Effective Eradication of Plaque Formation by Using *Psidium Guajava*

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ABSTRACT

The objective of the study was to reduce the dental plaque formation by the inhibitory effect of the extracts taken from *Psidium guajava* and eggshell against the pathogens present in the plaque. 10 plaque samples were collected and bacterial colony was isolated by using different media (Blood agar media, Nutrient media, and nutrient broth). The *Streptococcus mutans* have been the major dental plaque pathogen confirmed by 16srRNA sequencing. The inhibition of the *Streptococcus mutans* (HS SRM) by *Psidium guajava* and egg shell is proved by antibacterial activity test by antibiotic sensitivity test. Further, GC-MS determined the components of *P.guajava* which contains Tetrakis (2, 3-Ditert-Butylphenyl), 2, 6, 10, 14, 18, 22-Tetracosahexane, 1, 2-Benzendicarboxylic acid. Egg shell contains Oleic acid, Propyl ester, Squalene, 1, 2- Benzendicarboxylic acid, Pentadecanol. From this current research it has been proven that the *Psidium guajava* and egg shell is the effective curative agent against dental plaque by inhibiting *Streptococcus mutans*.

Index Terms: Plaque, *Psidium guajava*, egg shell, *Streptococcus mutans*, GC-MS, 16srRNA sequencing

I. INTRODUCTION

Dental plaque is a sticky colorless biomass of bacteria that grows on the surfaces of teeth leading to the formation of tartar or demineralization of teeth which is often pale yellow or brown (Darby M L *et.al*, 2010; James B. Summitt *et.al*). *Streptococcus mutans* and some other anaerobes invades the tooth surface which plays a major role in the formation of biofilm. (Kolenbrander P. *et.al*, 2010; Chaminda Jayampath Seneviratne *et.al*, 2014).

Facultative anaerobes like *Streptococcus mutans* are major contributor of tooth decay which are commonly found in human oral cavity (A.L. Coykendall *et.al*, 1974; Carlsson J. *et.*al, 1973). The demineralization of tooth is caused by cariogenic microorganism *S.mutans* by breaking down of sugar producing an acidic environment. (Howard K. Kuramitsu *et.al*, 1993).Water insoluble glycans, acid tolerance and production of lactic acid are the three main virulence factors found in *S.mutans.*

Huge amount of calcium and mineral content found in eggshell proves it to be an ideal treatment for healing cavities. Egg shell were found to lower the acidic content of teeth and to prevent white spot tooth disease. The

eggshells are widely used in restoring the mineral and calcium of teeth (Gaurav Balu Dafal *et.al*, 2017). *Psidium guajava* contains medicinally important phyto constituents that widely used in food technology and pharmacology. *P.guajava* is widely known for its antimicrobial, antigenotoxic, anti-inflammatory, antidiabetic. Hence, it can be preferred for treating diarrhoea, dental plaque, diabetes (Rosa Martha Perez Gutierrez *et.al*, 2008)

II. MATERIALS AND METHODS

Collection of sample:

Five to ten plaque sample were collected from SRM Hospital and Dental College. The samples were stored in the sterile tubes containing normal saline at 4℃ in the laboratory condition.

Isolation and screening of plaque sample*:*

Plaque samples were screened using various media with optimum conditions. The plaque samples were inoculated in 3 different media which include blood agar medium, Nutrient agar medium and Nutrient broth. Both Agar plates and broth test tubes were incubated at room temperature for 24 hours. After incubation single colonies were selected, subcultured and used for further studies and their biochemical and physiological characteristics has been evaluated (Nada H.A. Al-Mudallal *et.al*, 2008; Jean A. Setterstrom *et.al*, 1979)

Antibacterial activity:

The antibacterial activity of organic solvents of *Psidium guajava* and egg shell against bacterial colony isolated from plaque sample were determined by agar diffusion method where the pathogens are swabbed on Muller Hinton agar plates. The wells were made using gel puncture. The samples egg shell and psidium guajava, 10µl of Ampicillin (+Ve control), 10µl of petroleum ether (-Ve control) were added to the respective wells and incubated overnight at 37℃. After 24hrs of incubation thezone of inhibition was calculated. Finally the sample which showed higher zone of inhibition was identified by 16s rRNA sequencing (Nada H.A. Al-Mudallal *et.al*, 2008; Adhraa S *et.al*, 2016)

Solvent extraction by using soxhlet apparatus:

The *psidium guajava* and the egg shell was dried for 15 days in sunlight, then boiled for 8 hours to remove the unwanted substance from the eggshell. 500ml of petroleum ether was used an organic solvent for plant extraction. The soxhlet apparatus was allowed to proceed for 3 hours and the petroleum ether was used as solvent .The *Psidium Guajava* and egg shell extract was extracted (Gerard L *et.al*, 2011).

Gas chromatography and mass spectrometry:

The extracts of *Psidium Guajava* and egg shell was then subjected to GC-MS for the determination of mixture of components.

III. RESULTS

Isolation and screening of plaque sample:

Plaque samples cultured on the blood agar plates were subcultured repeatedly several times in order to obtain pure cultures. Gram staining test confirmed presence of gram positive, catalase and voges proskauer test confirmed the

presence of *Streptococcus mutans*. Zone of incubation of samples showed the maximum activity 13mm and 22 mm against to *S.mutans*. *Psidium guajava* show the highest incubation zone compare to the egg shell **(Table 1)**.

SAMPLE	ZONE OF INHIBITION (IN mm)
Egg shell	13
Sample (HSSRM)	**22**
Positive control	12
Negative control	0

TABLE 1: Zone of inhibition *S.mutans* stain

16S rRNA sequencing:

The screened plaque sample strain (HS SRM) was identified a molecular level by 16srRNA sequencing was confirmed *Streptococcus mutans* (NR 115733.1) and phylogenetic tree analysis using MEGA software confirmed the species as *Streptococcus mutans* **(Fig 1)**

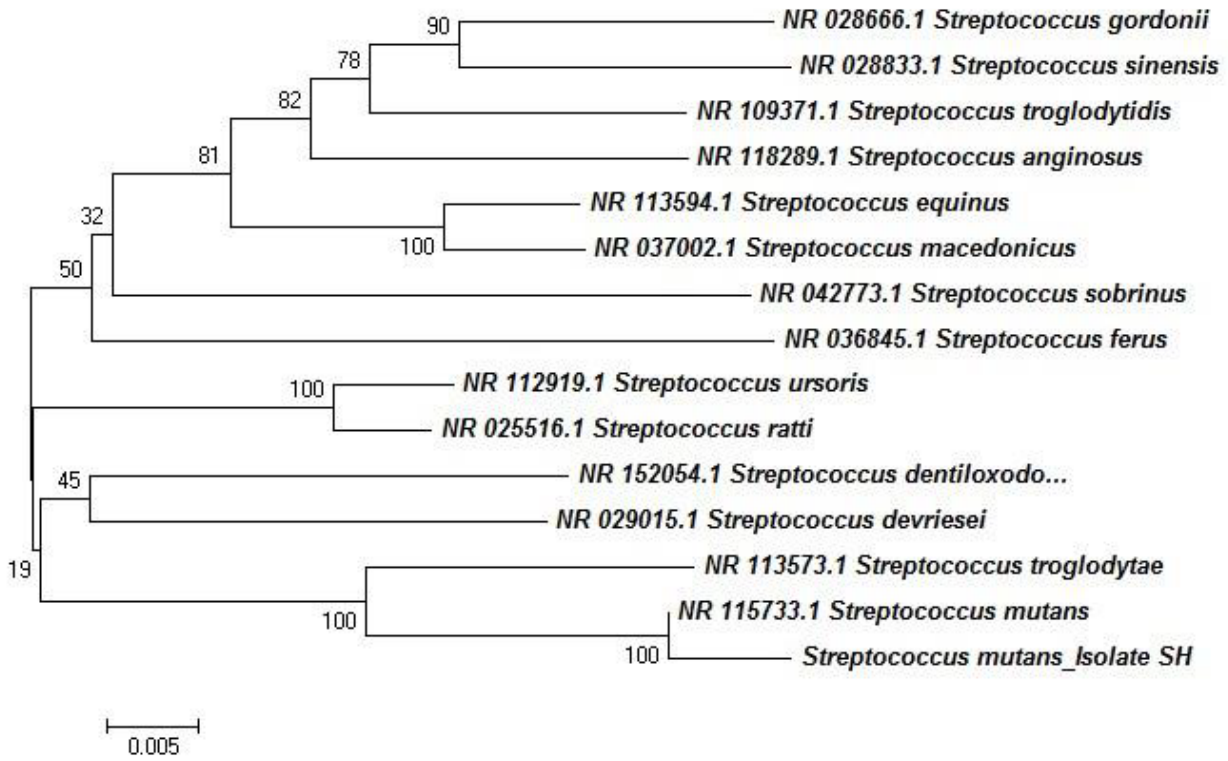


Fig 1 PHYLOGENRTIC TREE ANALYSIS OF PLAQUE SAMPLE HS SRM

Gas chromatography and mass spectrometer:

The pant extraction was done by using soxhlet apparatus. Two different kind of samples egg shell and bark of *Psidium guajava* were analysed by GC-MS technique in which the complex mixture of chemicals maybe separated, identified and quantified. Samples are usually analyzed as organic solutions, consequently materials of interest need to be solvent extracted and the extract subjected to various 'wet chemicals' technique before GC-MS analysis is possible. In this result show high retinal time compounds present in the bark of *Psidium Guajava* compared to the egg shell **(Table 2, 3; Fig 2, 3)**.

Table 2: GC –MS – EGG SHELL

Peak# R.Tim
e I.Time F.Time Area Area% Height Height% A/H Mark Name
1 11.407 11.383 11.425 4227 0.12 3673 0.23 1.15 MI N-ALLYLOXYMETHYLACRYLAMI
2 11.505 11.492 11.517 1212 0.03 1241 0.08 0.98 MI OXIRANE, 2,2'-[OXYBIS(METHYLE
3 12.950 12.917 12.992 28958 0.80 14933 0.94 1.94 MI PYRIMIDINIUM, 5-CARBOXY-4-(1,1
4 13.906 13.833 14.100 1356191 37.41 403229 25.48 3.36 MI 1,2-BENZENEDICARBOXYLIC ACID
5 15.920 15.883 16.000 47954 1.32 11858 0.75 4.04 MI Isoamyl nitrite
6 16.148 16.117 16.183 44532 1.23 29799 1.88 1.49 MI 2-DODECANOL, 1,1-DICHLORO-
7 16.218 16.200 16.258 8212 0.23 6374 0.40 1.29 MI 2-DECYLOXYETHANOL

16.898 16.867 16.925 18774 0.52 9311 0.59 2.02 MI 1,2-BENZENEDICARBOXYLIC ACID
17.392 17.375 17.417 6667 0.18 5086 0.32 1.31 MI Acetic acid, trifluoro-, 2,2-dimethylprop
17.509 17.475 17.533 14850 0.41 9019 0.57 1.65 MI DECANOIC ACID, 8-METHYL-, MET
17.855 17.817 17.908 71140 1.96 29026 1.83 2.45 MI 1,2-BENZENEDICARBOXYLIC ACID
17.960 17.925 18.000 50949 1.41 19800 1.25 2.57 MI HEXADECANOIC ACID
18.183 18.158 18.217 42723 1.18 27235 1.72 1.57 MI 1-TETRADECENE
19.656 19.583 19.733 86746 2.39 15935 1.01 5.44 MI .BETA.-D-RIBO-HEXOPYRANOSE,
20.047 19.983 20.075 34701 0.96 15338 0.97 2.26 MI 1-UNDECANOL
21.751 21.667 21.775 27841 0.77 8193 0.52 3.40 MI 1-PENTADECANOL
22.023 21.992 22.050 13359 0.37 6731 0.43 1.98 MI 3-HEXEN-1-OL, PROPANOATE, (Z) 18
22.887 22.850 22.925 102108 2.82 55517 3.51 1.84 MI 1,2-BENZENEDICARBOXYLIC ACID
24.958 24.917 25.008 478815 13.21 285835 18.06 1.68 MI Squalene
28.762 28.708 28.808 1098871 30.31 586385 37.05 1.87 MI Silane, dimethyl(docosyloxy)butoxy-
29.722 29.683 29.750 45972 1.27 19698 1.24 2.33 MI ETHYL ISO-ALLOCHOLATE
29.842 29.800 29.867 40828 1.13 18314 1.16 2.23 MI OLEIC ACID, PROPYL ESTER 3625630 100.00 1582530 100.00

Fig 2: EGGSHELL GC-MS

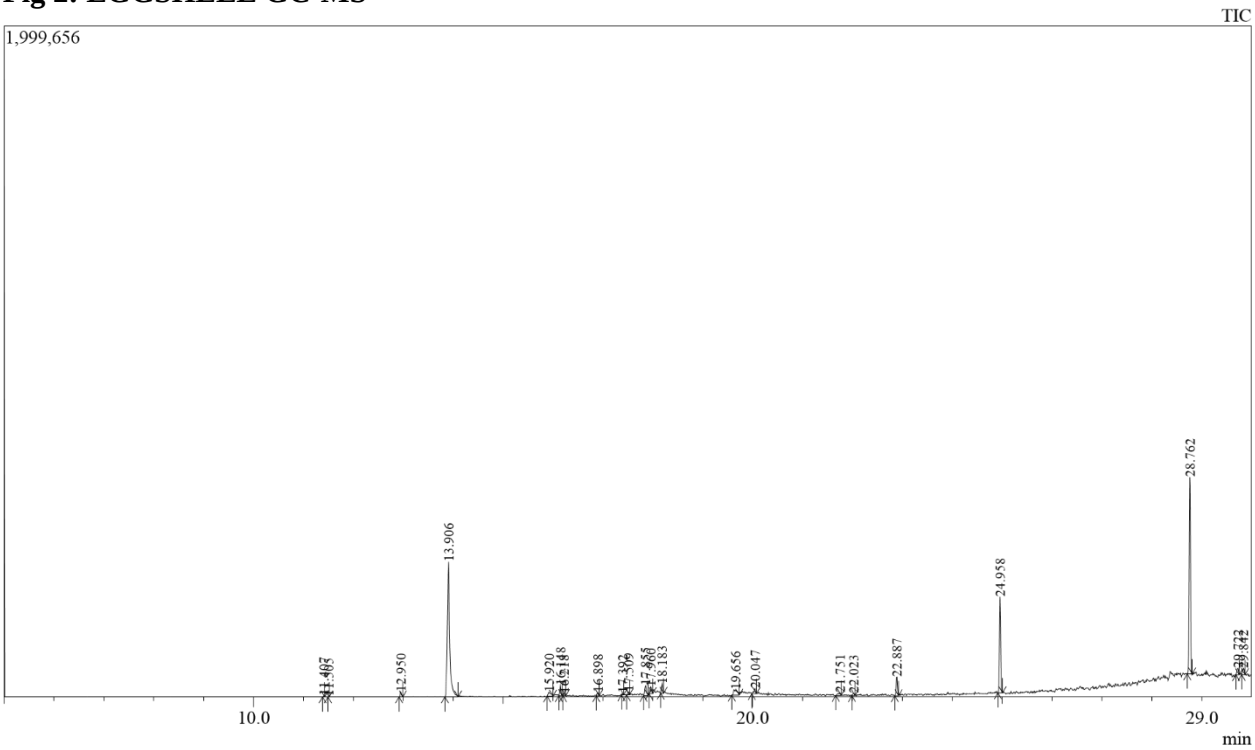
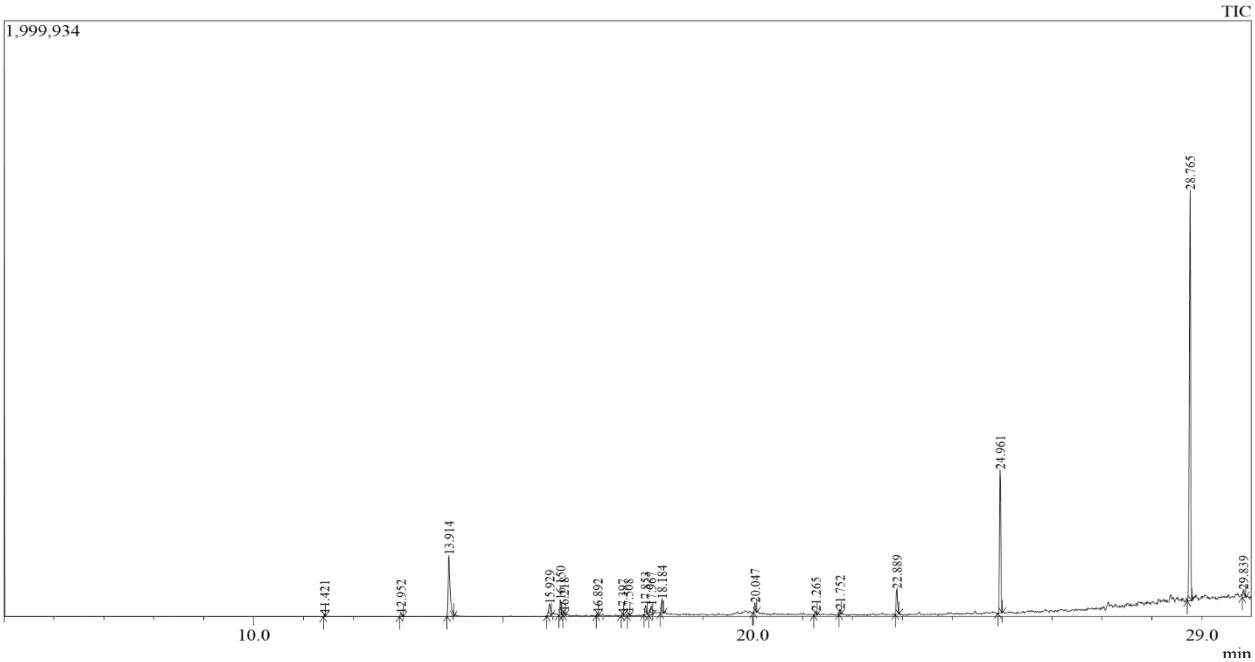


Table 3: GC-MS *PSIDIUM GUAJAVA*

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
•	11.421	11.400	11.442	11158	0.24	8696	0.35	1.28	MI	1-TETRADECANOL
•	12.952	12.925	13.000	21720	0.46	10248		0.41	2.12	MI PYRIMIDINIUM, 5-CARBOXY-4-(1,1
•	13.914	13.875	14.008	573450	12.16	204954		8.23	2.80	MI 1,2-BENZENEDICARBOXYLIC ACID
•	15.929	15.875	15.967	115813	2.46	39231	1.58	2.95	MI	HEXADECANOIC ACID
5	16.150	16.117	16.175	77643	1.65	49832	2.00	1.56	MI	1-DOCOSANOL
•	16.218	16.200	16.250	19502	0.41	12129		0.49	1.61	MI 2-PROPYLDECAN-1-OL
•	16.892	16.867	16.917	13375	0.28	9035	0.36	1.48	MI	1,2-BENZENEDICARBOXYLIC ACID
•	17.397	17.367	17.417	12933	0.27	7303	0.29	1.77	MI	2-OCTYLDODECAN-1-OL
•	17.508	17.483	17.542	18042	0.38	10269		0.41	1.76	MI TETRADECANOIC ACID, 12-METHY
•	17.853	17.825	17.892	51140	1.08	28772		1.16	1.78	MI 1,2-BENZENEDICARBOXYLIC ACID
•	17.967	17.917	18.000	85201	1.81	30865		1.24	2.76	MI HEXADECANOIC ACID
•	18.184	18.150	18.217	88435	1.87	47278		1.90	1.87	MI 1-TETRADECANOL
•	20.047	20.017	20.083	56764	1.20	32283		1.30	1.76	MI 1-TETRADECANOL
•	21.265	21.225	21.292	24497	0.52	12233		0.49	2.00	MI 2-t-Butylperoxy-2-ethylbutan-1-ol, buty
•	21.752	21.733	21.775	24284	0.51	16705		0.67	1.45	MI HEPTENAL
•	22.889	22.858	22.933	157635	3.34	84644		3.40	1.86	MI 1,2-BENZENEDICARBOXYLIC ACID
•	24.961	24.917	25.000	837354	17.75	481570		19.34	1.74	MI 2,6,10,14,18,22-TETRACOSAHEXAE
•	**28.765**	**28.708**	**28.800**	**2471969**	**52.41**	**1379752**	**55.40**	**1.79**	**MI**	**TETRAKIS(2,3-**
DITERT-BUTYLPHEN	**19**	**29.839**	**29.808**	**29.883**	**55725**	**1.18**	**24732**	**0.99**	**2.25**	
MI	**UNDECANENITRILE**	**4716640**	**100.00**	**2490531**	**100.00**					

Fig 3: *PSIDIUM GUAJAVA* GC-MS



IV. DISCUSSION

Psidium guajava have long been recognized for their antibacterial activity inhibiting both gram positive and gram negative bacteria such as *S.aureus, S.mutans, Pseudomonas aeruginosa, Salmonella enteritis and Bacillus cerans*. The bark of *Psidium guajava* can be regarded as the curative agent of dental plaques against dental plaque.

The identification of *S.mutans* is based on (Essam F. A. Al-Jumaily *et.al*.) distinctive colonial morphology on selective agar, Gram staining, distinctive cell shape on light microscopy, specific growth characteristics, and sugar fermentation. In addition to that *S.mutans* isolates was identified by the commercial biochemical test the identification also was depended the test results. So the bark of *Psidium guajava* have capacity for act as an antibacterial activity for against to *S.mutans* (Pongask Rattanachaikunsopon et al., 2010; T. Suman, *et.al*, 2010)

According to previous report, *Streptococcus mutans* was confirmed as one of the important bacteria for affected the tooth and it is form a biofilm .Also many bacteria present in the given plaque sample but *Streptococcus mutans* is the major cause the tooth decay. Therefore, this study aimed at analysis for identified the bacteria which one mostly involved the biofilm formation and it is reason for tooth decay.

The analysis of GC-MS indicates the bark of *Psidium guajava* have a high antibacterial activity against *Streptococcus mutans* compared to the egg shell. Therefore, aimed to focus the chemical compound playing a major role against the plaque sample.

V. CONCLUSION

Overall result suggested that the bark of *Psidium guajava* is an antibacterial agent against the plaque sample (*Streptococcus mutans)* compared to the eggshell. This study also prove the importance of *Psidium guajava* in the dental plaque hence can be regarded as the safe curative agent for dental plaques and further can be commercialized.

REFERENCES

1. A.L.Coykendall (1974). Four types of *Streptococcus mutans* based on their genetic, antigenic and biochemical characteristics. Journal of general microbiology.327-338
2. Adhraa S. Flayyih, Hayfa H. Hassani, Mohammed H. Wali. Identification of *Streptococcus mutans* from Human Dental Plaque and Dental Caries Using 16SrRNA Gene. Iraqi Journal of Science, 2016, Vol. 57, No.1C, pp: 552-557.
3. Akl Wan, WK Seow, LJ Walsh, PS bird (2002) Comparison of five selective media for the growth and enumeration of *Streptococcus mutans*. Australian dental journal; 47(1):21-26.
4. Carlsson J. (1973).A Medium for isolation of *Streptococcus mutans* .Archives of oral biol.12:1657-1658.
5. Howard K. Kuramitsu. Virulence Factors of Mutans Streptococci: Role of Molecular Genetics. Critical Reviews in Oral Biology and Medicine, 4(2):159-176 (1993)
6. Rosa Martha Perez Guitierrez, Sylvia Mitchell, Rosario Vargas Solis. *Psidium guajava:* A review of its traditional uses, phytochemistry and pharmacology, journal of ethnopharmacology. Volume 117 (1)1-27 (2008).
7. Essam F.A.Al-jumaily, Hashim M.Z.AL-Seubehawy and Faris A.Al-Toraihy (2014), Isolation and identification of *Streptococcus mutans* (H5) produced glucosyltransferase and cell- associated glucosyltransferase isolated from dental caries, International journal of current microbiology and applied science. Volume 3. 850-864.
8. Gaurav Balu Dafal and Navin K. Khare. Formulation and evaluation of toothpaste by using eggshell, World journal of pharmaceutical research .Volume 6, 534-543 (2017).
9. Gerard L, Penecilla and P. Mango (2011) Antibacterial activity of extract of twelve common medicinal plants from the Philippines. Journal of medicinal plants research vol 5(16), 3975-3981.
10. Jean A Setterstrom, Arthur gross, and Ronald S. Stanko (1979), Comparison of minitek and conventional methods for the biochemical characterization of oral streptococci. Journal of clinical microbiology .Volume 10. 409-414.
11. Kolenbrander P. “Oral Microbial Communities: Biofilms, Interactions, and Genetic systems”. Annu Rev Microbiol. 2000; 54(1):413-37.
12. Chaminda Jayampath Seneviratne, Cheng fei zhang, Lakshman Perera Samaranayake. Dental Plaque Biofilm in Oral Health and disease. The Chinese journal of Dental research. July 2014.
13. Nada H.A. Al-Mudallal, Essam F.A. Al-Jumaily, Nidhal.A.A.Muhimen and Abd Al-Wahid Al-Shaibany. Isolation and identification of mutan's streptococci bacteria from human dental plaque samples. Journal of Al-Nahrain University. Vol 11(3), December, 2008, pp.98-105.

14. Pongsak Rattanachaikunsopon and Parichat Phumkhachorn (2010). Journal of medicinal plants research vol 4(5), 393-396.

15. T. Suman, V. Anuradha and R. Hhemala Devi(2010),Bactericidal effect of certain plant extract on dental caries causing organism – *Streptococcus mutans* , journal of pure and applied microbiology.Vol.4(1), 231-235.

16. Wayne A. Little, Lynn A. Thomson, and William H. Bowen (1979), Antibiotic susceptibility of *Streptococcus mutans*: comparison of serotype profiles, National institute of dental research. Antibacterial agents and chemotherapy. 440-443.

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