Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [7] June 2021 : 57-62

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Comparison of efficacy of four fruit extracts on oral plaque : An *In Vitro* Comparative Study

[1]Akhilesh Shewale , [2]Sneha Puri , [3]Shweta Bhayade , [4]Nakul Mude , [5]Aruna Daware , [6]Indrajeet Deshpande
1-2Department of Periodontolgy, Swargiya Dadasaheb Kalmegh Smruti Dental College & Hospital , Nagpur
[2] Department of Pedodontics and Preventive Dentist ,Nanded Rural Dental College and Research Center, Nanded.
[3]Department of Orthodontics, Nanded Rural Dental College and Research Center, Nanded.
[4-5]Department of Periodontology,Nanded Rural Dental College and Research Center, Nanded.
[6]Dept of Pedodontics, Yogita Dental College, Khed.

ABSTRACT
To evaluate the efficacy and antimicrobial properties of a five herbal mouth rinses with chlorhexidine mouth rinse in vitro in healthy and periodontitis patients with established dental plaque. A total of 20 dental plaque samples were collected from patients diagnosed with gingivitis and healthy subjects and were streaked on blood agar plate. Well Diffusion method was used to compare freshly prepared fruit extracts of Punica granatum (Pomegranate), Vaccinium macrocarpon *(cranberry), Morinda Citrofilia L. (Noni) and Psidium guajava L (Guava) and distilled water as control. The streaked blood agar plate was incubated at 37° for 24 h and examined for the zones of inhibition. The present study resulted out statistically non significant differences in their zone of inhibitions between any of the tested fruit extracts (p>0.005). All the tested fruit extract were equally effective against the tested microorganism in vitro suggesting that phytotherapeutic agents may be used in the future to inhibit oral microbial growth.*
Keywords*: phytotherapeutic agent , dental plaque , dentistry , ayurveda*

Received 04.05.2021 Revised 20.05.2021 Accepted 22.06.2021

INTRODUCTION

Microbial plaque has been proved by extensive research to be a paramount factor in initiation and progression of periodontal diseases.[1] The results of the clinical trials and a re-analysis of literature data indicates a strong correlation between microbial plaque levels and severity of gingivitis.[2,3,4] Plaque control has long been considered as the cornerstone of its management.[5,6] Regular effective removal of microbial plaque by the personal oral hygiene protocol is the most rational methodology towards the prevention of periodontal diseases.[7,8]

Chemical plaque control approach is desirable to overcome the deficiency of mechanical plaque control. A number of chemical agents which have antimicrobial action have been used, with variable success, to inhibit supragingival plaque formation and the development of gingivitis ,chlorhexidine being the most widely used amongst all of them. [9]

In the midst of growing evidence of the connection between oral health and whole body health, phytotherapeutic agents with their 'naturally occurring' active ingredients offers a gentle and enduring way for restoration of health.[10] Various Phytotherapeutic agents have been used alone or in combination and have been scientifically proven to be safe against various oral health problems like bleeding gums, halitosis, mouth ulcers and decay.[11]

Fruits like Pomegrante (*Punica granatum*)[12,13],Cranberry (*Vaccinium macrocarpon*) [14,15], Noni (*Morinda citrifolia* L.)[16,17] and Guava(*Psidium guajava* L)[18,19] has shown antimicrobial properties in oral conditions.

Thus in view of this, the present study was carried out to compare the efficacy and antimicrobial properties of the aforementioned fruits invitro in healthy and gingivitis patients with established dental plaque.

MATERIAL AND METHODS

The study was designed and conducted in the Department of Periodontics, Swargiya Dadasaheb Kalmegh Smruti Dental college and Hospital ,Nagpur , India from November 2018 to March 2019. Approval from the Institutional Ethics Committee was obtained before initiating the study.

Subject selection:

A total of 20 adult patients between the age groups over 18 years of age were selected. All volunteers subjects were informed about the study protocol and informed consent was obtained. Participants were divided into two groups:

Group A: The healthy group comprised of 10 adult subjects with more than 3 teeth in each quadrant of the dentition and clinical features suggestive of Gingivitis

Group B: The Gingivitis group comprised of 10 adult untreated patients diagnosed as per World Workshop 2017 Classification

Exclusion criteria:

Patients who had received previous oral prophylaxis or any kind of periodontal treatment, patients with any history of systemic diseases or condition, or antibiotic and oral drug therapy, or had used chemical anti-plaque agents prior to six months of study initiation were excluded from the study.

Study design:

Plaque sampling:

Supragingival plaque samples was collected in the morning between 9:00 am to 11:00 am from 20 adult patients following the application of two – tone dye. Participants were instructed to refrain from eating, drinking, and oral hygiene habits two hours before sample collection.

Plaque sample was collected with a sterile scaler or curette from the buccal aspect of upper molar and lingual aspect of lower molar surface of 16, 36 either the left or the right side of the mouth. It was then placed in a sterile container and chilled until carried to the laboratory for microbial investigation.

Antimicrobial assay:

A total of 20 blood agar plates were used. Plaque samples were pooled, streaked on blood agar plate, incubated at 37°C for 48 hrs. Microorganisms were stained with Gram staining and were detected under high power microscope. Conventional test such as catalase, coagulase, oxidase, indole tests were used to identify specific microorganisms.

Preparation of test solution:

The extract of all the tested phytotherapeutic agents were prepared using the cold extraction or maceration procedure [13] .The powder of dried Noni fruit , Guava leaves , Craneberry and Pomegranate were placed into separate stoppered container with 70:30 hydro-ethanol (70% water and 30% alcohol) for a day, with frequent agitation, filtered and the marc was pressed to obtain a liquid extract .

Distilled water (D.W.) was used as control in the study. The various preparation used in the study is shown in figure 1.

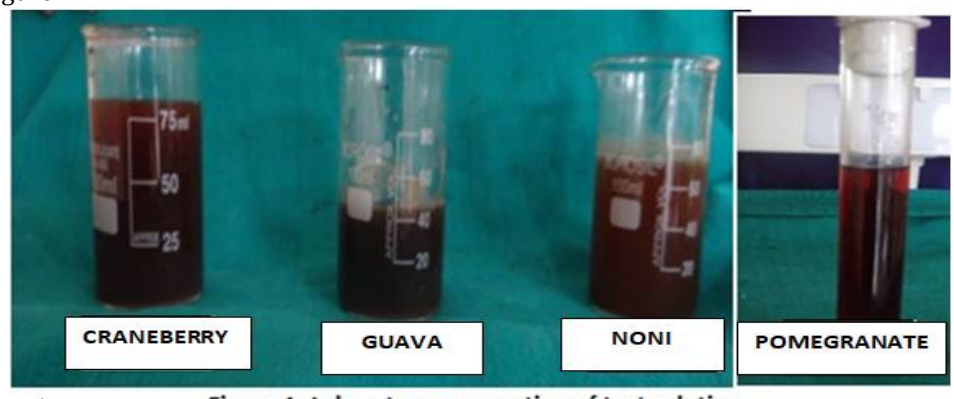

Figure 1: Laboratory preparation of test solution

Antimicrobial evaluation of the mouthwashes:

The modification of the disc diffusion method which is named as the "Well diffusion" method (WD) was used for antimicrobial susceptibility test in the present study. The streaked blood agar plate was incubated at 37° for 24 hrs. 7 wells were made equidistant to each other. 2 ml of test solution was poured in each well. Thereafter, the zones of inhibition were measured using an accurately calibrated measuring transparent scale. Results were recorded as the average diameter of inhibition zone surrounding the wells containing the test solution. The present study is an invito double blind study where an experienced investigator selected the patients in group A and group B and also poured drops of liquid

extracts in all the wells, thereafter a single trained investigator who was masked about the type of extract in each well, measured zone of inhibition in both the groups.

STATISTICAL ANALYSIS:

Statistical Analysis was performed using a statistical package for Social Sciences software (SPSS inc., Chicago, IL, windows version 16) by applying mean values. The test was considered statistically significant when the probability was less than 0.05(P<0.005). Student's t-test was used to compare the zones of inhibition in both the groups . Significance was reported at 95% confidence interval.

RESULTS

In the present study, extracts of Noni , guava , pomegranate and craneberry were selected based upon their medicinal uses in the treatment of oral diseases and their availability and distilled water as control as the tested preparations were aqueous in nature. The antibacterial activity and the effectivity of the mouth rinses were compared on dental plaque microflora.

The microorganisms detected in the plaque samples were namely *Staphylococcus aureus*, *Streptococcus salivarius, Candida albicans and Enterococcus faecalis*. Microorganisms which were detected under high power light microscope after Gram's staining and conventional tests in subjects from both the groups are shown in Table 1. Results of the present study showed the microorganisms detected under light microscope were aerobes and were similar in both the groups. However, Group A showed more Gram positive microorganism whereas Gram negative microorganisms were more in Group B subjects.

Table 1: Showing microorganisms detected in Group A and Group B.

Patients in group A	Microorganisms on blood agar	Patients in group B	Microorganisms on blood agar
1	*Gram positive cocci ++* *Gram negative cocci +* *Streptococcus salivarious,* *Staphylococcus aurous,*	*1*	*Gram positive cocci and bacilli ++* *Gram negative cocci ad bacilli++* *Streptococcus salivarious,* *Staphylococcus aurous,* *Candida albican*
2	*Gram positive cocci and bacilli ++* *Streptococcus salivarious,*	*2*	*Gram positive cocci and bacilli ++* *Gram negative cocci and bacilli ++* *Streptococcus salivarious,* *Staphylococcus aurous,* *Candida albican*
3	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Candida albican* *Enterococcus faecalis*	*3*	*Gram positive bacilli +* *Gram negative cocci ++* *Streptococcus salivarious,* *Staphylococcus aurous,*
4	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Candida albican*	*4*	*Gram positive cocci and bacilli ++* *Gram negative cocci ++* *Staphylococcus aurous,* *Candida albican* *Enterococcus faecalis*
5	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Candida albican*	*5*	*Gram positive cocci and bacilli +* *Gram negative cocci ++* *Staphylococcus aurous,* *Candida albican* *Enterococcus faecalis*
6	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Streptoccus salivarious* *Candida albican*	*6*	*Gram positive cocci and bacilli +* *Gram negative cocci ++* *Staphylococcus aurous,* *Enterococcus faecalis*
7	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Enterococcus faecalis*	*7*	*Gram positive cocci and bacilli +* *Gram negative cocci ++* *Staphylococcus aurous,* *Candida albican* *Enterococcus faecalis*
8	*Gram positive cocci and bacilli ++* *Gram negative cocci +* *Staphylococcus aurous,* *Candida albican*	*8*	*Gram positive cocci and bacilli +* *Gram negative cocci ++* *Staphylococcus aurous,* *Candida albican*

			Enterococcus faecalis
9	*Gram positive cocci and bacilli ++ Gram negative cocci + Steptococcus salivarious Candida albican*	*9*	*Gram positive cocci and bacilli ++ Gram negative cocci ++ Enterococcus faecalis*
10	*Gram positive cocci and bacilli ++ Gram negative cocci + Staphylococcus aurous, Staphyloccocus salivarius*	*10*	*Gram positive cocci and bacilli + Gram negative cocci ++ Staphylococcus aurous, Candida albican Enterococcus faecalis*

Comparison of extract preparations in Group A:

Comparative evaluation of the zones of inhibition with four extract preparations in healthy subjct is depicted in Table 2, Figure 2 .Results showed that the mean±S.D diameter of the zones of inhibition are Noni extract (2.5 ±0.17cm) , craneberry extract (1.99 ±0.10 cm), pomegranate extract (1.95±0.15 cm) and guava leaf extract (1.85±0.21 cm).

Non significant difference in zone of inhibition was observed between any of the tested extract preparations as depicted in table 3, suggesting overall the efficacy of all the tested preparations .

Comparison of extract preparations in Group B:

Comparative evaluation of the zones of inhibition with four extract preparations in healthy subjct is depicted in Table 2. Result of the present study showed mean diameter of zone of inhibition are Noni extract (2.8 ±0.19 cm), craneberry extract (1.96±0.12 cm), pomegranate (1.96 ±0.25cm) and guava leaf extract (1.86±0.14 cm) . Non significant difference in zone of inhibition was observed between any of the tested extract preparations as depicted in table 4, suggesting overall the efficacy of all the tested preparations .

Comparison of group A and group B showed non significant differences in the zone of inhibition as shown in table 2 (p>0.005), suggesting that antimicrobial efficacy of mouth rinses in both the group were similar.

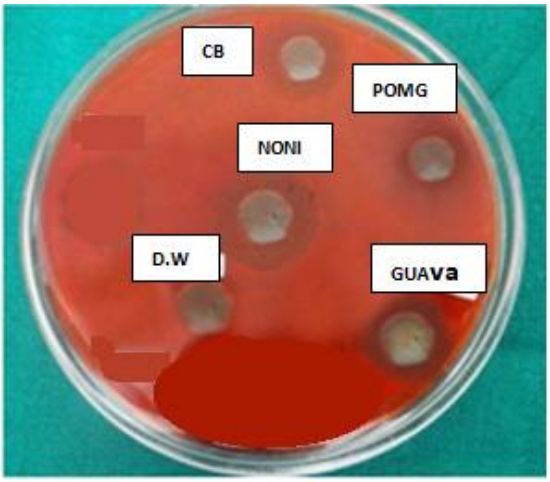

Figure 2: Zone of inhibition of mouthrinses in Group A

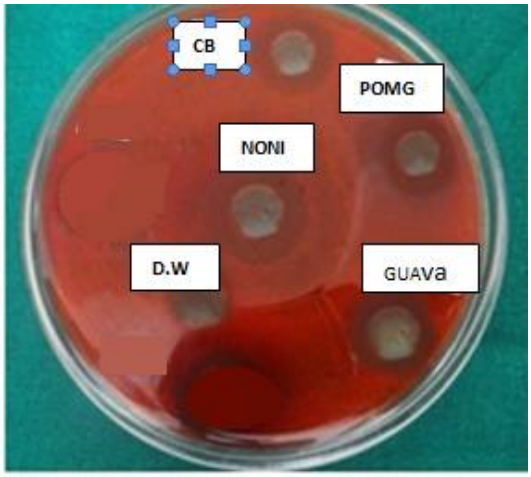

Figure3: Zone of inhibition of mouthrinses in Grup B

Table 2: Showing mean values of zone of inhibition in Group A and Group B

Mouthrinses	Group A Mean±S.D. (cm)	Group B Mean±S.D. (cm)	'p' Value	Significance
Noni	2.5 ±0.17	2.8 ±0.19	0.52 (p>0.05)	NS
craneberry	1.99 ±0.10	1.96±0.12	0.23 (p>0.05)	NS
Pomegranate	1.95±0.15	1.96 ±0.25	0.36 (p>0.05)	NS
Guava	1.85±0.21	1.86±0.14	0.37 (p>0.05)	NS

S.D.: Standard Deviation; NS: Non-Significant

Table 3: Comparison of CHX with other other mouthrinses in Group A.

Comparison of Fruit Extracts in Group A	'p' value	Significance
Noni & Craneberry	0.159 (p>0.05)	NS*
Noni & Pomegranate	0.310 (p>0.05)	NS
Noni & Guava	0.450(p>0.05)	NS

- One way ANOVA test ;p: Probability; NS: Non-Significant; S: Significant

Table 4: Comparison of CHX with other other mouthrinses in Group B

Comparison of Fruit Extracts in Group B	'p' value	Significance
Noni & Craneberry	0.07450 (>0.05)	NS*
Noni & Pomegranate	0.5445 (>0.05)	NS
Noni & Guava	0.625 (>0.05)	NS

- One way ANOVA test ;p: Probability; NS: Non-Significant; S: Significant

DISCUSSION

The present study was carried out to compare the efficacy of four different fruit extracts viz. Noni Fruit , Cranberry , Pomegranate and Guava on oral plaque. To best of our knowledge no study has compared the efficacy of aforementioned fruit extracts together, thereby the present study was carried out.

The study resulted out that all the prepared fruit extracts has antimicrobial activity against the tested organisms and showed no statistically significant difference in terms of their zone of inhibition .

Noni fruit extract has shown the non significant higher zone of inhibition as compared to other in our study . The results could be attributed to the presence of scopoletin, acubin, and alizarin in its fruit . The antibacterial activity of phenolic compounds like scopoletin is known to be better against Gram-positive bacteria than Gram-negative bacteria owing to a difference in their cell wall structure .

The antibacterial and anti-adhesion features of cranberry against oral bacteria has been demonstrated by the presence of certain components like phenolic acids, proanthocyanidins (particularly, A-type proanthocyanidins), anthocyanins, organic acids, and their microbial-derived metabolites which may limit dental caries by inhibiting the production of organic acids by cariogenic bacteria, the formation of biofilms by *Streptococcus mutans* and *Streptococcus sobrinus*, and the adhesion and coaggregation of a considerable number of other oral species of *Streptococcus* . Focusing on periodontal diseases, the non-dialyzable constituent fraction of cranberry (NDM) inhibits the formation of *P. gingivalis* and *Fusobacterium nucleatum* biofilms, two bacteria species associated with periodontitis. The NDM fraction may also inhibit the adhesion of *P. gingivalis* to various proteins, including type I collagen and may reduce bacterial coaggregation involving periodontal pathogens. [14,15]

Pharmacological properties of pomegranate have a long history, but, in the recent decades, the interest in evaluating therapeutic effects of pomegranate has increased noticeably. Studies show that pomegranate juice has potent antioxidant activity (capability to scavenge free radicals) due to its high polyphenols content, including ellagitannins (hydrolysable tannins) and anthocyanins (condensed tannins). There is a range of phytochemical compounds in pomegranate that have showed antimicrobial activity, but most of the researchers have found that ellagic acid and larger hydrolyzable tannins, such as punicalagin, have the most important activities.[12]

Recent study indicates that both pomegranate aril and peel extracts have an effective antimicrobial activity, as evidenced by the inhibitory effect on the bacterial growth of two important human pathogens, including *Staphylococcus aureus* and *Escherichia coli*, often involved in foodborne illness . In addition, experimental data strongly support the antibacterial activity of pomegranate extracts against oral pathogen such as *S. mutans* . However, little is known about the effect of pomegranate extracts on other pathogens involved in tooth decay such as *R. dentocariosa*, the first bacterium isolated from carious dentin [13]

Compounds of known antimicrobial activity in Guava includes 1,2-Benzenedicarboxylic acid, dibut, Alpha.-bisabolol, 1,2-Benzenedicarboxylic acid, buty, hexadeca-2,6,10,14-tetraen, caryophyllene, germacrene ,quercetin, quercetin-3-O-α-L-arabinofuranoside, quercetin-3-O-β-D-arabinopyranoside, morin-3-O-lyxoside, morin-3-O-arabinoside, quercetin and quercetin-3-O-arabinoside , 11-hydroxy-35-tricont-pentatriacontanoate, hexaeicosan-16-ol, tricosan-17-ene-5-ol, nonacosan-23-ene-3-ol, lupeol and betulinic acid.A recent study used aqueous extracts were tested against cariogenic and dental plaque causative microorganisms (Streptococcus sanguinis, Streptococcus mitis and Actinomyces sp.) and the MIC was determined, varying between 2.61 and 4.69 mg/mL. [18,19]

The various indications where our aforementioned phytotherapeutic agents can be substituted for commercially available anti plaque agents with equal efficacy are: Patient's less compliance to chlorhexidine ;Healthy, gingivitis and mild periodontitis patient ;After periodontal surgery as chemical

plaque control agents interferes with fibroblast activity ;Patient who are allergic to ingredients of chemical control agents ;Patient who have got anterior composite restoration so staining could be prevented.

Strength of the present study is that, this study has compared In interpreting the findings of the present study, it is important to acknowledge possible limitations. The present study has assessed only aerobic microorganism. The results may not correspond to the actual behavior of prepared extracts in I*n vivo* because they are not exposed to the same conditions found in the oral cavity. Substantivity exists or not could not be ascertained in this study. Comparison with the gold standard mouthwash has not been made in our study which could be the future scope of our study Further researches are needed which focus on various concentration of these fruit extracts .

CONCLUSION

In the present study, all the tested phytotherapeutic agent showed sufficient zone of inhibition against tested microorganisms. Hence could be used as an adjunct to non surgical periodontal therapy. Futhermore, laboratorial studies are needed to support the performance of further clinical investigations with much larger sample size and at various concentrations .

REFERENCES

1. Loe, H. (1965). Experimental Gingivitis in Man, J. Periodontol,36:177.
2. Breuer MM, Cosgrove RS. (1989). The relationship between gingivitis and plaque levels. J Periodontol, 60:172-1755.
3. Theilade E, Wright WH, Jensen SB, Löe H. (1966). Experimental gingivitis in man: A longitudinal clinical and bacteriological investigation. J Periodontal Res ;1:1-13.
4. Ramires-Romito ACD, Romito GA, Mayer MPA, Rodrigues RMD. (20050. Correlation study of plaque and gingival indexes of mothers and their children. J Appl Oral Sci, ;13:227-231
5. Darby I. (2009). Non-surgical management of periodontal disease. Aust Dent J, 54:86–95.
6. Blinkhorn A, Bartold PM, Cullinan MP, Madden, Marshall RI, Raphael SL. (2009). Is there a role for triclosan/ copolymer toothpaste in the management of periodontal disease? Br Dent J,3:117-125
7. Bassiouny G, Barrak HA. (2014). The Anti-plaque Effect of Miswak and Myrrh Mouthwashes versus Chlorhexidine in the Treatment of Chronic Gingivitis; A Comparative Clinical Trial. Medical Science ;9:28-31.
8. Mali AM, Behal R, Gilda SS. (2012). Comparative evaluation of 0.1% turmeric mouthwash with 0.2% chlorhexidine gluconate in prevention of plaque and gingivitis: A clinical and microbiological study. J Indian Soc Periodontol, 16:386-391.
9. Bayalty FH, kaubaisi AH, wahid NA, Abdulla MA. (2010). Effect of mouthwash extracted from salvodora persika on dental plaque formation:A clinical trial.J. Med. Plant. Res,4: 1446-1454.
10. Nagappan N, John J. (2012). Antimicrobial Efficacy of Herbal and Chlorhexidine Mouth rinse -A systematic review. JDMS2:5-10.
11. Shetty S, Pillai S, Sridharan S, Satyanarayana A, Rahul A, (2013). Comparative Efficacy of Chlorhexidine and a Herbal Mouth Rinse in Patients with Gingival Inflammation - A Clinical & Microbiologic Study. Asian Journal of Pharmaceutical Technology & Innovation;1:1-8.
12. P. Subramaniam, S. Dwivedi, E. Uma, and K. L. Girish Babu, (2012). "Effect of pomegranate and aloe vera extract on streptococcus mutans: An in vitro study," *Dental Hypotheses*, vol. 3, no. 3, pp. 99–105.
13. M. Onishi, (1949). "Study on actinomyces isolated from the deeper layers of carious dentin," *Shikagaku Zasshi*, vol. 6, pp. 273–318..
14. Sánchez MC, Ribeiro-Vidal H, Bartolomé B, Figuero E, Moreno-Arribas MV, Sanz M, Herrera D. (2020). New Evidences of Antibacterial Effects of Cranberry Against Periodontal Pathogens. Foods. 24;9(2):246.
15. Koo H., Duarte S., Murata R.M., Scott-Anne K., Gregoire S., Watson G.E., Singh A.P., Vorsa N. (2010). Influence of cranberry proanthocyanidins on formation of biofilms by ***Streptococcus mutans*** on saliva-coated apatitic surface and on dental caries development ***in vivo***. *Caries Res.* 44:116–126
16. Locher, C.P., Burch, M.T., Mower, H.F., et al. (1995) Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. Journal of Ethnopharmacology, 49, 23-32. doi:10.1016/0378-8741(95)01299-0
17. Leach, A.J. (1988) Antibacterial activity of some medicinal plants of Papua New Guinea. Science in New Guinea, 14, 1-7.
18. Kang, J.-H., & Song, K. B. (2019). Antibacterial activity of the noni fruit extract against Listeria monocytogenes and its applicability as a natural sanitizer for the washing of fresh-cut produce. Food Microbiology, 103260.
19. N GDe La Cruz-Sánchez, AG-Rivera, P Alvarez-Fitz, EV-Zapata, MDP García, MA-Flores, ASG Román, MG Cortazar, .(2019). Antibacterial activity of Morinda citrifolia Linneo seeds against Methicillin-Resistant Staphylococcus spp ;Microbial Pathogenesis.;128 :347-353

CITATION OF THIS ARTICLE

A Shewale , S Puri , S Bhayade , N Mude , A Daware , I Deshpande. Comparison of efficacy of four fruit extracts on oral plaque : An *In Vitro* Comparative Study*.* Bull. Env. Pharmacol. Life Sci., Vol10[7] June 2021 : 57-62