ANTIMICROBIAL, ANTI-INFLAMMATORY, AND CYTOTOXICITY EFFECT OF CHLORHEXIDINE AND POVIDONE-IODINE COMBINATION VS. USE OF THEM SEPARATELY

Sanjog Agarwal[1], Subhabrata Maiti[2*], Deepak Nallaswamy[3], S. Rajeshkumar[4], Vatika Agarwal[5]

Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Aim: The aim of this study is to evaluate efficacy of various combinations of Chlorhexidine And Povidone-iodine using anti-microbial ,anti-inflammatory and cytotoxicity tests.
Material and Methods: Commercially available pure compounds of 20% chlorhexidine gluconate and 99% Povidone Iodine were ordered from Sigma Aldrich.Out of which 0.2% concentration of both compounds were prepared by mixing 9.8ml of distilled water and 0.2ml of Chlorhexidine Gluconate and 10 ml of distilled water with 0.2gm of Povidone Iodine.Prepared samples went under anti-microbial testing in 3 wound pathogens and 3 oral pathogens, Anti Inflammatory testing was carried out in Bovine serum albumin each group had 5 samples or 10,20,30,40,50 microlitre, Cytotoxicity test was carried out in brine shrimps each group had 5 samples 5,10,20,40,80 microlitre ELISA plates were loaded with 10 shrimps each.
Results: There was a significant difference ($P<0.05$) of zone of inhibition , anti-inflammatory activity and cytotoxicity among all the groups against all microbes selected for the study where the combination of PI and CHX in different concentrations was showing better activity than PI or CHX alone and the difference was statistically significant ($p<0.05$, derived from post hoc TuKey).
Conclusion: Combination of chlorhexidine and povidone iodine has proven to be superior to using these antibiotics alone but as this is an in vitro study ,similar results have to be verified with clinical trials to prove efficacy of the combination over these antibiotics.

Keywords: Chlorhexidine gluconate; povidone iodine; antiseptic; anti inflammatory ;cytotoxicity, innovation

[1]Department of Prosthodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.
[2*]Assistant Professor, Department of Prosthodontics, Saveetha Dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.
[3]Professor Department of Prosthodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077,TamilNadu ,India.
[4]Professor, Nanomedicine Lab, Department of Pharmacology, Saveetha Dental College, SIMATS, Chennai-600077
[5]Department of Prosthodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University,

DOI: 10.31838/ecb/2023.12.s2.188

1. Introduction

Multidrug resistance will become a reality in the not-too-distant future. Multidrug-resistant organisms are becoming more common, emphasising the significance of utilising antiseptics to avoid infection.(1–3) .Antiseptics, on the other hand, offer a larger spectrum of activity than antibiotics. When antiseptics are applied, they reduce the possibility that the outer bacterial surface layers will be attacked, hence increasing its own uptake and attacking the cytoplasmic or inside membrane of the organism.(4–6).

Low quantities of chlorhexidine gluconate impair membrane fluidity, osmoregulation, and metabolic capacity. The membrane becomes liquid crystalline and loses its integrity at larger concentrations, such as those found in commercially available formulations..(7) Chlorhexidine gluconate has broad-spectrum activity against bacteria and yeast. It has low antiviral activity and is not sporicidal but can prevent spore development(5,8). Chlorhexidine Gluconate can adhere to the outermost layer of the epidermis and mucous membranes, providing a lingering or persistent antibacterial impact in addition to its immediate action on microorganisms.(9–11).

Povidone Iodine is a mixture of iodide and polyvinylpyrrolidone, a solubilizing carrier that acts as a reservoir of ‘‘free" iodine (the active component). Iodine is released slowly and transferred to the bacterial cell surface, where it penetrates the cell membrane and inactivates important cytosolic proteins, fatty acids, and nucleotides.(5,8,12) Iodine toxicity in mammalian cells is reduced by the slow release of iodine from the PI complex in solution. Iodine has antibacterial activity across a broad spectrum, as well as activity against fungi, protozoa, viruses, and some bacterial spores.(10,13–15).Different cellular targets and methods of action are found in CHG and PI. When these two antiseptics are used together, these distinctions may be advantageous. Damage to the outer membrane caused by CHG would increase access to the intracellular targets required for PI's bactericidal activity. Furthermore, the activity of PI is more quick than that of Chlorhexidine Gluconate,(9,16,17), implying that these 2 compounds may work cooperatively.Despite a lack of evidence demonstrating functional incompatibility, the use of CHG and PI in combination is typically avoided in clinical practise. Null Hypothesis suggests that there is no difference between antimicrobial , anti-inflammatory and cytotoxicity of chlorhexidine and povidone iodine when compared with the combination. Our team has extensive knowledge and research experience that has translate into high quality publications (18–27))

Hence the aim of this study was to determine whether the combined activity of CHG and PI against clinically relevant pathogens is inferior to the activity of either agent alone.

2. Material and Methods

Commercially available pure compounds of 20% chlorhexidine gluconate and 99% Povidone Iodine were ordered from Sigma Aldrich.Out of which 0.2% concentration of both compounds were prepared by mixing 9.8ml of distilled water and 0.2ml of Chlorhexidine Gluconate and 10 ml of distilled water with 0.2gm of Povidone Iodine,combinations were made by mixing 0.2% of both the chemicals using vortex at room temperature , in which were the pure groups and were divided into different groups by dividing it into various ratios.

GROUPS:
GROUP 1- 1:1(PI:CHX)
GROUP 2- 5:1(PI:CHX)
GROUP 3- 10:1(PI:CHX)
GROUP 4-1:5(PI:CHX)
GROUP 5-1:10(PI:CHX)
GROUP 6-CHX 0.2%
GROUP 7-PI 0.2%

Sample Testing:

Anti-Microbial Test

Prepared samples went under anti-microbial testing in 3 wound pathogens(E. coli;Pseudomonas;S. aureus) and 3 oral pathogens(S. mutans; E. fecalis, C. albus) adopting the agar well diffusion method. Roughly, 20 ml of sterilised and cooled Mueller-Hinton agar medium was filled with sterile Petri dishes and permitted to solidify at room temperature. The overnight growth test organisms were spread over the agar medium by a sterile cotton swab for each test, and then, the wells were made using a sterile polystyrene tip. Diverse concentrations of CHX AND PI were added to the wells. The inoculated plates were incubated for 24 h at 37°C. After that, the inhibition zone around the well was calculated using a vernier calliper and recorded. It was incubated for 24 hrs and then the zone of inhibition was read (Figure 1).

Anti-Inflammatory Test

Bovine serum was added to all the 5 test tubes along with distilled water.According to the markings, the prepared Combinations and controls were added to 5 test tubes 10,20,30,40,50 microlitre each and left for 10 minutes. After this they are transferred to a hot water(55 degree celsius) bath for 10 minutes. After this the end product absorbance was noted using a spectrophotometer .

Cytotoxicity Test

This test was carried out in brine shrimps each group had 5 samples 5,10,20,40,80 microlitre ELISA plates were loaded with 10 shrimps each and then samples were added to it, results were interpreted after 24hrs. Brine shrimps were counted against light using a dissection microscope (Figure 2).

Statistical Analysis :

All the three tests were repeated five times for all groups in order to eliminate the errors (total number of samples 105 obtained from G-Power calculation where the power of the study was 85%). Descriptive analysis was done for mean and SD and One way ANOVA was performed to analyse the difference among the groups, along with a post hoc TUKEY test for the comparison in between groups using SPSS software (IBM Corp. Released 2015,Statistics for Windows, Version 23.0. Armonk NY: IBM Corp.) with p value at the significant level of 0.05 and 95% Confidence Interval .

3. Results

There was a significant difference (P<0.05) of zone of inhibition where Group 4 was showing maximum antimicrobial activity for *S.mutans* (26.80±1.30) and *E.faecalis* (26.40±1.15) followed by *S.aureus* (25.4±1.17), *E.coli* (20.8-±0.85),*C.albicans*(20.40±1.17), *Pseudomonas* (19.80±1.30). anti-inflammatory activity among all the groups against all microbes selected for the study where the combination of PI and CHX in different concentrations was showing better activity in group 3[10:1(PI:CHX)](0.01±0.005) than PI(0.06±0.008 to 1.95±0.011) or CHX (0.09±0.007 to 0.19±0.007) alone and the difference was statistically significant (p<0.05, derived from post hoc TuKey). For cytotoxicity group 3 [10:1(PI:CHX)]combination was showing significant (p<0.05) outcome of alive shrimp (9.6±0.54) even in higher concentration .

For antimicrobial activity, Group 4 (1:5 ,PI:CHX)was showing a maximum zone of inhibition against all the organisms. and group 1(1:1,PI:CHX) was showing the minimum. Group 6 (CHX 0.2%) showed better antimicrobial activity than Group 7 (PI 0.2%)(P<0.05), when Group 6 (CHX 0.2%) was compared to Group 4 (1:5 ,PI:CHX) there was significant difference for S. mutans ,E. Faecalis and Pseudomonas(P<0.05)(Table 1).

For anti-inflammatory activity Group 3 (10:1,PI:CHX) was showing maximum anti-inflammatory effect. and group 6(1:1,PI:CHX) was showing the minimum anti inflammatory.Group 6 (CHX 0.2%) showed better antimicrobial activity than Group 7 (PI 0.2%)(P<0.05), when Group 6 (CHX 0.2%) was compared to Group 3 (10:1 ,PI:CHX) there was significant difference for anti inflammatory test for 10µl, 20µl,40µl,50µl(P<0.05). But between Group 4(1:5 ,PI:CHX) and Group 3(10:1 ,PI:CHX) there is no statistically significant difference(P<0.05)(Table 2).

For cytotoxicity , Group 3 (10:1,PI:CHX) was showing minimum cytotoxicity effect. and group 6(1:1,PI:CHX) was showing the maximum cytotoxicity effect .Group 6 (CHX 0.2%) showed better cytotoxicity activity than Group 7 (PI 0.2%)(P<0.05), when Group 6 (CHX 0.2%) was compared to Group 3 (10:1 ,PI:CHX) there was significant difference for cytotoxicity test for, 20µl,40µl,80µl(P<0.05). But between Group 4(1:5 ,PI:CHX) and Group 3(10:1 ,PI:CHX) there is no statistically significant difference (P<0.05)(Table 3).

4. Discussion

The combination of chlorhexidine and povidone iodine demonstrated the best results at 5:1 combination of chlorhexidine and povidone iodine for antimicrobial testing ,where as for anti inflammatory testing group 3[10:1(PI:CHX)] was best but there was no significant difference between group 3[10:1(PI:CHX)] and group 4[1:5(PI:CHX)], for cytotoxicity testing group 3 was best but there was no significant difference between group 3[10:1(PI:CHX)] and group 4[1:5(PI:CHX)]

In a study by (28) Combination skin preparation with CHG and PVI significantly reduced surgical site infection rates compared to CHG or PVI alone. in an in-vivo study by Anderson et al in 2010 not only did not support these negative effects but also found evidence of a synergistic effect.They postulate that the membrane disruption provided by CHG facilitates greater PVI uptake(29).Skin disinfection utilising the sequential application of chlorhexidine/alcohol and povidone-iodine was proven to be superior to any of the disinfectants alone in reducing the colonisation rates of CVCs (P=.006), according to a study by Langgartner et al(30). It has been demonstrated that skin cleaning followed by povidone-iodine and chlorhexidine/alcohol application is safe. A 1-minute application of povidone-iodine followed by a 1-minute application of chlorhexidine/alcohol had no negative effects. Chlorhexidine and povidone-iodine combined for skin antisepsis are secure and efficient. To lower skin bacterial flora before neurosurgical intervention, three minutes of preoperative chlorhexidine scrub followed by one washing with povidone-iodine may be sufficient. This procedure might offer a benchmark in the neurosurgical field(31).

The chemical CHG, which has two positive charges, is well known for being a potent antibacterial. The negatively charged bacterial cell wall is attacked by the positively charged CHG molecule, which easily damages its structure. When the cell wall is compromised, the contents may leak out and kill the cell. In addition to having a direct impact on

microorganisms, CHG can attach to human skin cells and hence contribute to a long-lasting antibacterial action.Iodine and CHG differ chemically from one another in a number of ways. Iodine molecules have no charge, but CHG molecules have a charge. Iodine does not interact with CHG's charge as a result. Iodine dissolves extremely little in water but very well in the majority of organic solvents. Most organic solvents are insoluble in CHG, which is primarily soluble in water. Iodine is a mild oxidizer, although it is unable to completely oxidise CHG. As a result, when used together, the two active substances will not chemically interact with one another.Iodine and CHG have several cellular targets and work through various methods. When combining the two antiseptics, these distinctions might be advantageous. Damage to the outer membrane caused by CHG would make it easier for iodine's antibacterial activity to access the intracellular targets it needs.

This combination can be used with a variety of products which are of importance such as mouth washes, gels,surgical scrubs , it can also be incorporated into dressing materials to protect the site from infections. Any condition related to the need for disinfection and control of infection , this can be a useful product.

Future research on this material can be done in the form of RCT to check the clinical efficacy of the material on a larger scale . Further clinical trials need to be done to verify the efficacy of the combination over the usage of these antimicrobials alone. As this study has been done on majorly aerobic bacteria we need to do this similar study on anaerobic bacteria and more resistant microorganisms to check the efficacy of the combination.

5. Conclusion

Combination of chlorhexidine and povidone iodine has proven to be superior to using these antibiotics alone but as this is an in vitro study ,similar results have to be verified with clinical trials to prove efficacy of the combination over these antibiotics. A this is a novel combination further studies need to be done with larger sample size and a variety of microorganisms such as anaerobic bacteria and other resistant bacteria to prove its superiority over the presently available antibiotics.

6. References

Jones RN. Resistance Patterns Among Nosocomial Pathogens [Internet]. Vol. 119, Chest. 2001. p. 397S – 404S. Available from: http://dx.doi.org/10.1378/chest.119.2_suppl.397s

Ajagannanavar SL, Battur H, Shamarao S, Sivakumar V, Patil PU, Shanavas P. Effect of aqueous and alcoholic licorice (glycyrrhiza glabra) root extract against streptococcus mutans and lactobacillus acidophilus in comparison to chlorhexidine: an in vitro study. J Int Oral Health [Internet]. 2014 Jul;6(4):29–34. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25214729

Aoun G, Saadeh M, Berberi A. Effectiveness of Hexetidine 0.1% Compared to Chlorhexidine Digluconate 0.12% in Eliminating Candida Albicans Colonizing Dentures: A Randomized Clinical In Vivo Study. J Int Oral Health [Internet]. 2015 Aug;7(8):5–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26464531

Jones CG. Chlorhexidine: is it still the gold standard? Periodontol 2000 [Internet]. 1997 Oct;15:55–62. Available from: http://dx.doi.org/10.1111/j.1600-0757.1997.tb00105.x

McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev [Internet]. 1999 Jan;12(1):147–79. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9880479

Zhao R, Liu S, Liu Y, Cui S. Adjunctive Use of Active Compounds such as Chlorhexidine in the Nonsurgical Treatment of Peri-Implant Mucositis for Oral Health: A Systematic Review and Meta-Analysis. Oxid Med Cell Longev [Internet]. 2022 Aug 27;2022:2312784. Available from: http://dx.doi.org/10.1155/2022/2312784

Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet [Internet]. Vol. 99, Journal of Applied Microbiology. 2005. p. 703–15. Available from: http://dx.doi.org/10.1111/j.1365-2672.2005.02664.x

Doyle AJ, Saab ME, McClure JT. Comparison of chlorhexidine and alcohol-based antisepsis on the paralumbar fossa in cattle. Vet Surg [Internet]. 2022 Aug 31; Available from: http://dx.doi.org/10.1111/vsu.13878

Fuursted K, Hjort A, Knudsen L. Evaluation of bactericidal activity and lag of regrowth (postantibiotic effect) of five antiseptics on nine bacterial pathogens. J Antimicrob Chemother [Internet]. 1997 Aug;40(2):221–6. Available from: http://dx.doi.org/10.1093/jac/40.2.221

Traoré O, Fournet Fayard S, Laveran H. An in-vitro evaluation of the activity of povidone-iodine against nosocomial bacterial strains [Internet]. Vol. 34, Journal of Hospital Infection. 1996. p. 217–22. Available from: http://dx.doi.org/10.1016/s0195-

6701(96)90069-9

Hasan F, Chiu HY, Salamanca E, Ridwan ES, Wiratama BS, Budi HS. Effects of Chlorhexidine and Povidone-Iodine on the SARS-CoV-2 Load: A Systematic Review and Meta-analysis. Eur J Dent [Internet]. 2022 Sep 8; Available from: http://dx.doi.org/10.1055/s-0042-1753470

Yaneva BK, Dermendzhieva YB, Mutafchieva MZ, Stamenov NV, Kavlakova LB, Tanev MZ, et al. Randomised controlled trial comparing the clinical effectiveness of mouthwashes based on essential oils, chlorhexidine, hydrogen peroxide and prebiotic in gingivitis treatment. Folia Med [Internet]. 2022 Aug 31;64(4):588–95. Available from: http://dx.doi.org/10.3897/folmed.64.e63528

Prasad KARV, John S, Deepika V, Dwijendra KS, Reddy BR, Chincholi S. Anti-Plaque Efficacy of Herbal and 0.2% Chlorhexidine Gluconate Mouthwash: A Comparative Study. J Int Oral Health [Internet]. 2015 Aug;7(8):98–102. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26464549

Goutham BS, Manchanda K, Sarkar AD, Prakash R, Jha K, Mohammed S. Efficacy of two commercially available Oral Rinses - Chlorohexidine and Listrine on Plaque and Gingivitis - A Comparative Study. J Int Oral Health [Internet]. 2013 Aug;5(4):56–61. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24155621

Hameed H, Babu BP, Sagir VMM, Chiriyath KJ, Mathias J, Shaji AP. Microleakage in Resin Composite Restoration following Antimicrobial Pre-treatments with 2% Chlorhexidine and Clearfil Protect Bond. J Int Oral Health [Internet]. 2015 Jul;7(7):71–6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26229374

Chaiyakunapruk N, Veenstra DL, Lipsky BA, Saint S. Chlorhexidine Compared with Povidone-Iodine Solution for Vascular Catheter–Site Care [Internet]. Vol. 136, Annals of Internal Medicine. 2002. p. 792. Available from: http://dx.doi.org/10.7326/0003-4819-136-11-200206040-00007

Hussain U, Alam S, Rehman K, Antonoglou GN, Papageorgiou SN. Effects of chlorhexidine use on periodontal health during fixed appliance orthodontic treatment: a systematic review and meta-analysis. Eur J Orthod [Internet]. 2022 Aug 24; Available from: http://dx.doi.org/10.1093/ejo/cjac044

Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol [Internet]. 2018 Oct;89(10):1241–8. Available from: http://dx.doi.org/10.1002/JPER.17-0445

Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol [Internet]. 2019 Dec;90(12):1441–8. Available from: http://dx.doi.org/10.1002/JPER.18-0673

Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: http://dx.doi.org/10.1016/j.archoralbio.2018.07.001

Teja KV, Ramesh S, Priya V. Regulation of matrix metalloproteinase-3 gene expression in inflammation: A molecular study. J Conserv Dent [Internet]. 2018 Nov-Dec;21(6):592–6. Available from: http://dx.doi.org/10.4103/JCD.JCD_154_18

Manohar MP, Sharma S. A survey of the knowledge, attitude, and awareness about the principal choice of intracanal medicaments among the general dental practitioners and nonendodontic specialists. Indian J Dent Res [Internet]. 2018 Nov-Dec;29(6):716–20. Available from: http://dx.doi.org/10.4103/ijdr.IJDR_716_16

Nandakumar M, Nasim I. Comparative evaluation of grape seed and cranberry extracts in preventing enamel erosion: An optical emission spectrometric analysis. J Conserv Dent [Internet]. 2018 Sep-Oct;21(5):516–20. Available from: http://dx.doi.org/10.4103/JCD.JCD_110_18

Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. J Dent Educ [Internet]. 2019 Apr;83(4):445–50. Available from: http://dx.doi.org/10.21815/JDE.019.054

Panchal V, Jeevanandan G, Subramanian E. Comparison of instrumentation time and obturation quality between hand K-file, H-files, and rotary Kedo-S in root canal treatment of primary teeth: A randomized controlled trial. J Indian Soc Pedod Prev Dent [Internet]. 2019 Jan-Mar;37(1):75–9. Available from: http://dx.doi.org/10.4103/JISPPD.JISPPD_72_18

https://bds.ict.unesp.br/index.php/cob/article/view/1617

Felicita AS. Orthodontic extrusion of Ellis Class VIII fracture of maxillary lateral incisor - The

sling shot method. Saudi Dent J [Internet]. 2018 Jul;30(3):265–9. Available from: http://dx.doi.org/10.1016/j.sdentj.2018.05.001

Davies BM, Patel HC. Does chlorhexidine and povidone-iodine preoperative antisepsis reduce surgical site infection in cranial neurosurgery? Ann R Coll Surg Engl [Internet]. 2016 Jul;98(6):405–8. Available from: http://dx.doi.org/10.1308/rcsann.2016.0143

Anderson MJ, Horn ME, Lin YC, Parks PJ, Peterson ML. Efficacy of concurrent application of chlorhexidine gluconate and povidone iodine against six nosocomial pathogens. Am J Infect Control [Internet]. 2010 Dec;38(10):826–31. Available from: http://dx.doi.org/10.1016/j.ajic.2010.06.022

Langgartner J, Linde HJ, Lehn N, Reng M, Schölmerich J, Glück T. Combined skin disinfection with chlorhexidine/propanol and aqueous povidone-iodine reduces bacterial colonisation of central venous catheters. Intensive Care Med [Internet]. 2004 Jun;30(6):1081–8. Available from: http://dx.doi.org/10.1007/s00134-004-2282-9

Guzel A, Ozekinci T, Ozkan U, Celik Y, Ceviz A, Belen D. Evaluation of the skin flora after chlorhexidine and povidone-iodine preparation in neurosurgical practice [Internet]. Vol. 71, Surgical Neurology. 2009. p. 207–10. Available from: http://dx.doi.org/10.1016/j.surneu.2007.10.026

Table 1: Showing difference among the groups for antimicrobial activity (Zone of Inhibition).

GROUPS	**MICRO-ORGANISMS(MEAN±SD)**					
	S. aureus	S. mutans	E. faecalis	C. albicans	E. colli	Pseudomonas
GROUP1	18.40±1.14	20.40±1.11	20.20±1.30	11.40±1.11	20.00±0.70	16.20±0.82
GROUP2	18.20±1.30	20.60±1.12	21.20±1.30	11.20±0.85	17.60±0.54	19.80±0.85
GROUP3	19.40±1.16	20.20±1.30	16.60±1.13	10.40±0.54	16.60±0.54	17.40±1.16
GROUP4	25.40±1.17	26.80±1.30	26.40±1.15	20.40±1.17	20.8-±0.85	19.80±1.30
GROUP5	22.00±1.58	25.00±0.70	26.00±1.58	16.20±1.30	20.8-±0.86	20.20±0.87
GROUP6	23.40±1.14	23.20±0.83	21.40±1.16	16.20±0.83	19.40±0.89	22.40±1.14
GROUP7	12.60±1.15	14.60±1.17	13.80±0.85	18.60±1.16	10.80±0.85	15.60±0.89
P-value	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
S.E	0.782	0.697	0.774	0.645	0.478	0.641
F	57.56	64.87	69.95	74.72	110.25	29.06
df	6	6	6	6	6	6

*P value at the level <0.05,derived from one way ANOVA

Table 2:Showing difference among the groups for anti-inflammatory activity.

GROUPS	**ANTI-INFLAMMATORY (MEAN±SD)**				
	10µl	20µl	30µl	40µl	50µl
GROUP1	0.01±0.005	0.02±0.005	0.02±0.005	0.11±0.008	0.44±0.010

GROUP2	0.06±0.005	0.09±0.008	0.14±0.007	0.02±0.005	1.18±0.005
GROUP3	0.01±0.005	0.01±0.005	0.02±0.005	0.01±0.005	0.01±0.005
GROUP4	0.04±0.007	0.11±0.007	0.02±0.005	0.04±0.005	0.94±0.010
GROUP5	0.01±0.004	0.03±0.004	0.18±0.004	0.04±0.005	0.06±0.005
GROUP6	0.11±0.007	0.09±0.007	0.11±0.004	0.11±0.008	0.19±0.007
GROUP7	0.06±0.008	0.10±0.008	0.06±0.007	0.81±0.019	1.95±0.011
P-value	0.000*	0.000*	0.000*	0.000*	0.000*
S.E	0.004	0.004	0.003	0.005	0.006
F	157.16	165.47	303.01	4517.0	36595.9
df					

*P value at the level <0.05,derived from one way ANOVA

Table 3:Showing difference among the groups for cytotoxicity activity.

GROUPS	**CYTOTOXICITY (MEAN±SD)**				
	5µl	10µl	20µl	40µl	80µl
GROUP1	4.40±0.54	1.6±0.54	1.8±0.44	0.8±0.83	0.6±0.54
GROUP2	9.00±0.70	2.6±0.54	3.0±0.70	0.4±0.54	0.2±0.44
GROUP3	9.40±0.89	9.4±0.89	8.4±1.14	9.4±0.89	9.6±0.54
GROUP4	8.40±0.89	6.6±0.54	6.8±0.44	6.6±0.89	6.6±2.40
GROUP5	4.60±0.54	4.6±0.54	7.4±1.44	5.0±0.70	6.4±1.51
GROUP6	9.00±0.70	9.0±1.00	5.0±0.70	2.2±0.83	0.4±0.54
GROUP7	9.4±0.89	6.6±0.54	0.4±0.54	0.6±0.54	0.4±0.54
P-value	0.979	0.966	0.000*	0.000*	0.000*
S.E	0.478	0.434	0.495	0.484	0.736
F	40.7	95.0	74.9	104.7	57.5
df					

*P value at the level <0.05,derived from one way ANOVA

Figure legend

Figure 1: Showing Zone of inhibition for antimicrobial test.

Figure 2: Showing cytotoxicity test

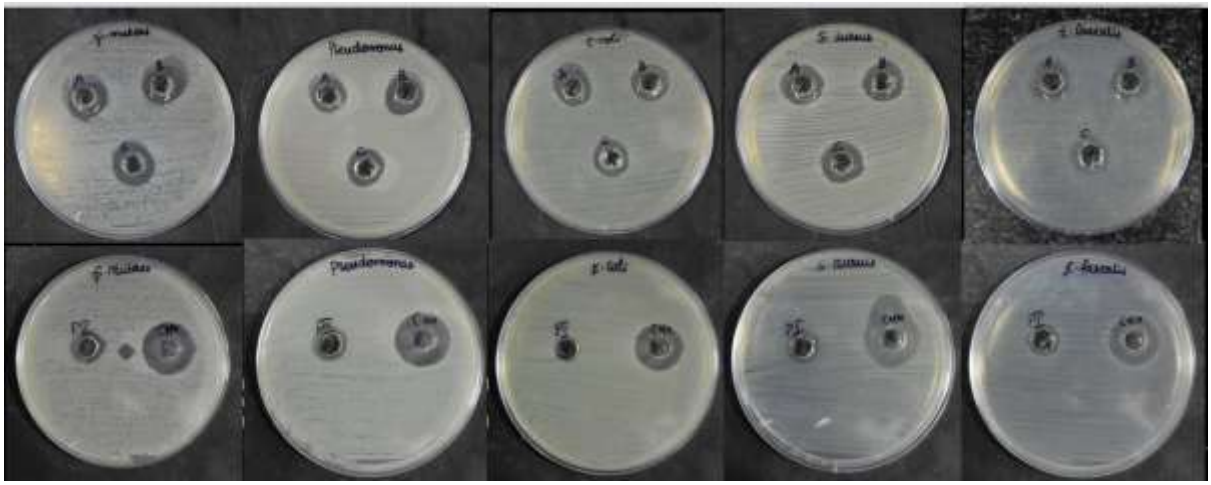

Figure 1: Showing Zone of inhibition for antimicrobial test.

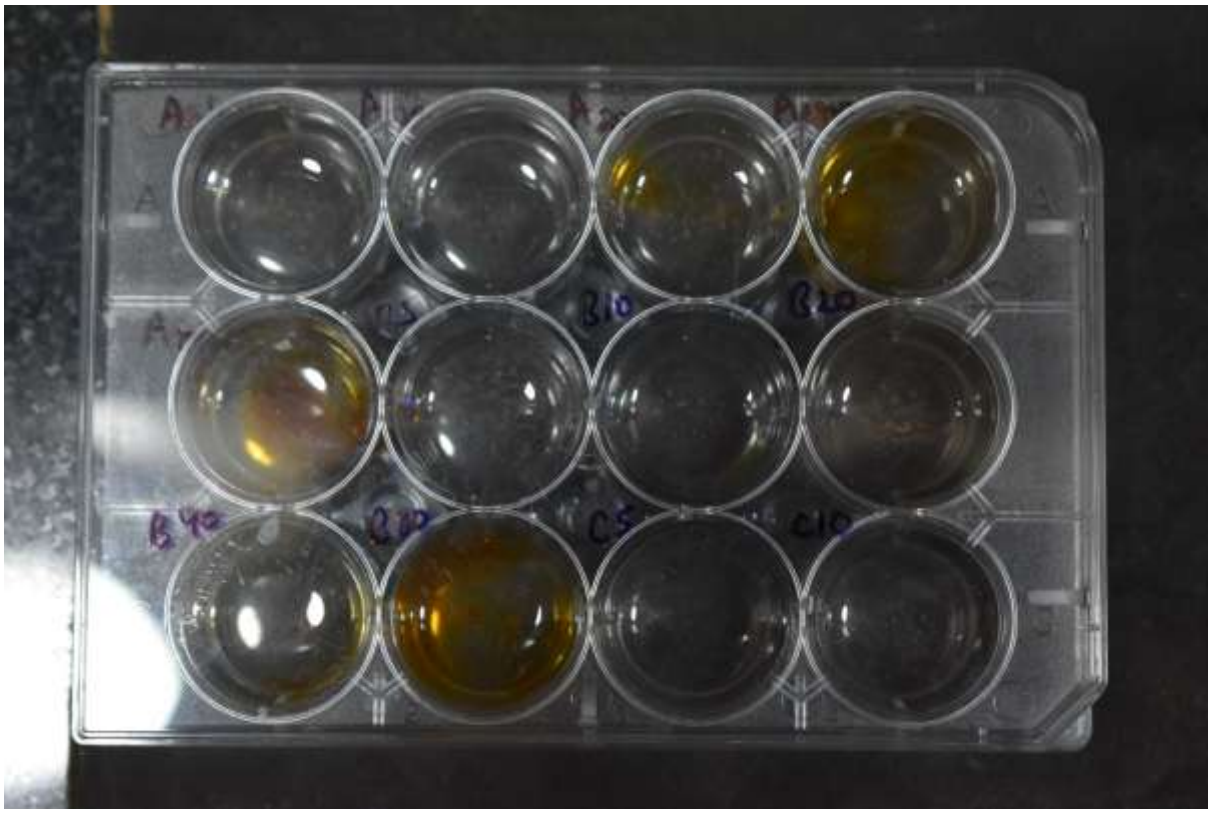

Figure 2: Showing cytotoxicity test