Estimation of antibacterial activity of ethanolic of Clitoria ternatea against St.mutans and E. Faecalis – an invitro study.

Tahoora Taskeen L
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences(SIMATS),
Saveetha University,Chennai - 600077,
Email- 151901044.sdc@saveetha.com
Corresponding author: Dr. Ramesh R
Department of Pedodontics& Preventive Dentistry,
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences(SIMATS),
Saveetha University,Chennai - 600077
Email- rameshkdc@gmail.com
Dr. Rajeshkumar S, Nanomedicine Lab,
Department of pharmacology,, Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences (SIMATS),
Saveetha University, Chennai-600077
Email- rajeshkumars.sdc@saveetha.com

Affiliation of Author
Saveetha Dental College and Hospital,
Saveetha Institute of Medical and Technical Sciences (SIMATS) University

INTRODUCTION:

Clitoria ternatea known as butterfly pea. It is an herbaceous perennial with oval, acute leaves. In neutral, moist soil, it thrives as a vine or creeper. The flowers of this plant are a remarkable deep blue hue, solitary, and marked with light yellow.[1] It is planted as a low-maintenance decorative plant and as a species for revegetation This plant is used to enhance soil quality by decomposing nitrogen-rich plant matter because, being a legume, its roots develop a symbiotic interaction with soil bacteria called rhizobia, which convert atmospheric N2 into a plant-usable form.

The bioactive chemicals found in plant extracts, such as polyphenols and carotenoids, which have antioxidant and antibacterial activity, particularly against low-density lipoprotein (LDL) and deoxyribonucleic acid, are now playing a larger role as food additives (DNA).[2,3] In medicine, the antimicrobial resistance is increasing leading to failure of allopathic medication given for certain treatment because of which the individuals are forced to choose herbal medications. In current study the usage of ethanolic extract of *Ternatea*waschosen as ethanol separates oil from plant extract giving bulk availability of extract and dissolves most non polar and polar compounds. Qualitatively and quantitatively, the methanol extract of the leaf contains enough phenol, carbohydrates, tannins, flavonoids and terpenoids. Agar well diffusion assay and minimum inhibitory concentration (MIC) were used to investigate the zone of inhibition measurements based on the methanol extract of C. ternatea leaves for bacterial treatment.[3-6]

The validity of *Clitoria ternatea* has been established by thorough examinations by numerous writers, a natural treatment that works for many different illnesses. The previous studies based on antimicrobial activity of Clitoria *Ternatea*regarding dental use is very minimal; that is the reason for the current study focusing on the antimicrobial property of the plant.[7,8]Because of the various therapeutic properties and easy availability, the plant were selected for the present study to evaluate the antimicrobial efficacy on pathogenic oral micro- organisms in different concentrations.[9-11]

Thus, the aim of the study was to find out the antibacterial activity of ethanolic extract of *Clitoris ternatea* against *E Faecalis and S.mutans.*

MATERIALS AND METHODS:

Plant collection: The roots of C. ternatea were washed, cleaned, and dried at 50 °C in hot air oven till complete removal of moisture. The dried material was ground in a grinder and passed through sieve 10 and 40 mesh size. After sieving the coarsely powdered material was stored in an airtight container till its further use.

Preparation of ethanolic extract of *Ternatea:* 1gm of dried powder of roots of C. ternatea (50 g) was extracted with ethanol with the help of Soxhlet extractor. Boiled it for 10-15mins at 40 degrees celsius (Figure: 1) The extracts were filtered using Whatman filter paper (0.45 µm) and the filtrate was then evaporated and dried at 45°C in a hot air oven. The dried extract was dissolved in a small amount (5 mL) of DMSO and stored in the freezer. Kept for condensation up to 5 ml and the solution was saved in the refrigerator for further use.(Figure: 2)

Antimicrobial Activity of the ethanolic extract of *Ternatea*:

Ethanol extracts of C. ternatea roots were studied against Streptococcus mutans and Enterococcus faecalis for antibacterial activity and Tetracycline was used as standard.

The agar well diffusion method was carried out to determine antimicrobial susceptibility against E. faecalis. The plates were designed for dispensing using isolates of S. mutans, E. faecalis (ATCC 29212) which were spread evenly over the Petridish followed by a sterile agar medium which was uniformly poured in each corresponding well of 6 mm diameter using a sterile well borer. The dishes were incubated overnight at 37°C in different concentrations, from 25, 50, 100 and 150 µg/L. The diameter of each well was subsequently measured and the zone of inhibition was compared individually.

The medium, Petri plates and glassware used were sterilized by autoclaving at 121°C (15 lb/in2) for 30 min. To each sterilized Petri plate, 30 mL of medium inoculated with respective strains of bacteria were transferred aseptically. A single well of 6 mm diameter was made in each plate by using sterile cork-bore. Test sample and control sample (0.5 mL) were placed into the well. The plate is kept for 2 h for diffusion. For antibacterial assay plates were incubated at 37±1 °C for 24 h. Tetracycline (50 µg/mL) was used as positive antibacterial control. The diameter of the zone of inhibition surrounding each well was recorded. The average of the lowest concentration showing no growth of the organism and the highest concentration showing visible growth by macroscopic evaluation was taken as MICs12-14. Each assay was performed in triplicate

Freeze dried forms of the micro-organisms *S. mutans* (MTCC No 3160), *S. aureus* (MTCC No 447), were obtained from Microbial type culture collection, Dept of microbiology, Saveetha Dental College, Chennai, India

Time-Kill Assay

The time-kill tests employed a bacterial concentration of 6–8 log colony-forming units (CFU)/mL.Overnight cultures of E. coli were added to test tubes containing Mueller-Hinton broth with 1, 2, or 4 MIC or without antibiotics. The cultures were subsequently incubated at 37°C for 2, 4, 6, and 24 hours in a shaker (Julabo, Allentown, PA, USA). At the conclusion of each time period, 100 L samples were plated in triplicate onto Mueller-Hinton agar plates using ten-fold serial dilutions made with PBS. Polymers 2022, 14, and 1499 5 of 13 The PBS dilution of the samples reduced the impact of antibiotic carryover on all three. The CFU for each strain during various time periods

Statistical Analysis

Using SPSS 21.0, the statistical analysis was performed (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov and Shapiro-Wilk tests were used in the normality test study, which revealed a non-parametric distribution. Friedman's test was used to analyze the data to compare absorbance between the treatment groups at various time points at 530 nm, and pairwise comparisons of value were carried out at various time points.

Figure 1 illustrates Boiling of extract for 10-15mins at 40 degrees Celsius.

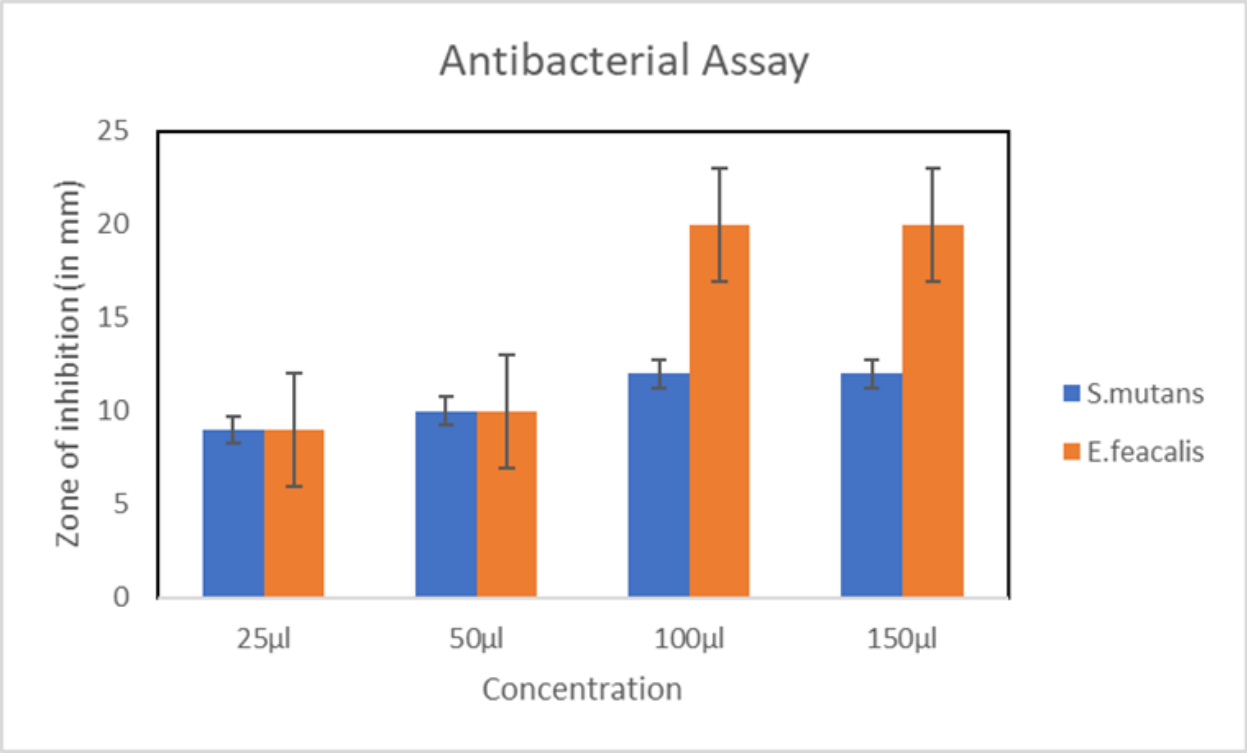
Figure 2 shows the prepared ethanolic extract of *Ternatea*

Figure 3 shows the antibacterial assay of C. Ternatea against S.mutans

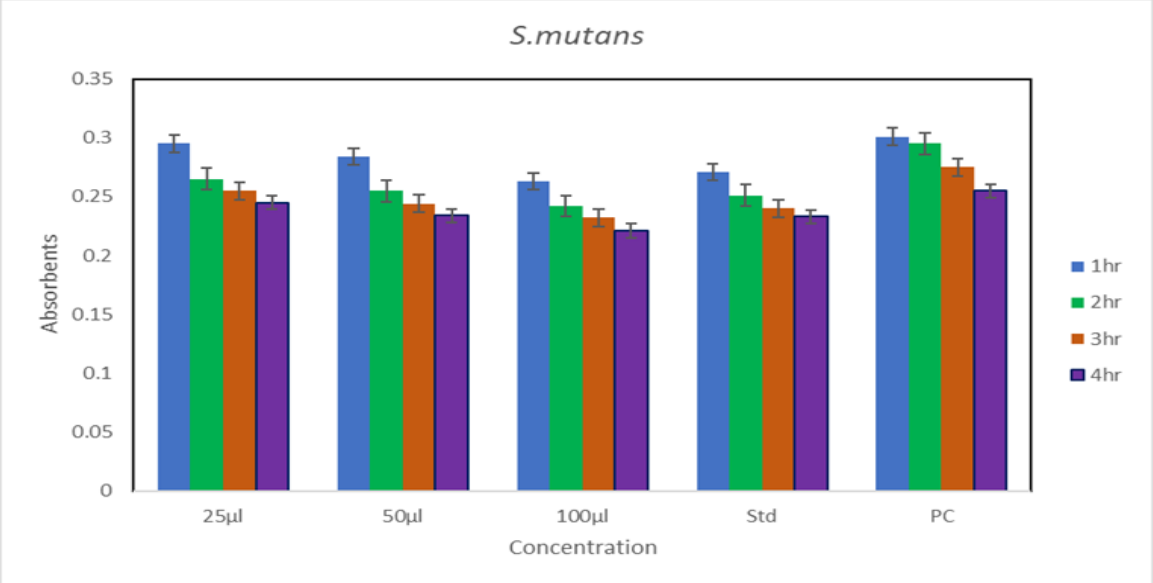
Figure 4 shows the antibacterial assay of Ternatea against Faecalis

RESULTS:

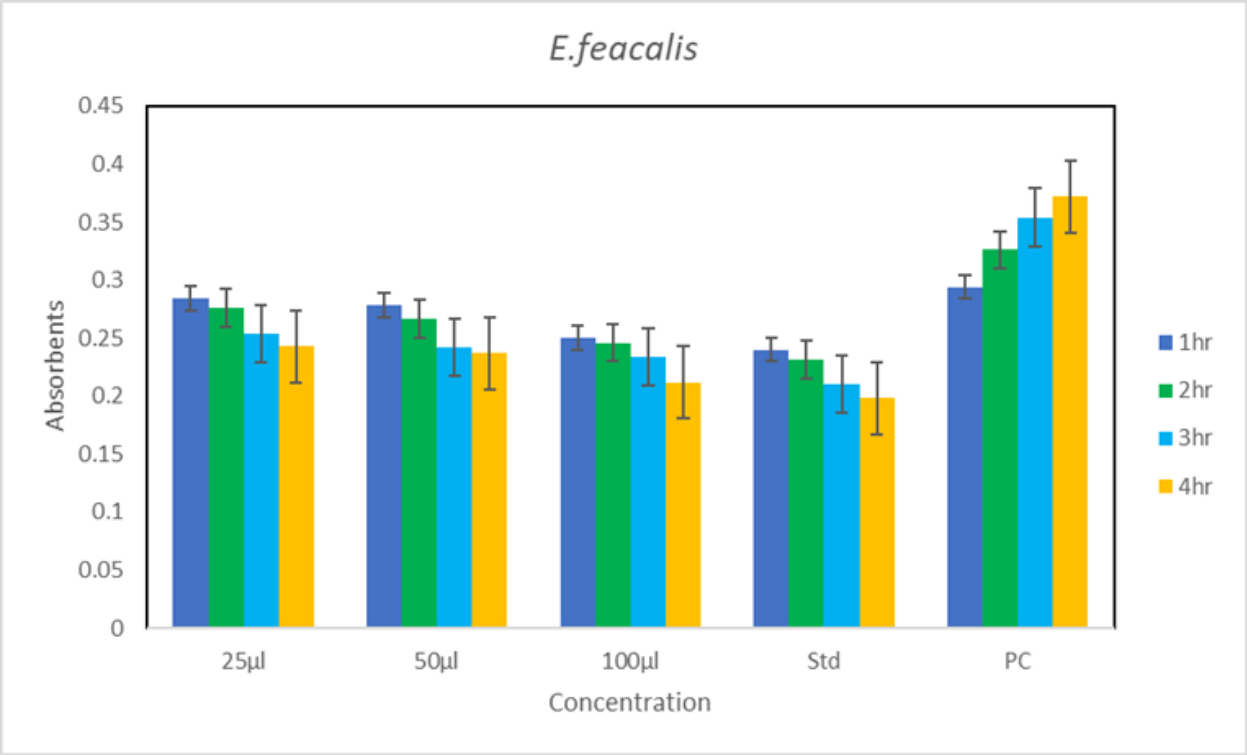
The results obtained are the zone of inhibition of C.ternatea against S.mutans and E.faecalis which shows that in 100 and 150 microlitres there is maximum inhibition shown by the ethanolic extract of the plant (graph 1). Minimum bacterial concentration against S. mutans and E.faecalis was more in first one hour of activity which gradually decreased in the four hours (graph 2.graph 3).



Graph 1: shows the zone of inhibition of ternatea against S.mutans and E. Faecalis which shows that in 100 and 150 microliters there is maximum inhibition shown by the ethanolic extract of plant.



Graph 2 shows that minimum bacterial concentration against S. mutans was more in first one hour of activity which gradually decreased in the four hours.



Graph 3 shows that the minimum bacterial concentration against E. Faecalis was more in first one hour of activity which gradually decreased in the four hours.

The results of the antimicrobial assay showed that the extracts of C. ternatea roots was effective against both streptococcus mutans and enterococcus faecalis and the ethanol extract exhibited better activity when compared to ethanolic extract against selected strain. However, the zone of inhibition exhibited by the plant extracts was more than that of the standard drug. Results for antimicrobial activity of extracts of C. ternatea against streptococcus mutans and e faecalis are shown in graph 2 and 3 respectively. C. ternatea extracts were potentially effective. Ethanolic extract of C. ternatea was found to be effective in concentration (25 µg/mL) against streptococcus mutans to prevent the microbial growth with the inhibition zones of 12, 20 mm respectively

DISCUSSION :

C. Ternatea, sometimes known as "Butterfly pea," is a member of the Fabaceae family. It is a herbaceous perennial with obtuse and elliptical leaves. It develops like a creeper or vine, doing well on neutral, moist soil. The most notable quality its striking deep blue blooms.[12-16] The plant is indigenous to the tropics. but it has been introduced to Africa, Australia, and equatorial Asia too, America. Tropical regions of India are home to it. particularly in southern India. It is a key component in A revitalizing concoction called "Medya Rasayana" (brain tonic) is utilize.[17-20] It is used as a brain tonic, neurological diseases treatment

Both a laxative and a nervine tonic. It is said that flower juice can be used for skin conditions andinsect bite. The roots are beneficial for allergies, burning.

One of the previous study states that ternatea is a good antimicrobial agent against*E, faecalis* alone but current study had determined that it has good antimicrobial activity against *S.mutans* also

One of the method to reduce antibiotics resistant in todays world is by utilizing antibiotic resistant inhibition from plant, similar plant is *Ternatea*which is active against drug resistant pathogens (Prabhat et al, 2020). In the present study the *Ternatea*showed 12mm and 20mm zone of inhibition in 100µl and 150µl against *S.mutans* and *E. Faecalis was*in accordance with study done by Pratap gowd *et al*, 2018.

There is scarce literature showing anti-microbial effects of *C. ternatea* on dental pathogens. The present study is a preliminary evaluation to explore the anti-microbial properties of these experimental plants for dental use.[21-26]

Since the tested extracts the plant was effective against pathogenic micro-organisms present in the oral cavity, purification and toxological studies of these plants and *in vivo* trials should be carried out. The anti-microbial efficacy can be enhanced if the phyto constituents of the plant extracts are purified using different solvents like ethanol, methanol, acetone, etc.[27-29] Anti-bacterial activity of this medicinal herb, if translated into clinical practice would lead to the development of indigenous, chemical free, cost effective, and holistic oral hygiene aids, which can be incorporated into various oral hygiene formulations like dentifrices, mouth rinses, gum paintsetc.[30-32]

CONCLUSION:

The outcome of this research work reaffirms the antimicrobial potential of the roots of C. ternatea against streptococcus and e faecalis. The antimicrobial assay of the ethanol extract of C. ternatea roots showed a significant antimicrobial activity. Present antimicrobial study of the C. ternatea roots showed that this plant possesses better antibacterial activity in ethanol extract and we can conclude that the *C. Ternatea* can be used as a potent anti-microbial agent against of caries causing microorganisms like *S.mutans and E Faecalis* .

REFERENCES

1. Lakhera S, Devlal K, Rana M, Celik I. Study of nonlinear optical responses of phytochemicals of by quantum mechanical approach and investigation of their anti-Alzheimer activity with in silico approach. Struct Chem. 2022 Jun 16;1–16.
2. Jeyaraj EJ, Lim YY, Choo WS. Antioxidant, cytotoxic, and antibacterial activities of Clitoria ternatea flower extracts and anthocyanin-rich fraction. Sci Rep. 2022 Sep 1;12(1):14890.

3. Santos LG, Alves-Silva GF, Martins VG. Active-intelligent and biodegradable sodium alginate films loaded with Clitoria ternatea anthocyanin-rich extract to preserve and monitor food freshness. Int J Biol Macromol. 2022 Aug 20;220:866–77.
4. Zhang H, Li S, Cheng Y. Antibiofilm Activity of Allicin and Quercetin in Treating Biofilm-Associated Orthopaedics Infection. Appl Biochem Biotechnol [Internet]. 2022 Feb 10; Available from: http://dx.doi.org/10.1007/s12010-022-03845-4
5. Batool SQN. Tissue Culture and Antimicrobial Activity of Clitoria Ternatea L. LAP Lambert Academic Publishing; 2012. 128 p.
6. Sapiee S. The Extraction of Anthocyanin from Clitoria Ternatea (Blue Pea Flower) by Using Spray Dryer. 2013. 59 p.
7. Swathi KP, Jayaram S, Sugumar D, Rymbai E. Evaluation of anti-inflammatory and anti-arthritic property of ethanolic extract of. Chin Herb Med. 2021 Apr;13(2):243–9.
8. Chayaratanasin P, Adisakwattana S, Thilavech T. Protective role of Clitoria ternatea L. flower extract on methylglyoxal-induced protein glycation and oxidative damage to DNA. BMC Complement Med Ther. 2021 Mar 1;21(1):80.
9. Zhao J, Ge G, Huang Y, Hou Y, Hu SQ. Study on activation mechanism and cleavage sites of recombinant butelase-1 zymogen derived from Clitoria ternatea. Biochimie. 2022 Aug;199:12–22.
10. Sreekala S, Muraleedharan UD. Cationic Clitoria ternatea Seed Peptide as a Potential Novel Bioactive Molecule. Protein Pept Lett. 2021;28(11):1259–71.
11. Jamil N, Pa'ee F. Antimicrobial activity from leaf, flower, stem, and root of Clitoria ternatea – A review [Internet]. AIP Conference Proceedings. 2018. Available from: http://dx.doi.org/10.1063/1.5050140
12. Archana H, Geetha Bose V. Evaluation of phytoconstituents from selected medicinal plants and its synergistic antimicrobial activity. Chemosphere. 2022 Jan;287(Pt 4):132276.
13. Jacob B, Malli Sureshbabu N, Ranjan M, Ranganath A, Siddique R. The antimicrobial effect of pomegranate peel extract versus chlorhexidine in high caries risk individuals using quantitative real-time polymerase chain reaction: a randomized triple-blind controlled clinical trial. International Journal of Dentistry. 2021 Aug 30;2021.
14. Paulraj J, Nagar P. Antimicrobial efficacy of Triphala and propolis-modified glass ionomer cement: An in vitro study. International Journal of Clinical Pediatric Dentistry. 2020 Sep;13(5):457.
15. Deepika BA, Ramamurthy J, Girija S, Jayakumar ND. Evaluation of the Antimicrobial Effect of Ocimum sanctum L. Oral Gel against Anaerobic Oral Microbes: An In Vitro Study. World Journal of Dentistry. 2022 Oct 1;13(S1):S23-7.
16. Solanki LA, Sundari KS, Muralidharan NP, Jain RK. Antimicrobial effect of novel gold nanoparticle oral rinse in subjects undergoing orthodontic treatment: An ex-vivo study. Journal of International Oral Health. 2022 Jan 1;14(1):47.
17. Gulzar RA, Ajitha HS. Comparative evaluation of antimicrobial efficacy of Moringa oleifera extract and calcium hydroxide against E. faecalis. Int J Dentistry Oral Sci. 2021 May 30;8(05):2605-9.
18. Sushanthi PK, Arumugham M. Antimicrobial Efficacy Of Oregano Oil, Thyme Oil and Helichrysum Oil Against Oral Pathogens: An In Vitro Study. Int J Dentistry Oral Sci. 2021 May 30;8(05):2615-9.
19. Sabarathinam J, Madhulaxmi R. Development of anti inflammatory and antimicrobial silver nanoparticles coated suture materials. International Journal of Dentistry and Oral Science. 2021 Mar 17;8(3):2006-13.
20. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. Clinical oral investigations. 2020 Sep;24:3275-80.
21. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Archives of oral biology.

2021 Jun 1;126:105132.

22. Kambalyal PB, Shanmugasundaram K, Rajesh V, Donthula S, Patil SR. Comparative Evaluation of Antimicrobial Efficacy of Silver, Titanium Dioxide and Zinc Oxide Nanoparticles against Streptococcus mutans. Pesquisa brasileira em odontopediatria e clinica integrada. 2018 Aug 27;18(1):4150.
23. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artificial Cells, Nanomedicine, and Biotechnology. 2020 Jan 1;48(1):1153-8.
24. Maliael MT, Jain RK, Srirengalakshmi M. Effect of Nanoparticle Coatings on Frictional Resistance of Orthodontic Archwires: A Systematic Review and Meta-analysis. World. 2022;13(4).
25. Pandiyan I, Sakthi SD, Indiran MA, Rathinavelu PK, Rajeshkumar S. Mediated Selenium Nanoparticles, Characterization and its Antimicrobial Activity-An In Vitro Study. Thymus Vulgaris. 2021;7:3516-21.
26. Jaju K, Nasim I. Evaluation Of Tooth Discolouration Following The Use Of Silver Nanoparticle Based Intracanal Medicaments-An In Vitro Study. Int J Dentistry Oral Sci. 2021 Jul 15;8(6):3214-8.
27. NivedaRajeshwaran JR, Rajeshkumar S. Evaluation of Antioxidant and Anti Inflammatory Activity of Grape Seed Oil Infused With Silver Nano-particles an In Vitro Study. Int J Dentistry Oral Sci. 2021 Jul 15;8(7):3318-22.
28. Behera K, Nasim I. Effect Of Nanoparticles Based Root Canal Disinfectants On Enterococcus Faecalis-A Systematic Review. Int J Dentistry Oral Sci. 2021 Jun 30;8(5):2898-904.
29. Teja KV, Kaligotla AV, Gummuluri S. Antibacterial efficacy of conventional versus herbal products on Streptococcus mutans in adult population-a systematic review & meta-analysis. Brazilian Dental Science. 2020 Sep 30;23(4):18p-.
30. Tatekalva P, Subbaiyan H, Kumar SR. Comparative evaluation of antimicrobial potential of herbal extracts on Streptococcus mutans and Enterococcus faecalis: An in vitro study. Brazilian Dental Science. 2021;24(1):7-p.
31. Mahtani AA, Jayashri P. Comparing the effect of natural and synthetic sugar substitutes on salivary pH–a double-blinded randomized controlled study. Drug Invention Today. 2019 Aug 1;11(8).
32. SRİNİVASAN M, Nivedhitha MS, POORNİ S. Comparing the effect of probiotic Streptococcus salivarius K12 and M18 on the Streptococcus mutans Count, salivary pH and buffer capacity: a randomized double blinded clinical trial. Cumhuriyet Dental Journal. 2022 Jan 3;24(4):346-54.

FIGURES

Figure 1 illustrates Boiling of extract for 10-15mins at 40 degrees Celsius.

Figure 2 shows the prepared ethanolic extract of *Ternatea*

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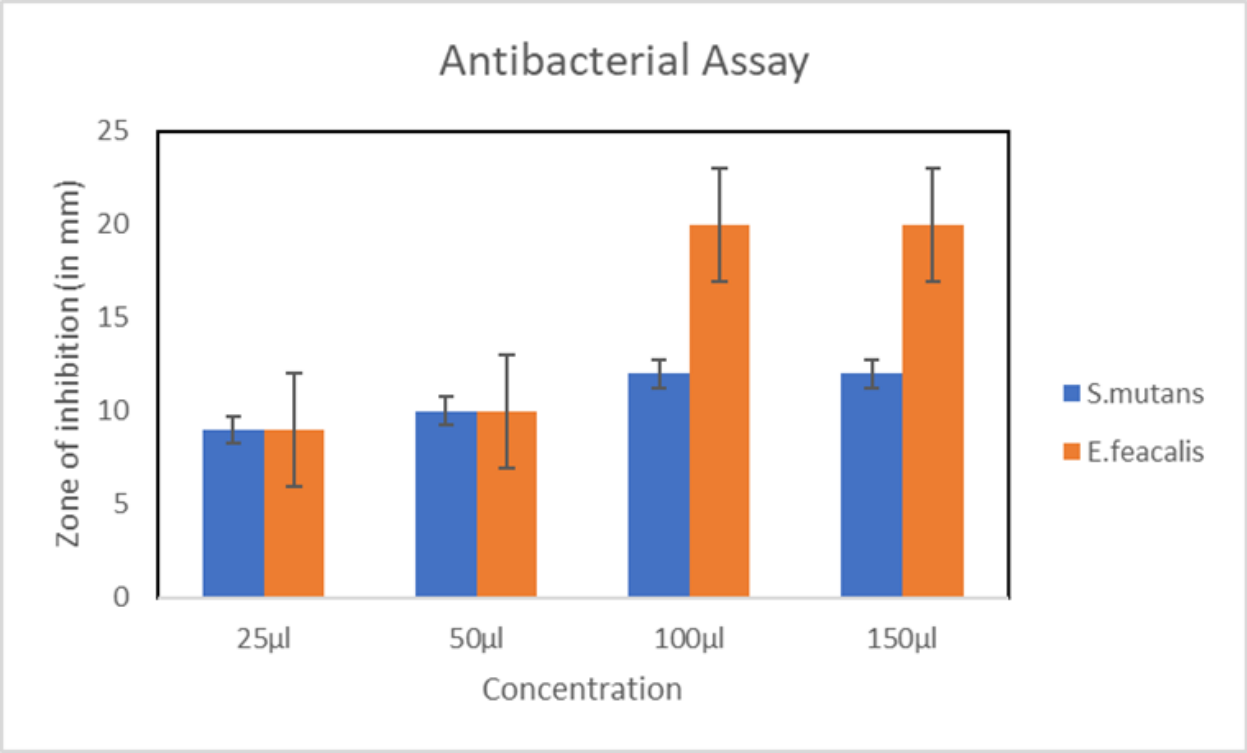

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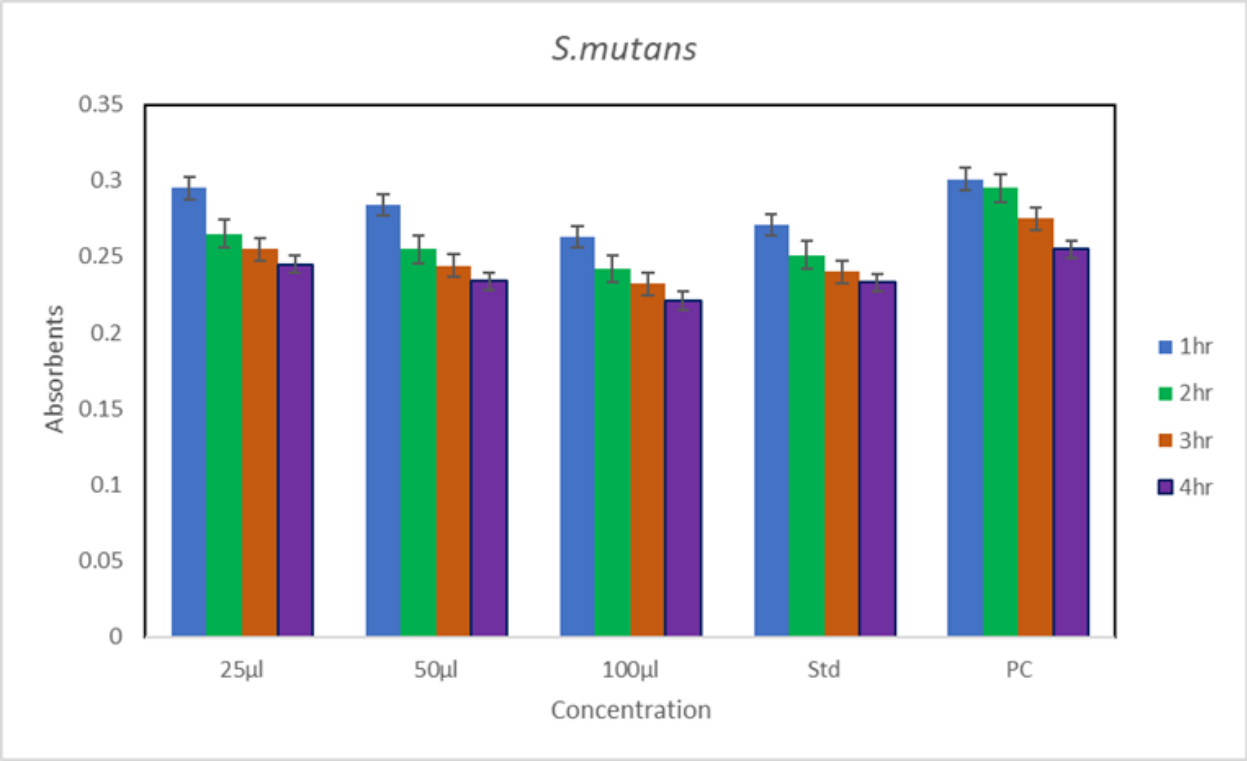
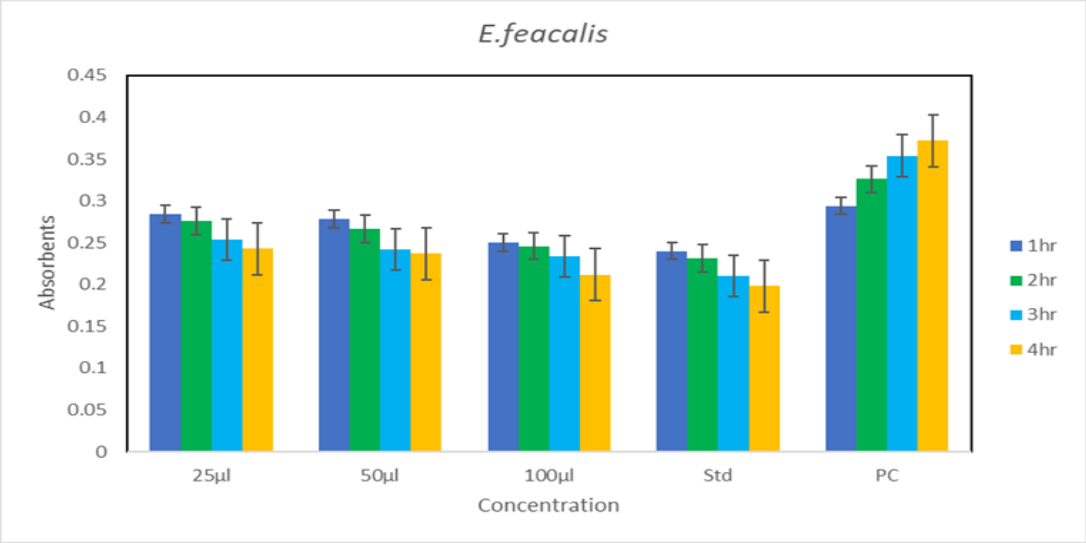


Graph 2 shows that minimum bacterial concentration against S. mutans was more in first one hour of activity which gradually decreased in the four hours. Standard control :

Positive control:



Graph 3 shows that the minimum bacterial concentration against E.faecalis was more in first one hour of activity which gradually decreased in the four hours.