INTERNATIONAL JOURNAL OF MEDICAL AND APPLIED SCIENCES
Earthjournals Publisher
E-ISSN:2320-3137
www.earthjournals.org

RESEARCH ARTICLE

IN-VITROANTIBACTERIAL EFFICACY OF HOUSEHOLD HERBS AS ADJUNCTS AGAINST ORAL – BACTERIA

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Publication history: Received on 23/3/2016 Published online 17/04/2016

ABSTRACT:
Aqueous extract from the leaves of well known medicinal herbs viz ocimum sanctum (basil), Mentha spicata (mint),Murraya koenigii(curry leaves) and Mangifera indica(mango leaves) were evaluated for sensitivity against heterogenous oral bacteria. Extract of each herb was prepared by standard method and their antibacterial activity was studied by disc-diffusion method in which aliquots of oral bacterial suspension was inoculated on nutrient agar plates.6mm paper discs impregnated with herbal extract were placed concentrically.Clear zones of bacterial inhibition were measured after 48 h and their means calculated. Chlorhexidine (0.2%) was used as positive control.Minimum inhibitory concentration(MIC) was obtained for each herb conducting turbidity test using spectrophotometer.Results were analysed statistically using ANOVA and post-hoc HSD test for multiple comparisons..O.sanctum revealed maximum bacterial inhibition followed by mint.The lowest MIC was also exhibited by basil leaves. Based on the findings of present study,chewing of fresh leaves as adjuncts in oral health care has been discussed.

Key words: Medicinal herbs, water extract, oral bacteria, zones of inhibition, antibacterial activity.

INTRODUCTION

The oral cavity is a very favourable and an ideal place for heterogenous microflora where in a large number of varied bacterial, fungi and protozoa etc. grow continuously as it provides an excellent medium for their growth. In addition continuous and abundant supply of nutrients thru ingested food, moisture, temperature, and suitable niche for aerobic and anaerobic and facultative micro-organisms, help them grow continuously and rapidly. Moreover, the saliva, crevicular fluids, palate surfaces of buccal cavity, gums and hard surfaces of teeth provide diverse environment favouring the accomplishment of about750 types of microbes[1] which vary qualitatively and quantiatively.moreover intra tooth and gingiva-tooth spaces provide permanent niche for specific and indigenous gram negative bacteria.

Many of the oral bacteria belonging to genera streptococci, Lactobacilli and actinomycetes cause dental caries as they are acidogenic and acidoduric in nature. These bacteria produce extra-cellular polysaccharides like glucans, dextrans and fructans[2] resulting from carbohydrate breakdown of food particles engulfed in tooth spaces. These by-products

enable the bacteria to adhere to pellicle on the tooth surface. This intracellular matrix forms a hydrated gel in which bacteria can survive and proliferate. This bio-film attaches firmly to the tooth surface and is resistant to mechanical removal as well as antibiotic-action.

The transition from bio-film to dental plaque is extremely rapid. These bacteria invade the tooth surface in succession. First of all streptococci attach along the surface and form monolyer within few hrs followed by prevotellasp, canocytophaga, porphyromonas, and gingivalis. The ability of these bacteria to adhere to different species and genera of micro-organisms called co-aggregation results in building up of multilayer pellicle finally forming plaque.

The acid production by these bacteria dissolves calcium phosphate causing decalcification leading to tooth decay followed by periodontitis. Numerous epidemiological studies have also shown that dental diseases continue to be the major health problem throughout the world. Oral health is integral to the general well-being and relates to quality of life beyond the functions of craniofacial complex [3]. Therefore control and prevention of these pathogens is of utmost necessity which can be achieved by good oral hygiene practices and regular visits to the dentist. The dental services are either scarce, costly or unavailable in the remote and rural areas of the developing countries. Moreover antibacterial agents used in the prevention and treatment of oral diseases such as chlorhexidine, cetylpyridinium, amine fluorides etc. are toxic with undesirable side-effects such as vomiting diarrhoea and tooth staining is [4,5] and the role of alcohol in oral-carcinogenesis with particular reference to alcohol-containing mouthwashes has been reported[6,7]

Hence global need of search for alternate, safe, effective and economical prevention and treatment options for oral pathogens continues. The natural phytochemicals isolated from medicinal plants which are abundant source of biologically active compounds[8] is now one of the major interest - areas of research.

The present study was therefore conducted to study the antibacterial efficacy of well known medicinal plants to prevent and inhibit the growth of fast multiplying oral bacteria which primarily cause halitosis or malodor with particular focus on the use of fresh herbal-leaves in dental hygiene.

MATERIALS AND METHODS

Procurement of herbal leaves : Fresh leaves of following herbs were collected from our kitchen-garden and got approved from a certified botanist (Professor in a Agricultural University) and an agriculturist.

	Common Names	**Botanical Name**
1.	Basil leaves	*Ocimum sanctum*
2.	Mint leaves	*Mentha spicata*
3.	Curry leaves	*Murraya koeniggi*
4.	Mongo leaves	*Mangifera indica*

Preparation of herbal extract

The fresh leaves of each herb were plucked from mid of the plant shoots , washed and dried in shade at about 35±2°C. Dried leaves were crushed and powdered in sterilized Pestle-Morter. The aqueous extract of each herb was prepared by standard method[9] Where in 10 g powdered herb was infused in 4ml of sterilized water and boiled for 4 h. Sterilized water was added at the end to make the volume 4 ml. The mixture was cooled and filtered. This aqueous extract produced 2.5 g/ml of the herbal extract.

Sample collection of oral bacteria

Person aged between 18-25 yrs was randomly selected with following parameters:

- was non-smoker who never chewed tobacco.
- had full complement of teeth
- did not have medical disorders or undergone any recent antibiotic or any other medical treatment
- was checked for not having cavitated caries lesions, naso-pharyngeal alterations mouth breathers, orthodontic or dental appliances.
- The absence of plaque was confirmed by careful examination of teeth.
- Scaling was done and also examined for peridontal diseases or any other oral-disorders.

Sterilized cotton swabs were wiped on all surfaces of the teeth and mixed in 100 ml sterilized water in 250 ml Erlenmeyer flask andshaken This inoculm was stored in refrigerator at 4±2°C and its bacterial count (cfu/ml) was obtained by standard plate count.

Screening for antibacterial activity

Antibacterial assay of herbal extracts was conducted against oral bacteria by disc-diffusion method using standard protocol[10] sterilized petri plates was poured with about 20 ml of nutrient agar medium and inoculated with 20 µl or 0.02 ml of oral bacterial suspension. Plates were allowed to solidify .6 mm sterile filter paper discs (Whattman #1) impregnated with 0.02 ml of different conc. of herbal extract placed concentrically in the petri plate with the help of sterilized forceps. Discs impregnated with 0.2% chlorhexidine was used as positive control. Inoculated plates were kept in refrigerator for about 2 h (pre-diffusion time) fallowed by incubation at 37°C for 48 h. Tetrazolium red was used as an indicator in the medium to help visualize the zones of inhibition which appeared white to yellow against red background. The diameter of inhibition zones were measured at three equidistant points and the mean calculated.

Minimum inhibitory concentration of herbal extracts

Simple turbidity test was conducted using spectrophotometer to determine the MICof the herbs under study. The test tubes containing 9.8 ml of nutrient broth were sterilized and inoculated with 0.1 ml of oral bacterial suspension followed by adding of 0.1 ml of each herbal extract concentration. All the herbal extracts were diluted two - fold from 156 mg/ml to 1.15 mg/ml. For each dilution test tubes were inoculated in triplicates and observed visually for turbidity and color change. The highest dilution which prevented the visible

turbidity/growth of inoculum used under defined in-vitro conditions within a definite period of time was recorded as MIC of the herbal extract.

Statistical Analysis

The data are represented as mean±SD. Statistical significance of difference between the herbs and concentration was analyzed using SPSS version 17. ANOVA followed by post-hoc honest significant difference (HSD) were used for analysis. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Bacterial Count of Oral-Cavity

The oral bacteria sampled from mouth cavity by sterilized cotton swabs and plated on nutrient agar resulted in growth of bacterial colonies. Visual count revealed that oral cavity inhabited 28 x 10^4 CFU/ml

Antibacterial Activity

Four house-hold herbs were tested for their efficacies against heterogenous oral bacteria which could cause dental infections leading to caries and periodontal diseases. The anti-bacterial effect of these herbs viz. *Ocimum sanctum, Menthaspicala, Murrayakoengii* and *Mangiferaindica* determined by disc diffusion method was analysed statistically applying ANOVA (table 1) The aqueous extract of all the herbs was found to be effective against bacteria but exhibited significantly different activity at $P < 0.05$ level.

Table 1. ANOVA of bacterial inhibition by herbs

Source	Type III sum of squares	df	Mean squar e	F	Sig.
Corrected Model	1364.628*	23	59.332	330.856	0.000
Intercept	11457.448	1	11457.448	63891.090	0.000
Concentration	201.696	5	40.339	224.946	0.000
Herbs	1142.782	3	380.927	2124.196	0.000
Concentration * Herbs	20.151	15	1.343	7.491	0.000
Error	8.608	48	0.179		
Total	12830.684	72			
Corrected Total	1373.236	71			

a. R Squared = 0.994 (Adjusted R Squared = 0.991)

Anti-bacterial activity which is directly proportional to the inhibition zone was measured and statistica mean values as mentioned in table 2B reveal that, *O. sanctum* exhibited maximum activity of 17.889 mm followed by significantly different 15.033 mm recorded with mint

(P<0.05) *Murrayakoengii*and *Mimangifera*were also found to be effective as inhibitary zones of 9.10 mm and 8.42mm respectively were measured against indigenous bacteria sampled from oral cavity especially these inhabiting the tooth surfaces but their activity was about 50% less than *O. sanctum* which is also statistically different (P < 0.05).

Table 2-A: Post-hoc HSD test for multiple comparisons
Multiple ComparisonsDependent Variable: Inhibition Tukey HSD

(I) Herbs	(J) Herbs	Mean difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
	2	2.856*	.1412	.000	2.480	3.231
1	3	9.780*	.1412	.000	2.404	9.156
	4	9.461*	.1412	.000	9.085	9.837
	1	-2.856*	.1412	.000	-3.231	-2.480
2	3	5.924*	.1412	.000	5.549	6.300
	4	6.606*	.1412	.000	6.230	6.981
	1	-8.780*	.1412	.000	-9.156	-8.404
3	2	-5.924*	.1412	.000	-6.300	-5.549
	4	.681*	.1412	.000	.305	1.057
	1	-9.461*	.1412	.000	-9.837	-9.085
4	2	-6.606*	.1412	.000	-6.981	-6.230
	3	-.681*	.1412	.000	-1.057	-0.305

Based on observed means.
The error term is Mean Square (Error) = .179
*The mean difference is significant at the 0.05 level.
(I) & (J) designations according to post-hoc analysis by SPSS
Herbs 1.*O. sanctum* 2.*M. spicata*
3. *M. koenigii* 4. *M. indica*

Table 2-B: Mean values

Herbs	Inhibition
1	17.889^{a}
2	15.033^{b}
3	9.109^{c}
4	8.428^{d}
C	20.108

Values followed with different superscripts are significantly different (p < 0.05)
Hence, Herb 1 shows maximum inhibition as compared to other three herbs used.

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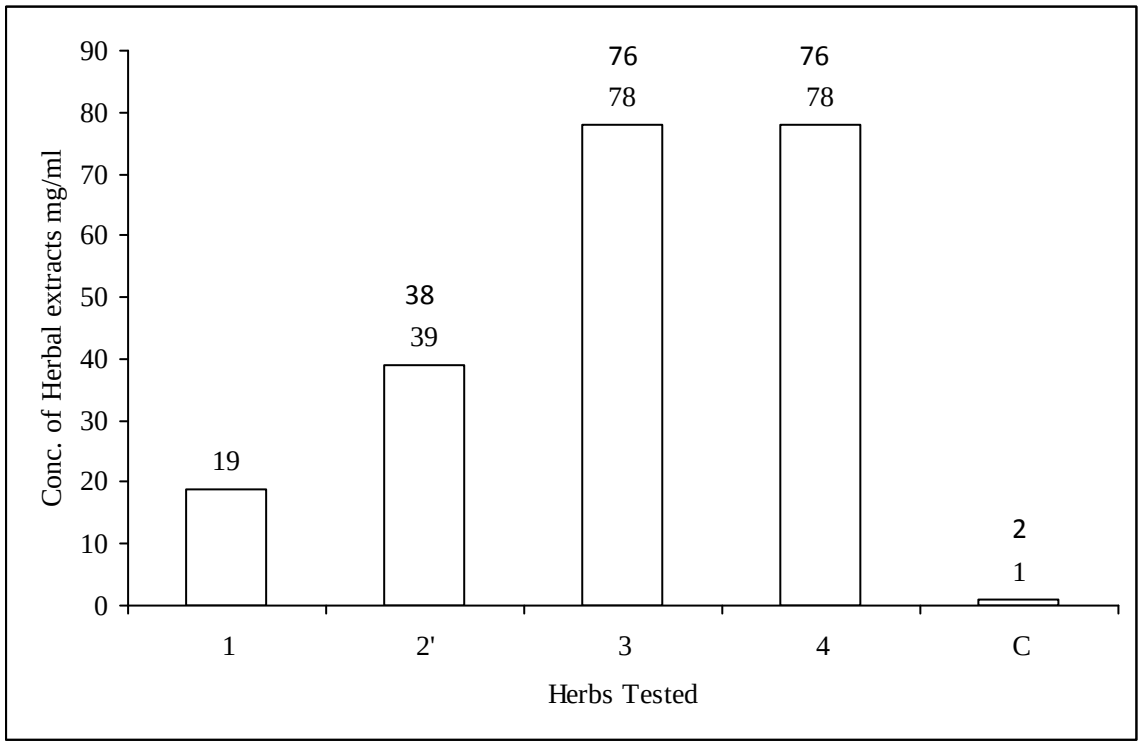

Herbs 1.*Ocimum sanctum* 2.*Menthaspicata*
3. *Murrayakoengii* 4. *Mangiferaindica*
C cholrhexidine(0.2% w/v)

Antimicrobial activity for all the herbs increased with increase in the conc. and highest inhibition recorded at 300 mg/ml. However the minimum inhibitory concentration (MIC) varied for all the tested herbs (Fig. 1). *O. sanctum* was found to inhibit bacterial growth at 19 mg/ml(fig 2) while double strength of 38 mg was recorded with *M. spicata.* For *Mukoengii* and*M. indica*same levels of 76 mg/ml were observed as MIC.

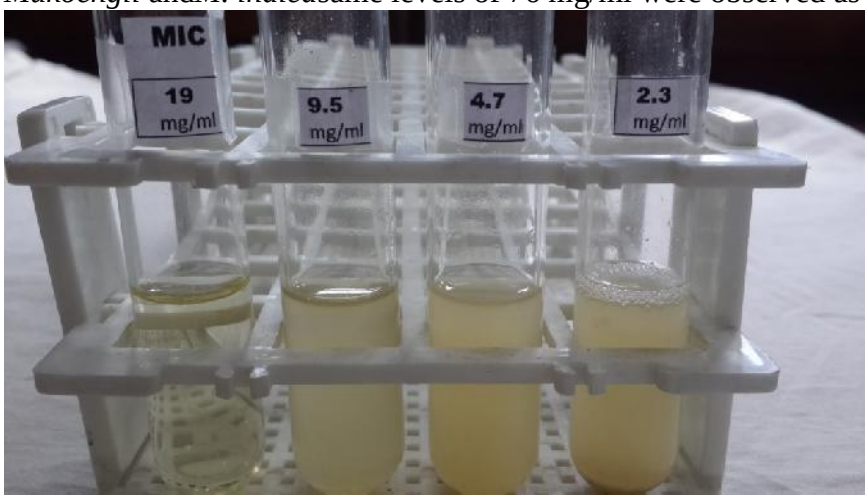

Fig.2. Minimum inhibitory concentration of ocimum sanctum(basil leaves) against oral bacteria

The bacterial inhibition achieved with *O. sanctum*<u>*somewhat*</u> corresponded to inhibition activity of chlorhexidinewhich was compared as positive control.

DISCUSSION

Among the four medicinal herbs tested for anti bacterial activity against oral bacteria, *Ocimum sanctum* showed strongest inhibition which may be due to the reported complex assortment of phytonutrients ,essential oils and antibacterial agents[11] such as Eugenol (1-hydroxy-2methoxy-4-allylbenzene) Ursolic acid (tetradecahydro-1H-picene-4-a-carboxylic acid) and carvacrol (5-isopropyle-z-methylphenol), Linolool(3,7-dimethylocta-1,6-dren-3-ol), Limatrol, caryophyllene (4,11,11-allyl-4-methoxybenzene). In addition, saponins, flavonoids, triterpenoids and tannins especially present in the leaves form complexes with soluble proteins in saliva which inhibit bacterial adherence to the tooth surface and prevent the growth of cariogenic bacteria[12,13].

This biennial shrub of family Lamiaceae is worshipped as religious plant and grown in almost every home and temple of India for its multivarious uses since anticient times. This adaptogenic herb is among few plants which purifies atmosphere, imparts freshnesss in mouth which lasts longer. In addition to its antibacterial, anti-inflammatory [12,13] and anti-caries properties [12,14]*O. sanctum* is also reported to cure mouth ulcers and oral cancer caused by tobacco chewing[15]Its astringent property[14] bonds the teeth more strongly in the gums. Its use not only improves/cures dental and oral health but also improves overall health of individuals due to its well known medicinal uses..

Mint also called mentha (*Menthaspicata*) is a genus of family Limiaceae (Mint family). It has well known medicinal, cosmetic and industrial applications. It is extensively used as antiseptic freshner in mouth rinses, tooth pastes, chewing gums, desserts and candies etc. Being an antibacterial agent, it improves oral health, helps curing halitosis[16].Its recorded antimicrobial activity of 15.033 mm in this study may be due to presence of monoterpenes (65.8%) sesquiterpenes (12.4%). Monoterpenes hydrocarbons (9.7%), oxygenated sesquiterpenes (2.8%), and oxygenated diterpentenes (1.2%). However characteristic flavour and aroma of mints is due to menthol and pulegone[17]

Curry leaves (Murrayakoenigii) most popular for its distinct aroma in south-east Asia,is a green leafy shrub.Its sensitivity to oral bacteria obtained in the present study may be due to water soluble volatile essential oils(2.6%) Its anticariogenic and antibacterial properties have also been reported [18,19]due to its mahanimbine,murrayanol and mahaninecontents.The antibacterial property of mango leaves demonstrated in this study may be due to compound mangeferin which has been reported[20,21] to be highly effective against cariogenic bacteria. Its twigs and leaves are being used for oral hygiene especially in remote and rural areas of the developing countries.

The use of water extract of the herbs studied in present investigation is based on the recent research reports[(19,21,22,23,24)]from different countries for their regional medicinal plants where significantly higher antibacterial activity was achieved with aqueous extracts as compared to the solvent extracts.These studies indicate thatantibacterial component in the such plants may be water soluble.

The water soluble property of antibacterial component in these herbs envisages their use in fresh form .The preliminary study related to this aspect i.e " effect of chewing fresh herbal leaves on oral bacteria & halitosis"-an unpublished work conducted on five participants

revealed encouraging response. However, more elaborated in-vitro study with higher number of participants has been tgiouo obtain detailed intensive scientific data.

CONCLUSION

The findings of present investigation reveal that the common herbs easily available in our kitchen gardens/ as pot herbs possess anti-bacterial substance. Its isolation, qualitative & quantitative analysis is further needed to specify their usage as disease curing agents to counteract the increasing multi-drug resistance of the allopathic antibiotics.

REFERENCES

1. Jenkinson H F, Lamont R J. Oral microbial communities in sickness and in health. Trends in Microbiology, 2005; 13(12): 589-595.
2. Arora D R, Arora B B. Text book of microbiology for dental students. 3rded, CBS Publishers New Delhi 2011: 361-5.
3. Petersen P E. The burden of disease: challenges to improving oral health in 21st century. Bulletin of WHO 2005; 83(1) p. 3.
4. Park K M et al. Kuwanon G: An antibacterial agent from the root bark of *Morusalba*against oral pathogens. J of Ethnopharmacology. 2003; 84(2-3): 181-185 (PubMed).
5. Chang J Y et al. Anticariogenic activity of macelignan isolated from *Myristicafragrans*(nutmeg) against *Streptococcus mutans.* Phytomedicine, 2006; 13(4): 216-266 (PubMed).
6. Lachenmeier D W. Safety evaluation of topical applications of ethanol on skin and inside the oral cavity. *Journal of Occupational Medicine and Toxicology*. 2008; 3(1, article 26) (PubMed).
7. McCullough M J, Farah C S. The role of alcohol in oral carcinogenesis with particular reference to alcohol-containing mouth washes. Australian Dental Journal. 2008; 53(4): 302-306.
8. Kim H S. Do not put two much value on conventional medicines. J of Ethnopharmacology, 2005; 100(1-2) 37-39.
9. Hu J P, Takahashi N , Yamada T. Capitidisrhizoma inhibits growth and proteases of oral bacteria, oral disease, Vol. 6, No. 5, September 2000, pp. 297.
10. Mothana R A, Lindequist U. Antimicrobial activity of some medicinal plants of island Soqotra. J Ethnopharmacol, 2005; 96: 177-181.
11. Pattanayak P et al. *Ocimumsanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev. 2010; 4: 95-105.
12. Singh S. Comparative evaluation of antiinflamatory potential of fixed oil of different species of Ocimum and its possible mechanism of action. Indian J Exp Biol. 1998; 36: 1028-31.
13. Prakash P, Gupta N. Therapeutic uses of *O. sanctum* Linn with a note on eugenol and its pharmacological actions: A short review. India J PhysiolPharmacol, 2005; 49; 125-31.
14. Devanand Gupta et al. A randomized controlled clinical trial of *O. sanctum* andchlorhexidine mouth wash on dental plaque and gingival inflammation.
15. Benefits of Tulsi (Holy Basil) Ayurveda-for=you.com/ayurveda-herb/benefits of tulsihtml. (Last accessed on 2015, Dec. 5).
16. Health benefits of mint available from : http:/www.organicfacts.net/health-benefits of mint.html. Last accessed on Dec. 11, 2015.
17. Znini M, et al. Chemical composition and inhibitory effect of *Menthaspicata*essential oil on the corrosion of steel in molar hydrochloric acid. Int J ElectrochemSci 2011; 6: 691-704.
18. Math M V, Balasubramaniam P. Curry leaves. Br Dent J. 2004; 197: 519.
19. Parul S, et al. Curry leaves - A medicinal herb. Asian J Pharm Res, 2012; 2: 51-3.
20. Prashant G M et al. The effect of mango and neem extract on organisms causing dental caries: *S. mutans, S. salivarus, S. mitis,* and *S. sangins.*An In-vitro Study. Indian J Dent Res. 2007; 18: 148-51.
21. Bairy I et al. Evaluation of anti-bacterial activity of *Mangiferaindica* on anaerobic dental microflora based on *in-vivo* studies. Indian J Pathal Microbial. 2002; 45: 307-10.
22. Michelle K Z Yuen et al. Antimicrobial activity of traditional chinese medicines on common oral bacteria. Chinese Medicine, 2011; 2: 37-42.
23. Bukola T. Aluko and Omotade I Oloyede. In vitro antibacterial activity of extracts from leaves of *Ocimumomericanum*against some pathogenic micro-organisms. World J of Pharmacy and Pharmaceutical Sciences, 2016; 5, 3:1-7.

24. Bayati F A and sulaiman KD. In vitro antibacterial activity of S. Parsical extracts against some isolated oral pathogens in Iraq. Turk J Biol 2008, 32:57-62

BIOGRAPHY

Dr Arpit Singh : Dr Arpit Singh received his primary school education in punjab, north India and completed his secondary school studies(high school) from California, USA. He obtained his bachelor degree in dental Surgery from Baba Farid University of Health Sciences Faridkot, Punjab.He studied core dental health problems currently prevailing in punjab especially the remote rural areas where dental services are either scarce or unavailable.So he worked to evolve low cost & easily available adjuncts to improve general oral health and control most of the dental diseases in such under developed areas of the society.

Dr. Seerat S Sekhon: Dr. Seerat took his school education from punjab.He obtained his bachelor of dental surgery degree from Baba Farid University of health Sciences ,Faridkot, Punjab.His research paper was accepted for presentation in 5th American dental conference, at Philadelpia USA in 2015 .He is member of World Health Organisation's Generation Saviour for implementing anti-Tobacco programmes in which he organises camps to create awareness about the ill effects of tobacco in the economically weaker sections of the society especially the slum areas.He is focussed to conduct research to explore low cost organic alternatives to conventional chemical based mouth washes which have many side-effects.

Paper cited as: Arpit Singh, Seerat Singh Sekhon. IN-VITROANTIBACTERIAL EFFICACY OF HOUSEHOLD HERBS AS ADJUNCTS AGAINST ORAL – BACTERIA. International Journal of medical and applied Sciences, 5(1), 2016, pp.7-15