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Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of *Acacia nilotica* extract

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Oral diseases are one of the major public health issues. Due to acquisition of pathogenic resistance over conventional antimicrobials, the search for natural alternatives continues. In the present study, thirty two methanol and ethyl acetate extracts prepared from 14 different plant species were screened against oral pathogens. Principal Component Analysis indicated that methanol extract of *Acacia nilotica* twig was the most influential with highest F1 score and showed almost 2 fold higher antimicrobial activity in comparison to others. GC-MS analysis of *Acacia nilotica* twig revealed the presence of various bioactive such as limonene, stigmasterol, linoleic acid, ricinoleic acid, santalol, undecylenic acid. Evaluation of antimicrobial potential of medicinal plants may thrive a safe, inexpensive and efficient therapeutic in developing formulation for oral care products.

Keywords: Antimicrobial activity, GC-MS, Medicinal plants, Oral pathogens, Principal Component Analysis (PCA)

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Introduction

Oral health and hygiene includes every aspect of keeping the mouth clean and germ free. Tongue scraping removes the white or yellow coating of bad breath-generating pathogens[1]. Poor oral hygiene may lead to many diseases such as dental caries, tooth sensitivity, toothache, bad breath, oral sores, bleeding gums etc[2]. Oral infections may show the symptoms of many systemic diseases earlier in life including diabetes, high BP, heart disease, cancer, gastrointestinal infections and autoimmune diseases[3].

A number of factors can be responsible for poor oral health such as having too hot and cold beverages, sweet eatables, smoking, hormonal changes, genetic susceptibility, prolonged illnesses and their medications, acidity, dry mouth, overgrowth of pathogenic microbes etc. Pathogens such as *S. mutans* are the most common causal agent of dental caries[4]. *E. faecalis* was found the most commonly isolated species from the root canals of teeth[5] whereas *S. aureus* was most prevalent in gingivitis suffering patients[6]. Overgrowth of fungus *C. albicans* causes oral candidiasis or thrush especially in immuno-compromised individuals, which in turn may lead to bad breath and biofilm formation[7]. Many antimicrobial agents including chlorhexidine, fluorides, sodium lauryl sulfate etc. are used for prevention and cure of oral diseases. There are reports on their side effects such as hypersensitivity reactions, toxicity, tooth staining and desquamation of oral mucosa etc[8-9].

India ranks the second largest exporter of medicinal plants after China. Many herbs and spices are conventionally used for oral care in different regions of the country such as cloves oil for dental caries[10], turmeric for pyorrhea[11] and cardamom is used as mouth freshener. *Aloe vera* mouthwashes are used to reduce the risk of dental plaque and gingivitis[12]. A decoction triphala ingredients namely harad, baheda and amla can be effectively used to manage dental caries, gingival and periodontal diseases[13]. Even the use of neem, miswak and babul datun is a proficient way of dental care[14]. For identification of compounds gas chromatography with mass spectrometry is widely used in quality control of active pharmaceutical ingredients, bulk drugs and their formulations[15]. Therefore, the main objective of the present study was to screen the antimicrobial potential of different medicinal plants against the selected oral pathogens and the GC-MS analysis of most effective extract.

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Methodology

Collection of plant samples

Locally available plant samples were collected from Rohtak district of Haryana, India (Table 1). The plant material was identified and authenticated by comparing the herbarium specimen available in Department of Genetics, M. D. University, Rohtak (India).

Preparation of extracts

Thirty two plant extracts of methanol and ethyl acetate were prepared by cold percolation method[16]. 10 gram of dried extract was dissolved in 100 mL solvent and rotated in incubator shaker for 3 days. After that it was filtered, lyophilized and stored for further use.

Antibacterial activity

Antibacterial activity of 40 µl of sample solution was added separately to each well of agar plate i.e. (2 mg/well) from prepared plant extracts of 50 mg/mL (stock solution) was evaluated by agar well diffusion method[17]. Pathogenic strains namely; *Streptococcus mutans* (MTCC 497), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 259323) and *Candida albicans* (ATCC 10231) were used for the study. Pure Dimethyl Sulfoxide (DMSO) and 2% Chlorhexidine diacetate were used as negative and positive control respectively.

Minimum Inhibitory Concentration

The most effective plant extracts which exhibited strong antimicrobial activity at 10 mg/mL was used to determine their MIC using 96 well plate methods[18].

Statistical analysis

Experiment was done in triplicates. The data from zone of inhibition of different extracts were represented as mean ± standard error. Principal Component Analysis (PCA) was performed[19] using statistical software XLSTAT version 2018.1.

GC-MS conditions

Methanol extract of *Acacia nilotica* twig which showed the most significant activity were identified using GC-MS at AIRF centre, JNU, New Delhi. GC conditions are shown in Table 2. All data were obtained by collecting the full scan mass spectra with scan speed of 3333 within the scan range 40 to 650 m/z. Various components were identified by the software libraries viz. WILEY8, NIST11.

Results

Antimicrobial activity

In the present study, zone of inhibition of all plant extracts against oral pathogens was ranged between 10-40 mm (Fig. 1 and Table 3). DMSO (-ve control) showed negligible antimicrobial activity while 2% Chlorhexidine diacetate (+ve control) have zone of inhibition range from 17-25 mm against the tested oral pathogens. In Principal Component Analysis, F1

Table 2 — GC-MS method for extract analysis

Column Oven Temp. : 50.0 °C	Purge Flow : 3.0 ml/min
Ion Source Temp : 230.00 °C	Flow Control Mode : Linear Velocity
Injection Temp. : 260.00 °C	Linear Velocity :40.1 cm/sec
Split Ratio :10.0	Total Flow :16.4 ml/min
Pressure : 69.8 kPa	Column Flow :1.22 ml/min

Table 1 — Details of selected medicinal plants tested against oral pathogens

Scientific name	English name	Local name	Family	Part used
Acacia nilotica L., Delile	Gum Arabic	Babool/Kikar	Fabaceae	Twig, Leaf
Amomum subulatum Roxb.	Large Cardamom	Badi Elaichi	Zingiberaceae	Seed pod
Azadirachta indica A. Juss.	Indian lilac	Neem	Meliaceae	Twig, Leaf
Cinnamomum zeylanicum Nees. Pl. Asiat. Rar. (Wallich).	Cinnamon	Dalchini	Lauraceae	Bark
Curcuma longa L.	Turmeric	Haldi	Zingiberaceae	Rhizome
Elettaria cardamomum L., Maton.	Small Cardamom	Chhoti Elaichi	Zingiberaceae	Seed pod
Emblica officinalis Gaertn.	Indian Gooseberry	Amla	Euphorbiaceae	Fruit
Glycyrrhiza glabra L.	Licorice	Mulethi	Fabaceae	Rhizome
Murraya koenigi L., Spreng.	Curry leaf	Curry patta	Rutaceae	Leaf
Punica granatum L.	Pomegranate	Anar	Punicaceae	Fruit peel
Psidium guajava L.	Guava	Amrood	Myrtaceae	Leaves
Terminalia bellerica Gaertner, Roxb.	Bastard Myrobalan	Baheda	Combretaceae	Fruit
Terminalia chebula Retz.	Black Myrobalan	Harad	Combretaceae	Fruit
Syzygium aromaticum L., Merr. & L.M. Perry	Clove	Laung	Myrtaceae	Flower bud

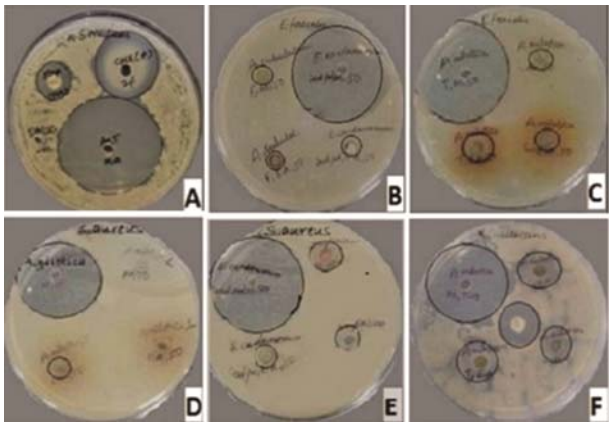

Fig. 1 — Zone of inhibition of different plant extracts against oral pathogens: (A) *A. nilotica* against *S. mutans*, (B) *E. cardamomum* & (C)) *A. nilotica* against *E. faecalis*, (D) *A. nilotica* & (E) *E. cardamomum*, *C. zeylinicum* against *S. aureus*, (F) *A. nilotica* against *C. albicans*

represents horizontal axis which is positively correlated with activity against *S. aureus* followed by *E. faecalis*, *S. mutans* and *C. albicans*. F2 represents vertical axis that is positively linked with activity against *C. albicans* only (Fig. 2). Twig of *A. nilotica* extract is most influential extract with highest F1 score. Extract of *E. cardamomum* is positively correlated with a very high activity against *E. faecalis* and *S. aureus* but negatively correlated against *C. albicans* as F2 is negative (Table 4).

Minimum Inhibitory Concentration

The MIC of the effective plant extracts viz. *A. nilotica*, *E. cardamomum*, *P. gujava and G. glabra* were employed against specific oral pathogens by 96 well plate method (Fig. 3). Lowest MIC was shown

Table 3 — Zone of inhibition of different plant extracts against oral pathogens

Plant Name	Solvent	Code	Zone of Inhibition(mm) at 2mg of plant extract/well			
			E. faecalis	*S. aureus*	*S. mutans*	*C. albicans*
A. nilotica(T)	M	S1	38.01±0.33**	40.20±0.25**	40.21±0.32**	27±0.45**
	EA	S2	16.23±1.21*	10.78±1.45	-	14.67±1.03
A. nilotica (L)	M	S3	10.56±0.76	-	11.24±0.46	11±0.35
	EA	S4	13.54±0.87	-	-	15.36±1.02
A. subulatum	M	S5	10.44±1.24	-	9.56±1.56	-
	EA	S6	-	-	-	-
A. indica (L)	M	S7	-	-	-	-
	EA	S8	9.89±0.78	-	11.38±0.56	-
A. indica (T)	M	S9	10.33±0.87	-	-	-
	EA	S10	-	-	-	-
C. zeylanicum	M	S11	13.25±1.56	11±0.67	10.56±1.45	-
	EA	S12	10.45±1.34	11.87±1.14	15.6±0.45 *	13.32±0.89
C. longa	M	S13	8.45±0.54	9.67±1.43	-	9.65±1.47
	EA	S14	9.12±0.56	11.23±1.56	-	8.78±1.45
E. officinalis	M	S15	-	11.08±0.67	-	10.44±1.34
	EA	S16	-	10.56±1.58	-	8.55±1.34
E. cardamomum	M	S17	40.32±0.11**	40.02±0.65**	-	-
	EA	S18	10.45±1.23	-	10.68±1.43	-
G. glabra	M	S19	12.45±1.32	18.32±0.45*	12.45± 0.68	14.51±1.34
	EA	S20	14.56±0.45	18.4±0.5*	14.38±0.79	13.54±1.54
M. koenigi	M	S21	9.34±1.32	13.86±0.78	16.43±0.69*	-
	EA	S22	9.65±0.56	10.45±0.67	12.45±0.59	-
P. guajava	M	S23	-	12.11±0.45	-	20.34±0.59*
	EA	S24	-	11.34±1.57	-	-
P. granatum	M	S25	12.2 ±1.65	16.34±0.56*	-	14.49±1.43
	EA	S26	9.34±0.56	12.54±1.56	-	-
S. aromaticum	M	S27	10.53±0.67	10.45±0.48	12.27±1.45	10.39±1.56
	EA	S28	9.86±0.45	-	10.67±0.69	-
T. chebula	M	S29	12.34±0.45	13.76±0.87	-	15.58±1.03*
	EA	S30	13.3±0.56	12±1.35	-	16.6 ±0.79*
T. bellerica	M	S31	8.45±0.78	13.85±1.01	11.34±1.53	15.45±1.57*
	EA	S32	10.56±0.56	12.23±1.67	9.67±0.78	11.54±1.37
Chlorhexidine diacetate		S33	24±0.23*	23±0.34*	25±0.53**	17±0.44*

Abbreviations: *S. mutans- Streptococcus mutans, E. faecalis- Enterococcus faecalis, S. aureus -Staphylococcus aureus, C. albicans- Candida albicans*, M- Methanol extract, EA- Ethyl acetate extract, T- Twig, L- leaf, - no activity, * moderate activity (15-25) mm, ** very high activity (25-40) mm

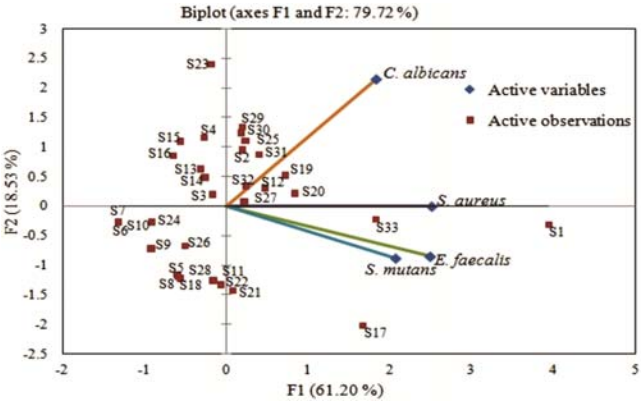

Fig. 2 — Principal Component Analysis of antimicrobial activity of selected plant extracts

Table 4 — Correlations between sample's activity and factors

Plant Samples	Code	F1	F2
A. nilotica	S1	6.157	-0.266
E. cardamomum	S17	2.614	-1.736
G. glabra	S20	1.308	0.186
P. guajava	S23	-0.297	2.064
P. granatum	S25	0.365	0.948
T. chebula	S30	0.302	1.154

by *A. nilotica* extract against *C. albicans* and *E. cardamomum* against *E. faecalis* at concentration of 0.19 mg/ml respectively.

GC-MS analysis

The results pertaining to GC-MS analysis of the methanol extract of *Acacia nilotica* twig lead to the identification of a number of compounds. A total of 46 peaks were observed revealing 25 possible phytochemicals with their retention time and area percentage (Fig. 4 and Table 5).

Discussion

Numerous studies have been done on antimicrobial potential of different parts of *A. nilotica* including its leaves[20,21], bark[22], pod[23] but very less studies are on the antimicrobial potential of its twig. Many bioactives found in the *A. nilotica* extract already have been reported in other medicinal plants and proved to exhibit antimicrobial activities such as Ricinoleic acid against *S. aureus*[24], Santalol against *C. albicans* and *S. aureus*[25], Undecylenic acid inhibits the biofilm formation in *C. albicans*[26], Limonene against E. faecalis and *S. aureus*[27], Stigmasterol against S. aureus[28] and *C. albicans*[29]. The most abundant phytochemical found in the study is a polyunsaturated fatty acid i.e. 9, 12-Octadecadienoic acid (linoleic acid)

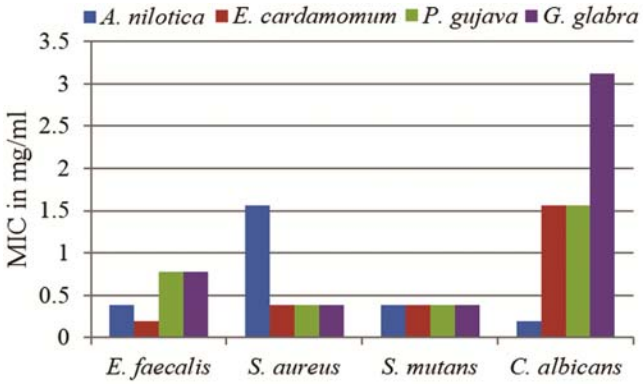

Fig. 3 — Minimum Inhibitory Concentration of effective plant extracts against oral pathogens

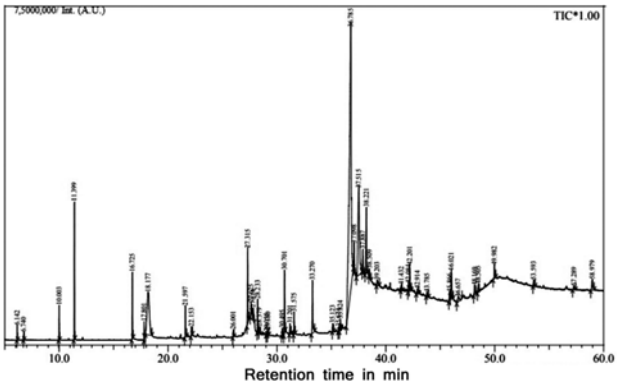

Fig. 4 — GC-MS Chromatogram for the methanol extract of *A. nilotica* twig.

with an area 42.79%. The fatty acid has evidenced to inhibit significant growth of S. mutans and *C. albicans* by penetrating and disrupting normal function of the cellular membranes[30]. Compounds such as mome inositol[31], lanceol[32] exhibits anti-proliferatory activity, neophytadiene possesses larvicidal property[33], cis-9-octadecenamide is a natural sleep inducing lipid[34], N-Hexadecanoic acid and Octadecanoic acid anti-inflammatory activity[35].

Polar extract of small cardamom also showed very high antimicrobial activity in comparison to its non polar extract but most of the pharmacological analysis reports are on the role of its essential oil or non polar metabolites against oral microbes[36]. The spice is rich in terpenoids which can disrupt microbial membrane or alteration in the proton-motive force that could affect their biofilm formation[37]. *P. guajava* exhibited high activity against *C. albicans* followed by *T. chebula* and *P. granatum*. Due to the presence of phenolic compounds, they have an influence on virulence factors of Candida[38]. *G. glabra* extract showed moderate inhibition against *S. aureus* while *C.*

Table 5 — Chemical profile of Methanol extract of Babul twig using GC-MS

Peak	R. Time	Area%	Possible Compound
1	6.142	0.34	Ethanol,2,2'-oxybis-
2	6.740	0.19	1,3,5,7-Tetroxane
3	10.003	0.99	1-Methyl-4-(1-methylethenyl)cyclohexene; limonene
4	11.399	3.69	Ethanol,2,2'-oxybis-
5	16.725	2.08	Ethanol,2,2'-oxybis-
6	17.801	0.11	Oct-2-ynoic acid
7	18.177	8.52	3-Tetradecyn-1-ol
8	21.597	1.15	Ethanol,2,2'-Oxybis-
9	22.153	0.46	Undecylenic acid
10	26.001	0.26	Ethanol,2,2'-Oxybis-
11	27.315	2.94	Alpha.-Santalol
12	27.625	0.46	Bergamotol,Z-.Alpha.-Trans-
13	27.814	0.58	Momeinositol
14	28.233	1.14	Beta.-Santalol
15	28.379	0.21	6-(P-Tolyl)-2-Methyl-2-Heptenol,Trans-
16	29.055	0.15	14-Methyl-8-Hexadecyn-1-ol
17	29.180	0.19	Cis – Lanceol
18	30.495	0.23	Neophytadiene
19	30.701	2.30	Neophytadiene
20	31.201	0.42	Neophytadiene
21	31.575	0.84	Neophytadiene
22	33.270	2.72	N-Hexadecanoic acid
23	35.123	0.32	13-Hexyloxacyclotridec-10-En-2-one
24	35.674	0.16	9,12-Octadecadienoic acid(Z,Z)-,Methylester
25	35.824	0.48	9,12-Octadecadienoic acid,Methylester
26	36.785	42.79	9,12-Octadecadienoic acid(Z,Z)-; linoleic acid
27	37.098	1.11	Octadecanoic acid
28	37.515	10.64	9,12-Octadecadienoicacid(Z,Z)-
29	37.887	1.26	9,12-Octadecadienoicacid(Z,Z)-
30	38.221	3.88	9,12Octadecadienoicacid(Z,Z)-
31	38.509	0.40	9,17-Octadecadienal,(Z)-
32	39.203	0.47	9-Octadecenoic acid
33	41.432	0.22	9,12-Octadecadienoylchloride,(Z,Z)-
34	42.084	0.28	1,E-6,Z-11-Hexadecatriene
35	42.201	1.04	9,12-Octadecadienoylchloride,(Z,Z)-
36	42.914	0.42	1,8,11-Heptadecatriene,(Z,Z)-
37	43.785	0.35	9,12-Octadecadienoylchloride,(Z,Z)-
38	45.866	0.42	Trans,Trans-9,12-Octadecadienoic acid
39	46.021	1.75	9,12-Octadecadienoic acid(Z,Z)-
40	46.657	0.66	Octadecanoicacid,2,3-Dihydroxypropylester
41	48.169	0.65	Trans-9,12 Octadecadienoic acid
42	48.505	0.37	9-Octadecenamide
43	49.982	0.69	12-Hydroxy-9-Octadecenoic acid (Ricinolic acid)
44	53.593	0.36	Stigmast-5-En-3-ol,Oleate
45	57.289	0.33	Stigmasterol
46	58.979	0.97	Stigmast-5-En-3-ol,(3.Beta.)-

zeylinicum exhibited medium activity against *S. mutans*. Extracts of licorice root, cinnamon bark, harad fruit exhibited moderate activity against oral pathogens is also supported by other investigators[39]. Extracts of neem, turmeric, amla, large cardamom showed very weak or negligible activity against all the strains. However, these plants have very good antioxidant activity[40] and may be helpful in holistic oral care.

Conclusion

Screening of plant extracts and GC-MS analysis will be beneficial in development of oral care products such as in tooth paste, endodontic irrigants, dental gel, mouthwash, mouth fresheners etc. Isolation of individual bioactive compounds and evaluation of their pharmacological potency will open a new area of investigation.

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References

1 Matsui M, Chosa N, Shimoyama Y, Minami K, Kimura S & Kishi M, Effects of tongue cleaning on bacterial flora in tongue coating and dental plaque: A crossover study, BMC Oral Health, 14 (1) (2014) 1-8.

2 Kuppuswamy VL, Murthy S, Sharma S, Surapaneni KM, Grover A & Joshi A, Oral hygiene status, knowledge, perceptions and practices among school settings in rural South India, Oral Health Dent Manag, 13 (1) (2014) 146-154.

3 Mougeot JC, Stevens CB, Paster BJ, Brennan MT, Lockhart PB & Mougeot FB, *Porphyromonas gingivalis* is the most abundant species detected in coronary and femoral arteries, J Oral Microbiol, 9 (1) (2017) 1281562.

4 Chu J, Zhan T & He K, Cariogenicity features of *Streptococcus mutans* in presence of rubusoside, BMC Oral Health, 16 (1) (2016) 54-59.

5 Kumar H, An In vitro evaluation of the antimicrobial efficacy of *Curcuma longa*, *Tachyspermum ammi*, chlorhexidine gluconate and calcium hydroxide on *Enterococcus faecalis*, J Conserv Dent, 16 (2) (2013) 144-147.

6 Abbas MH, Al-Yasseen AK & Alhamadi WW, Prevalence of *Staphylococcus aureus* among Gingivitis in Patient with Orthodontic and Non-Orthodontic Wires in Kufa City/Iraq, Pak J Biotechnol, 14 (1) (2017) 91-96.

7 Paulone S, Malavasi G, Ardizzoni A, Orsi CF, Peppoloni S, Neglia RG & Blasi E, *Candida albicans* survival, growth and biofilm formation are differently affected by mouthwashes: an in vitro study, New Microbiol, 40 (1) (2017) 45-52.

8 Jain P & Ranjan M, Role of herbs in root canal irrigation-A review, J Pharm Biol Sci, 9 (2) (2014) 6-10.

9 Nocca G, Ahmed HMA, Martorana GE, Callà C, Gambarini G, Rengo S & Spagnuolo G, Chromographic analysis and cytotoxic effects of chlorhexidine and sodium hypochlorite reaction mixtures, J Endod, 43 (9) (2017) 1545-1552.

10 Jeeva S & Femila V, Ethnobotanical investigation of Nadars in Atoor village, Kanyakumari District, Tamilnadu, India, Asian Pac J Trop Biomed, 2 (2) (2012) S593-600.

11 Velayudhan K, Dikshit N & Abdul M, Ethnobotany of turmeric (*Curcuma longa* L.), Indian J Tradit Know, 11 (4) 2012 607-614.

12 Karim B, Bhaskar DJ, Agali C, Gupta D & Gupta RK, Effect of *Aloe vera* mouthwash on periodontal health: triple blind randomized control trial, Oral Health Dent Manag, 13 (1) (2014) 14-19.

13 Shanbhag VK, Triphala in prevention of dental caries and as an antimicrobial in oral cavity-A review, Infect Disord Drug Targets, 15 (2) (2015) 89-97.

14 Sagar S, Role of natural toothbrushes in containing oral microbial flora- A review, Asian J Pharm Clin Res, 8 (4) (2015) 29-33.

15 Hussain SZ & Maqbool K, GC-MS: Principle, Technique and its application in Food Science, Int J Curr Sci, 13 (2014) 116-126.

16 Azwanida NN, A review on the extraction methods use in medicinal plants, principle, strength and limitation, Med Aromat Plants, 4 (196) (2015) 1-6.

17 Perez C, Pauli M & Bazerque P, An antibiotic assay by the agar well diffusion method, Acta Biol Med Exp, 5 (1) (1990) 113-115.

18 Sarker SD, Nahar L & Kumarasamy Y, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, Methods, 42 (2) (2007) 321-324.

19 Malik SA, Arain RA, Khatri Z, Saleemi S & Cherif C, Neural network modeling and principal component analysis of antibacterial activity of chitosan/AgCl-TiO 2 colloid treated cotton fabric, Fibers and Polymers, 16 (5) (2015)1142-1149.

20 Yadav A, Yadav M, Kumar S & Yadav JP, Bacterial effect of *Acacia nilotica*: In Vitro antibacterial and time kill kinetic studies, Int J Curr Res, 7 (11) (2015) 22289-22294.

21 Shekar BC, Nagarajappa R, Jain R, Singh R, Thakur R & Shekar S, Antimicrobial efficacy of *Acacia nilotica*, *Murraya koenigii* (L.) Sprengel, Eucalyptus hybrid, *Psidium gua*java extracts and their combination on *Streptococcus mutans* and *Lactobacillus acidophilus*, Dent Res J, 13 (2) (2016) 168-173.

22 Deshpande SN & Kadam DG, Phytochemical analysis and antibacterial activity of *Acacia nilotica* against *Streptococcus mutans*, Phytochem Anal, 5 (1) (2013) 236-238.

23 Saini ML, Saini R, Roy S & Kumar A, Comparative pharmacognostical and antimicrobial studies of Acacia species (Mimosaceae), J Med Plant Res, 2 (12) (2008) 378-386.

24 Persson C, Robert E, Carlsson E, Robo C, López A, Godoy-Gallardo M, Ginebra MP & Engqvist H, The effect of unsaturated fatty acid and triglyceride oil addition on the mechanical and antibacterial properties of acrylic bone cements, J Biomater Appl, 30 (3) (2015) 279-289.

25 Bommareddy A, Brozena S, Steigerwalt J, Landis T, Hughes S, Mabry E, Knopp A, VanWert AL & Dwivedi C, Medicinal properties of alpha-santalol, a naturally occurring constituent of sandalwood oil, Nat Prod Res, 2017 (2017) 1-17.

26 Shi D, Zhao Y, Yan H, Fu H, Shen Y, Lu G, Mei H, Qiu Y, Li D & Liu W. Antifungal effects of undecylenic acid on the biofilm formation of *Candida albicans*, Int J Clin Pharmacol Ther, 54 (5) (2016) 343-353.

27 Van Vuuren SF & Viljoen AM, Antimicrobial activity of limonene enantiomers and 1, 8 cineole alone and in combination, Flavour Fragr J, 22 (6) (2007) 540-544.

28 Edilu A, Adane L & Woyessa D, In vitro antibacterial activities of compounds isolated from roots of *Caylusea abyssinica*, Ann Clin Microbiol Antimicrob, 14 (2015) 1-8.

29 Moshi M, Joseph C, Innocent E & Nkunya M, In vitro antibacterial and antifungal activities of extracts and compounds from *Uvaria scheffleri*, Pharm Biol, 42(4-5) (2004) 269-273.

30 Huang CB, George B & Ebersole JL, Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms, Arch Oral Biol, 55 (8) (2010) 555-560.

31 Neda GD, Rabeta MS & Ong MT, Chemical composition and anti-proliferative properties of flowers of *Clitoria ternatea*, Int. Food Res J, 20 (3) (2013) 1229-1234.

32 Chang CC, Hsu HF, Huang KH, Wu JM, Kuo SM, Ling XH & Houng JY, Anti-proliferative effects of *Siegesbeckia orientalis* ethanol extract on human endometrial RL-95 cancer cells, Molecules, 19 (12) (2014) 19980-19994.

33 Aboaba SA, Aiyelaagbe OO & Ekundayo O, Chemical composition, toxicity and larvicidal activity of the essential oil from the whole plant of *Acalypha segetalis* from south-west Nigeria, Nat Prod Commun, 5 (3) (2010) 481-483.

34 Cravatt BF, Lerner RA & Boger DL, Structure determination of an endogenous sleep-inducing lipid, cis-9-octadecenamide (oleamide): A synthetic approach to the chemical analysis of trace quantities of a natural product, J Am Chem Soc, 118 (3) (1996) 580-590.

35 Othman AR, Abdullah N, Ahmad S, Ismail IS & Zakaria MP, Elucidation of in-vitro anti-inflammatory bioactive compounds isolated from *Jatropha curcas* L. plant root, BMC Complement Altern Med, 15 (2015) 1-10.

36 Thosar N, Basak S, Bahadure RN & Rajurkar M, Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study, Eur J Dent, 7 (1) (2013) S71-77.

37 Nogueira JWA, Costa RA, da Cunha MT & Cavalcante TTA, Antibiofilm activity of natural substances derived from plants, Afr J Microbiol Res, 11(26) (2017) 1051-1060.

38 Morais-Braga MF, Carneiro JN, Machado AJ, Sales DL, Dos Santos AT, Boligon AA, Athayde ML, Menezes IR, Souza DS, Costa JG & Coutinho HD, Phenolic composition and medicinal usage of *Psidium guajava* Linn.: Antifungal activity or inhibition of virulence?, Saudi J Biol Sci, 24 (2) (2017) 302-313.

39 Kumar D & Sidhu P, The antimicrobial activity of *Azardirachta indica*, *Glycyrrhiza glabra*, *Cinnamum zeylanicum*, *Syzygium aromaticum*, *Acacia nilotica* on *Streptococcus mutans* and *Enterococcus faecalis*—An in vitro study, Endodontology, 23 (2011) 18-25.

40 Devasagayam TPA & Sainis KB, Immune system and antioxidants, especially those derived from Indian medicinal plants, Indian J Exp Biol, 40 (6) (2002) 639-655.