidine etc. Yet there is always a quest for effective herbal mouth wash. Herbal medicine is India's one of the age old forms of medicine that has been highly reliable. In our study we have prepared mouthwash based on Acacia nilotica and Thymus vulgaris.

Acacia nilotica is also called babul tree or gum arabic tree. It is known for its antibacterial,

antioxidant, anticarcinogenic and anti-inflammatory activities.(10)

Thymus vulgaris is a herb which has leaves of distinct smell. It has been used since ancient times to achieve healing, cure chest congestion, and induce saliva, the fresh leaves are taken to relieve sore throat. It is also known to have antimicrobial, anti inflammatory and anti cariogenic actions. (11)Thymus and Acacia have been used separately and two different mouthwashes have been self prepared. The cytotoxicity, antimicrobial action of both separately and combined mouthwash has also been evaluated.

Zone of inhibition was the indicator used for antimicrobial activity. Zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. This can be used to measure the susceptibility of the bacteria towards the antibiotic. Larger the zone is, more sensitive the bacteria is to that antibiotic, smaller the zone, more resistant is the bacteria to that particular product.Control used for antimicrobial test was commercially available mouthwash. Acacia nilotica showed good antimicrobial action. Zone of inhibition of Acacia against S mutans at 25µl was 16mm, at 50µl was 20mm, at 100µl was 22mm whereas ZOI of control commercially available mouthwash was only 16mm. Against S aureus, ZOI at 25µl was 14mm, at 50µl was 18mm at 100µl was 20mm whereas that of commercially available mouthwash was only 14mm. Against E faecalis, ZOI at 25µl was 14mm, at 50µl was 18mm and at 100µl ZOI was 20mm whereas that of control was 20mm. Against C albicans, ZOI was equal to control at all concentrations, that is 10mm. This shows that Acacia nilotica mouthwash is most effective against S mutans, also better results were shown against S aureus, E faecalis and C albicans compared to commercially available mouthwash. Similar study was conducted by Chandrasekhar et al., in which antimicrobial action was seen against Porphyromonas Gingivalis. (6). According to the author, results were in favor of herbal mouthwash.The same author also evaluated the efficacy of Acacia on S mutans. Better antimicrobial activity was recorded.(12)

Zone of inhibition of Thymus against S mutans at 25µl was 20mm, at 50µl was 21mm, at 100µl was 25mm whereas ZOI of control mouthwash was only 18mm. Against S aureus, ZOI at 25µl was 14mm, at 50µl was 18mm, at 100µl was 22mm whereas that of commercially available mouthwash was only 15mm. Against E faecalis, ZOI at 25µl was 8mm, at 50µl was 12mm and at 100µl ZOI was 28mm whereas that of control was 15mm. Against C albicans, ZOI was equal to control at all concentrations, that is 15mm. These results show that compared to control, Thymus vulgaris showed better results against Smutans, E faecalis and also S aureus and C albicans. Thymus vulgaris essential oil has been studied and stated in literature, its usage as mouthwash has not been extensively studied. Antibacterial property of Thymus vulgaris has been studied against S mutans by Damtie et al. The study favoured the use of thyme(13) Also thyme essential oil has been studied against Lactobacillus by Oliveria et al. According to the author, this essential oil can be used and formulated to act against cariogenic biofilms.(14)

Zone of inhibition of mixed mouthwash against S mutans at 25µl was 15mm, at 50µl was 21mm, at 100µl was 25mm whereas ZOI of control mouthwash was only 16mm. Against S aureus, ZOI at 25µl was 15mm, at 50µl was 18mm at 100µl was 22mm whereas that of commercially available mouthwash was only 15mm. Against E faecalis, ZOI at 25µl was 12mm, at 50µl was 15mm and at 100µl ZOI was 18mm whereas that of control was 15mm. Against C albicans, ZOI was equal to control at all concentrations, that is 10mm. Mixed mouthwash also showed better results against oral microbes compared to that of the control group. Mixes or combinations of drugs have been studied in literature. Kulaksiz et al. studied mouthwash which was a combination of Laurus, rosemary and origanum. Significant antimicrobial activity and better synergistic activity was seen while combining herbal extracts. (15)

Cytotoxicity test done was brine shrimp lethality assay. The number of live nauplii were checked for after incubation with mouthwash at various concentrations. For acacia, at concentrations of 5,10 and 20µl,9 nauplii were alive, at 40µl concentration, 7 were alive and at higher concentration of 80µl , 4 shrimp larvae were alive.The results of Thymus mouthwash showed that, at 5µl, 10µl, 20µl, 9 larvae were alive, at 40µl 8 were alive and at higher concentration of 80µl, 4 were alive. In the case of mixed mouthwash, at 5µl, 10µl, all 10 nauplii were alive, at 20µl and 40µl, 9 were alive, at higher concentration of 80µl, 5 nauplii were alive. Cytotoxicity results of a solution is of utmost importance. The results showed that both Acacia and Thymus were less cytotoxic , however no significant difference was seen between both the groups.Of the three herbal mouthwashes, mixed mouthwash showed better results as all nauplii were alive at various concentrations.

Our team has extensive knowledge and research experience that has translate into high quality publications (Neelakantan et al. 2013; Aldhuwayhi et al. 2021; Sheriff et al. 2018; Markov et al. 2021; Jayaraj et al. 2015; Paramasivam et al. 2020; Li et al. 2020; Gan et al. 2019; Dua et al. 2019; Mohan and Jagannathan 2014)

## 5. Conclusion

Acacia nilotica & Thymus vulgaris based mouthwash is more antimicrobial and less cytotoxic than chlorhexidine mouthwash.

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