Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2019;9:3s:50-57.

Identification of an Herbal Ingredient that could be Incorporated In Oral Topical Cleansing Aids and Performs the Action of Caries Prevention: A Clinical Study

Vaibhav Patni[1*] Aniket Gupta[2] Siddharth Swarup[3] Vikram Belkhode[4] Sharayu Nimonkar[5] Swapnil Kurhade[5]

[1]Private practitioner, Patni Dental Clinic, Wardha, India.
[2]Assistant Professor, Department of Prosthodontics & Crown & Bridge, Dr. D Y Patil, Vidyapeeth, Pune, India.
[3]Associate Professor, Department of Prosthodontics & Crown & Bridge, Dr. D Y Patil, Vidyapeeth, Pune, India.
[4]Assistant Professor, Department of Prosthodontics & Crown & Bridge, Sharad Pawar Dental College, Wardha, India.
[5]Assistant Professor, Department of Prosthodontics & Crown & Bridge, Sharad Pawar Dental College, Wardha, India.
[6]Assistant Professor, Department of Prosthodontics & Crown & Bridge, Dr. D Y Patil, Vidyapeeth, Pune, India.

ABSTRACT

Background: Dental caries and periodontal disease are complex multifactorial diseases with dental plaque as their primary cause. Gram-positive bacteria such as *Streptococcus mutans, Streptococcus sobrinus*, *Lactobacillus* species and some nonmutans streptococci are closely associated with caries formation. The aim of this study was to identify a herbal ingredient that could be incorporated in oral topical cleansing aids and perform the action of caries prevention.

Materials & methods: The present study consisted of two phases; Phase 1: In vitro experiment carried out to calculate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic and nutmeg extract, Phase 2: In vitro experiment carried out to test the antimicrobial property of prepared garlic and nutmeg mouth wash against test microorganism. The antimicrobial activity of garlic and nutmeg extract were determined against oral pathogens by cup plate diffusion method. The data generated during the study was processed using various statistical tests.

Results: The low MIC and MBC value indicate that extract has strong antibacterial activity. The results revealed that hydroethanolic extract of garlic showed MIC of 4mg/ml and MBC of 8mg/ml against *Streptococcus mutans* and MIC of 0.125 and MBC of 0.25mg/ml against *Candida albicans*. For *Streptococcus mutans*, statistically significant difference was found among garlic and nutmeg group, garlic and chlorhexidine group, garlic and negative control group, nutmeg and negative control group, chlorhexidine and negative control group, and no statistically significant difference was found between nutmeg and chlorhexidine group.

Conclusion: Garlic mouthwash has shown better antimicrobial properties as compared to nutmeg and chlorhexidine mouthwash and can be considered as safe and effective alternatives to chlorhexidine for plaque control and thereby prevention of dental caries.

Keywords: Antimicrobial, Garlic mouthwash, Streptococcus mutans.

INTRODUCTION

Dental caries and periodontal disease are complex multifactorial diseases with dental plaque as their primary cause[1]. Gram-positive bacteria such as *Streptococcus mutans, Streptococcus sobrinus*, *Lactobacillus* species and some nonmutans streptococci are closely associated with caries

Received: Mar. 9, 2019**: Accepted**: May. 7, 2019
*Correspondence **Dr. Vaibhav Patni.**
Patni Dental Clinic, Wardha, India.
Email: Not Disclosed

pISSN 2278-0076
eISSN 2321-9270

formation[2,3].Although *Streptococcus mutans* and *Lactobacillus acidophilus* are considered the main etiological agents of human dental caries, fungal oral microflora has been found to be involved by numerous studies of dental decay. A strong correlation between high prevalence of yeast *Candida albican* species in dental plaque and saliva and the development of active carious lesions have been demonstrated in numerous studies[4].

Practicing good oral hygiene with the use of oral care products such as toothpaste, toothbrush, mouthwash, and oral paste that contain antimicrobial and anticariogenic properties can aid in the prevention of caries and periodontal diseases.Nowadays, there are high interests in oral care products that are incorporated with medicinal plant extracts and are used extensively by the consumers due to low toxicity compared to oral care products containing antimicrobial agents such as triclosan, cetylpyridinium chloride, chlorhexidine, and amine fluorides that are reported to exhibit toxicity and cause staining of the teeth[4]. It is well established that many metabolites produced by plant extracts such as tannins, terpenoids, alkaloids, and flavonoids provide new source of antimicrobial substances that help in combating new developing drug resistant pathogens[5,6].

Garlic (*Allium sativum*) is one of the most extensively researched medicinal plants and its typical odor and anti bacterial activity depends on allicin produced by enzymatic activity of allinase (a cysteine sulfoxidelyase) on alliin after crushing or cutting garlic clove. Allicin and other thiosulfinates have reported to inhibit growth of various gram-positive and gram-negative bacteria in the oral cavity[7].

Myristica fragrans Houtt (Family: Myristicaceae) is composed of the skin, flesh, seed, and mace. Nutmeg is the seed kernel inside the fruit and mace is the fleshy red, net-like skin covering (aril) on the kernel[8]. In traditional medicine, the seed kernel (nutmeg) is widely used as carminative, astringent, hypolipidaemic, antithrombotic, antiplatelet aggregation, antifungal, aphrodisiac[9], treating flatulence, nausea, and dyspepsia[10].Mace is widely used as a flavouring agent, a hair dye, and a folk medicine. It also possesses antipapillomagenic, anticarcinogenic[11], and anti-inflammatory activities[12]. Macelignan, an active compound from seed has strong anticariogenic activity and possesses antibacterial effect against oral microorganisms such as Streptococcus and Lactobacillus species[13].

The aim of this study was to identify a herbal ingredient that could be incorporated in oral topical cleansing aids and perform the action of caries prevention.

MATERIAL AND METHODOLOGY

The present in vitro study was carried out at Department of Paedodontics and Preventive Dentistry, Sharad Pawar Dental College, Sawangi (Meghe), Wardha; Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, and Department of Bio-processing and Herbal laboratory, Mahatma Gandhi Institute of Rural Industralization (MGIRI), Wardha. Ethical approval for the study was obtained from the Institutional Ethics Committee.

The present study consisted of two phases

Phase 1: In vitro experiment carried out to calculate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of garlic and nutmeg extract

Phase 2: In vitro experiment carried out to test the antimicrobial property of prepared garlic and nutmeg mouth wash against test microorganism.

Phase 1:

Extract Preparation

A total of 1kg of garlic bulb and nutmeg seeds were obtained from local market were identified,authenticated,tray dried at 45-50 ^{0}Cfor 8 days and grounded in a kitchen blender to make fine powder. Two solvents (hydroethanolic and ethanol) were used for extraction procedure. Fifty grams of fine powder of the seed wasmixed in 250 ml of 99% ethanol and 250 ml of double distill water to obtain hydroethanolic extract and 500 ml of 99% ethanol to obtain ethanolic extract. The obtained solutions were kept for 48 hours at room temperature (25^{0}C ± 2^{0}C). After 48 hours, solvent extracted maximum amount of medicinalconstituents in it. Whole mixture was

subjected to filter twice by using Whatmann filter paper no.1 and the solvents were evaporated using rotary distillation apparatus.Moisture analyzer Sartorius MA-150 was used to calculate the amount of moisture present in the test material and the final concentration of the material was calculated.

Inoculum preparation:

Streptococcus mutans

Indicator strains of *Streptococcus mutans* (MTCC 890) was obtained in lyophylized from. They were grown in Brain heart infusion broth for 48 hours at 37^0C according to physiological characteristics of the microorganism and then subcultured on Blood agar for further processing. The resultant bacteria were again placed in 5 ml of BHI (Brain Heart Infusion) broth for 24 hrs at 37^0C to form a suspension (inoculum), corresponding to 10^6 CFU/mL using Mc Farland scale.

Candida albicans

Candida albicans was inoculated in 5ml nutrient broth and incubated for 24 hours at 37^0C to form a suspension (inoculum), corresponding to 10^6 CFU/mL using Mc Farland scale.

Determination of MIC and MBC against *Streptococcus mutans*

Eight mg of hydroethanolic extract of garlic and nutmeg and ethanolic extract of nutmeg were dissolved in BHI broth respectively and serially diluted to achieve the concentration from 8mg/ml to 0.125mg/ml by broth double dilution method. These tubes were incubated at 37^0C for 24 hours. MIC was taken as the lowest concentration of test material that inhibited bacterial growth onto Brain heart infusion agar plates. Inoculated plates were kept in candle jar and following overnight incubation, the plates were examined for colony growth. Lack of growth indicated that the particular test material concentration is bactericidal.

Determination of MIC and MFC against *Candida albicans*

In order to obtain a standard inoculum, a single colony of test organism (*Candida albicans*) was passed in nutrient broth and was incubated at 37^0C for 4-6 hours. MIC was determined using broth dilution method as described above and to determine MFC, tubes were subcultured on sabaurad's dextrose agar plate.

Phase 2:

Preparation of Mouthwash

To prepare garlic mouthwash, 4 mg of the garlic extract i.e approximately 15ml was dissolved in 75 ml distilled water and gently stirred with a stirrer till it was completely dissolved. To this 10 ml of sorbitol was added which acted as a sweetener, and 10 mg of peppermint was added in order to mask the smell of the garlic and will also act as a flavouring agent. The prepared mouthwash was then transferred to 100 ml plastic bottles.

To prepare nutmeg mouthwash, 0.125 mg of the nutmeg extract i.e approximately 10ml was dissolved in 80 ml distilled water and gently stirred with a stirrer till it was completely dissolved. To this 10 ml of sorbitol was added which acted as a sweetener, and 10 mg of peppermint was added in order to mask the smell of the nutmeg and will also act as a flavouring agent. The prepared mouthwash was then transferred to 100 ml plastic bottles.

Positive control

Hexidine mouthwash (ICPA health products ltd., Batch no.L30145) was used as a positive control.

Preparation of negative control mouthwash

This mouthwash was prepared in a similar manner and contained the very same ingredients as the garlic and nutmeg extract mouthwash, without addition of the garlic and nutmeg extract.

Antimicrobial activity

The antimicrobial activity of garlic and nutmeg extract were determined against oral pathogens by cup plate diffusion method as described by Gavin J J et al[14]. Test cultures were swabbed on the top of the solidified media i.e brain heart infusion agar for *Streptococcus mutans* and Sabourud's dextrose agar for *Candida albicans*. The agar plates were allowed to dry and wells of 6 mm diameter were made with a sterile standard device. The wells were designated as Garlic, Nutmeg, CHX and Neg and were

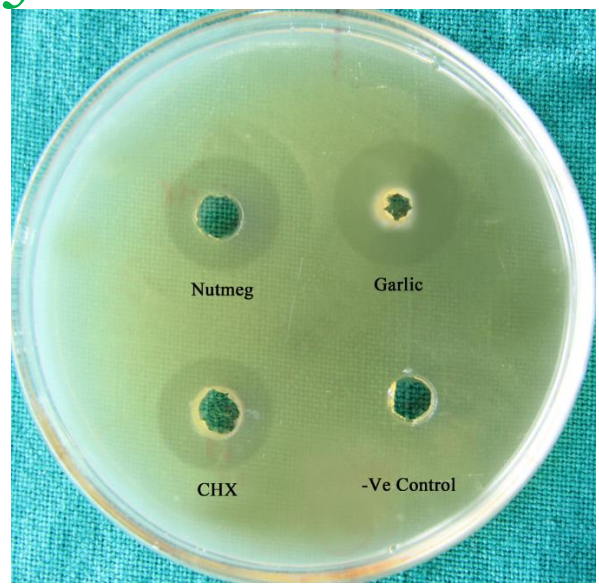

Fig 1: Mutant zone.

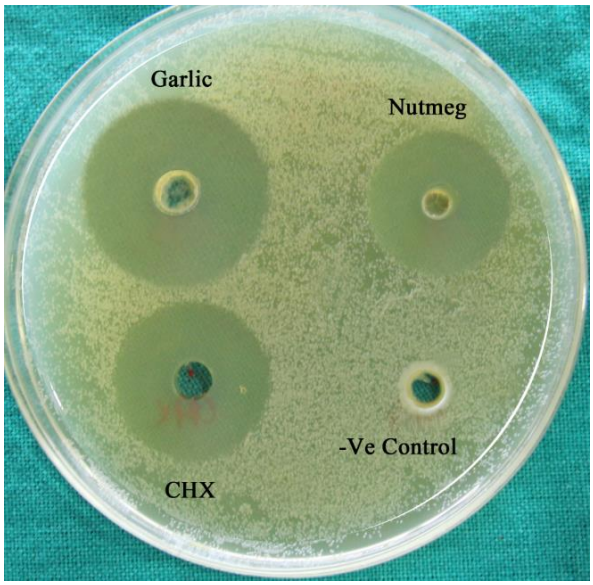

Fig 2: Candida zone.

completely filled with garlic mouthwash, nutmeg mouthwash, hexidine mouthwash, and negative control mouthwash respectively. A 100µl volume of each extract was propelled directly into the wells of the inoculated specific media agar plates. The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37°C for 24 hours for *Streptococcus mutans* in closed lid candle jar and 48 hours for *Candida albicans* in an incubator. The experiment was performed for 30 sample plates under strict aseptic conditions to ensure consistency of all findings. After incubation for 24 hrs for *Streptococcus mutans* and 48 hours for *Candida albicans*, the plates were observed for clear zones of growth inhibition surrounding the well containing the extract indicating the antimicrobial property. The zone of inhibition was measured using vernier callipers and expressed in millimetres.

Statistical Analysis

The data generated during the study was processed using various statistical tests with the aid of PASW 18.0 Software (formerly known as SPSS). Statistical analysis of the comparison of zone of inhibition of garlic, nutmeg, chlorhexidine and control mouthwashes was carried out to find the significant difference between those values. The statistical test used for the analysis of the result were one way ANOVA, and scheffe multiple comparison test was applied as post hoc test.

RESULTS

The low MIC and MBC value indicate that extract has strong antibacterial activity. The results revealed that hydroethanolic extract of garlic showed MIC of 4mg/ml and MBC of 8mg/ml against *Streptococcus mutans* and MIC of 0.125 and MBC of 0.25mg/ml against *Candida albicans* (Table 1).

Ethanolic extract of nutmeg showed MIC and MBC of 0.125mg/ml and 0.25 mg/ml respectively for *Streptococcus mutans* and MIC of 0.06mg/ml and 0.125mg/ml respectively for *Candida albicans*. Hydoethanolic extract of nutmeg showed MIC range above 8mg/ml for both the oral pathogens. The results revealed that ethanolic extract of nutmeg possessed better antibacterial activities against tested bacteria compared to its Hydroethanolic extract. (Table 2)

Mean zone of inhibition for garlic was 16.10mm with a standard deviation of 1.29, for nutmeg it was 13.46mm with a standard deviation of 2.02 and for CHX it was 13.53mm with a standard deviation of 1.22 for *Streptococcus mutans*. For *Candida albicans*, the mean zone of inhibition was found to be 16.93mm with a standard deviation of 3.16 for garlic, 15.43mm with a standard deviation of 1.69 for nutmeg and 14.03mm with a standard deviation of 1.18 for CHX. (Table 3 & Table 4)

For *Streptococcus mutans*, statistically significant difference was found among garlic and nutmeg group (P= 0.000), garlic and chlorhexidine group

(P= 0.000), garlic and negative control group (P= 0.000), nutmeg and negative control group (P= 0.000), chlorhexidine and negative control group (P= 0.000), and no statistically significant difference was found between nutmeg and chlorhexidine group (P= 0.998).For Candia albicans, statistically significant difference was found among garlic and nutmeg group (P= 0.028), garlic and chlorhexidine group (P= 0.000), garlic and negative control group (P= 0.000), nutmeg and chlorhexidine group (P= 0.046), nutmeg and negative control group (P= 0.000), chlorhexidine and negative control group (P= 0.000).(Table 5 & Table 6)

DISCUSSION

The natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds, many of which have been the basis for the development of new chemicals for pharmaceuticals and have been in widespread use since prehistoric times[15]. There is a long history of the use of plants to improve dental health, promote oral hygiene andthe identification of phytocompounds having potential uses in dental practice will change dentistry to "green dentistry".Henceforth the present study was undertaken to evaluate and compare the antimicrobial activity of garlic, nutmeg and chlorhexidine mouthwash against *Streptococcus mutans*and *Candida albicans*. In our study, we selected garlic and nutmeg as they are easily available, biocompatible, cost effective and possessed strong antimicrobial properties[16].

Prior to evaluation of efficacy of garlic against *Streptococcus mutans*and *Candida albicans*, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) value of garlic was evaluated, as different studies have given different MIC and MBC ranges for garlic. The MIC for *Streptococcus mutans*has been found to range from 4 µg/ml to 71.4 mg/ml[17,18,19],while MIC range against *Candida albicans*was reported between 0.8-48.3 mg/ml[20,21]. In this study, the MIC value for hydroethanolic exract of garlic against *Streptococcus mutans*and *Candida albicans*was found to be 4mg/ml and 0.125 mg/ml respectively. The minimum inhibitory concentration, at which garlic would inhibit the activity of both *Streptococcus mutans*and *Candida albicans*i.e 4 mg/ml, was selected for the formulation of mouthwash and the antibacterial effect of the prepared mouthwash was evaluated. In the garlic mouthwash, sorbitol and peppermint oil were used as sweetening and flavoring agents as these properties have been found to be used unduly as sweetening and flavoring agents in cosmetics, mouthwash and toothpaste preparations[22]. Negative control mouthwash was also prepared without addition of extract to rule out any antibacterial property possessed by active ingredients. The study showed that garlic possessed an antibacterial effect against *Streptococcus mutans*and *Candida albicans*which is in accordance with the study conducted by Ghannoum MA[20] and Groppo FC et al[19].

The antibacterial effect of garlic can be attributed to its composition and presence of various active ingredients. It was not until 1944 that Cavallito C and hiscolleagues isolated and identified the component, Allicin responsible for the remarkable antibacterial activity of crushed garlic cloves[23]. The antibacterial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, for example, alcohol dehydrogenase, thioredoxin reductase and RNA polymerase, which could affect the metabolism of essential bacteria[24].Ankri S et al studied activity of allicin of freshly crushed garlic and showed that garlic has a broad spectrum inhibitory activity against gram-positive and gram-negative bacteria and *Candida albicans*, by inhibiting both germination of spores and growth of hyphae[25, 26].

The biological property ofgarlic have been studied and reported that thesteroid saponins present in garlic exhibited antifungal, antitumor, preventing firming clot and cytotoxicity effects[27].Ajoene present in garlic has been reported to inhibit Candida albicans and Aspergillus niger[28]. In a study, allivin and lillin, special proteins isolated from garlichave shown to exhibit strong antifungal effects[29].The presence of theseconstituents all together could be the reason for garlic possessing antibacterial activity against Streptococcus mutans and Candida albicans in the present study.

Ethanolic extract of nutmeg in the present study showed a MIC of 0.06 mg/ml and 0.125 mg/ml against *Streptococcus mutans*and *Candida albicans* respectively, where as hydroethanolic extract of nutmeg showed MIC greater than 8 mg/ml. Since

the ethanolic extract of nutmeg showed MIC at lower value, it was selected for further study for the formulation of mouthwash. Findings of the MIC in our study paralleled with that of Valchos et al, who reported that aqueous extract of Myristica fragrans showed no inhibitory activity against any strain of bacteria, whereas antibacterial activity of ethanolic extract can be attributed to the availability of active constituents of Myristica fragrans in the solvent. The other factors may include respective composition of these compounds, the structural configuration and possible synergistic interactions between the components[30].

The higher MIC i.e 0.125 mg, at which nutmeg would inhibit the growth of *Streptococcus mutans*and *Candida albicans*, was selected to prepare the mouth wash. The results of the finding suggest that nutmeg possessed an antibacterial effect against *Streptococcus mutans*and *Candida albicans.* Previous studies with nutmeg have shown similar findings[13,31]. The antimicrobial activity of nutmeg can be attributed to presence of macelignan, an active compound isolated from seed of Myristica fragrans which have shown to exhibit strong antibacterial activity against*Streptococcus mutans*[13]. Studies have shown that the presence of essential oil i.e trimyristin, myristic acid and myristicin, isolated from the seed exhibited good antibacterial activity against selected gram-positive and gram-negative bacteria[32]. These essential oils sensitize the phospholipid bi layer of the microbial cytoplasmic membrane causing increased permeability to reduce the availability of vital intercellular substance thereby depriving the cell of nutrients which led to impaired bacterial enzymes function and eventual overall cellular collapse and death[33, 34]. The presence of all these constituents could be the reason for nutmeg exhibiting antibacterial activity in the present study.

In our study, the mean zone of inhibition which is indicative of effectiveness of antimicrobial activity was compared for garlic, chlorhexidine and nutmeg against *Streptococcus mutans*and *Candida albicans*. The mean zone of inhibition of garlic mouthwash when compared to chlorhexidine mouthwash showed significant difference. Garlic possessed better antibacterial properties against *Streptococcus mutans*and *Candida albicans*compared to chlorhexidine which is in accordance with the study conducted by Zohreh Dalirsani et aland Mansour amin et al.[35, 36]. However, Dalirsani Z et al in his study against *Streptococcus mutans*found that garlic did not possessed antibacterial activity as compared to chlorhexidine mouthwash[37]. Even though nutmeg showed antibacterial properties against *Streptococcus mutans*and *Candida albicans*, the antibacterial property was comparable and equal to that of chlorhexidine mouthwash with respect to *Streptococcus mutans*, whereas for *Candida albicans*, results were found to be statistically significant.

CONCLUSION

According to the results obtained in the present study, garlic mouthwash has shown better antimicrobial properties as compared to nutmeg and chlorhexidine mouthwash and can be considered as safe and effective alternatives to chlorhexidine for plaque control and thereby prevention of dental caries.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Microbiology, Jawaharlal Nehru Medical College, Sawangi (Meghe), Wardha, and Department of Bio-processing and Herbal laboratory, Mahatma Gandhi Institute of Rural Industralization (MGIRI), Wardha, for the assistance given in all phases of this research.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

REFERENCES

1. Bowen WH, Koo H. Biology of *Streptococcus mutans*derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms.Caries Research 45(1):69–86, 2011.

2. Ellen RP, Banting DW,Fillery E.D. Longitudinal microbiological investigation of a hospitalized population of older adults with a high root surface caries risk.Journal of Dental Research 64(12):1377–1381, 1985.

3. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiological Reviews 50(4): 353–380, 1986.

4. Signoretto C, Burlacchini G, Facciono F, Zanderigo M, Bozzolo N, Canepari P. Support for the role of *Candida* spp. in extensive caries lesions of children. New Microbiologica 32:101-107, 2009.

5. Knoll-K¨ohler E, Stiebel J. Amine fluoride gel affects the viability and the generation of superoxide anions in human polymorphonuclear leukocytes: an in vitro study. European Journal of Oral Sciences 110(4):296–301, 2002.

6. CowanMM.Plant products as antimicrobial agents.Clinical Microbiology Reviews 12(4):564–582, 1999.

7. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants.Journal of Phytology3(12): 10–14, 2011.

8. Jaiswal P, Kumar P, Singh VK, Singh DK. Biological effects of Myristica fragrans.Annual Review of Biomedical Sciences 11: 21–29, 2009.

9. Sonavane GS, Sarveiya VP, Kasture VS, Kasture SB. Anxiogenic activity of Myristica fragrans seeds. Pharmacology Biochemistry and Behavior 71: 239–244, 2002.

10. Zaidi SFH, Yamada K, Kadowaki M, Usmanghani K, Sugiyama T. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori.Journal of Ethnopharmacology 121(2): 286–291, 2009.

11. Hussain SP, Rao AR. Chemopreventive action of mace (*Myristica fragrans*, Houtt) on methylcholanthrene-induced carcinogenesis in the uterine cervix in mice. Cancer Letters56(3): 231–234, 1991.

12. Ozaki Y, Soedigdo S, Wattimena YR, Suganda AG. Antiinflammatory effect of Mace, aril of *Myristica fragrans* HOUTT., and its active principles. Japanese Journal of Pharmacology 49(2):155–163, 1989.

13. Chung JY, Choo JH, Lee MH, Hwang JK.Anticariogenic activity of macelignan isolated from Myristica fragrans (nutmeg) against *Streptococcus mutans*. Phytomedicine13(4):261-6, 2006.

14. Gavin JJ. Analytical microbiology. II. The diffusion methods. Appl. Microbiol 5: 25-33, 1957.

15. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evidence-Based Complementary and Alternative Medicine Volume 2011, Article ID 680354, 15 pages.

16. De M, Krishna DA, Banerjee AB. Antimicrobial screening of some Indian spices. Phytother. Res 13:616-618, 1999.

17. Bakri IM, Douglas CW. Inhibitory effect of garlic extract on oral bacteria. Arch of Oral Biology 50:645-51, 2005.

18. Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans.* J Ind Soc Pedod Prevent Dentistry 25:164-8, 2007.

19. Groppo FC, Ramacciato JC, Motta RHL, Ferraresi PM, Sartoratto A. Antimicrobial activity of garlic against oral Streptococci. Int J Dent Hygiene 5:109–15, 2007.

20. Ghannoum MAStudies on the Anticandidal Mode of Action of *Allium sativum* (Garlic). Journal of General Microbiology134: 2917-2924, 1988.

21. Iwalokun BA, Ogunledun A, Ogbolu DO, Bamiro SB, Jimi-Omojola J. In Vitro Antimicrobial Properties of Aqueous Garlic Extract Against Multidrug-Resistant Bacteria and Candida Species from Nigeria. J Med Food 7(3): 327-333, 2004.

22. Peppermint. http://en.wikipedia.org/wiki/peppertmint.

23. Cavallito C, Bailey JH. Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. Journal of American Chemical Society 66(11): 1950–1951, 1944.

24. Zakaria El. The inhibitory action of aqueous garlic extract on the growth of certain pathogenic bacteria. European Food Research and Technology 218(5): 460-464, 2004.

25. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes and Infection 2:125–129, 1999.

26. Yamada Y, Azuma K. Evaluation of the in vitro antifungal activity of allicin. Antimicrob. Agents of Chemotherapy 11(4):743–749, 1997.

27. Matsuura H. Saponins in garlic as modifiers of the risk of cardiovascular disease. J Nutr 131(3):1000S-1005, 2001.

28. Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H, Nakagawa S. Antifungal activity of ajoene derived from garlic. Applied Environmental Microbiology 53(3): 615-17, 1987.

29. Wang HX, Ng TB. Purification of allivin a novel antifungal protein from bulbs of the round, clove, garlic. Life sciences 70: 357-65, 2001.

30. Vlachos V, Critchely AT, Von HA. Establishment of a protocol for testing antimicrobial activity in Southern African macroalagae. Microbios 88: 115-123, 1996.

31. Ogu GI, Ekeanyanwu RC, Madagwu EC, Eboh OJ, Okoye J. In Vitro antimicrobial evaluation of African Nutmeg (Monodora Myristica) seeds. International Journal of Tropical Agriculture and Food Systems 5(1):55-60, 2011.

32. Narasimhan B, Dhake AS. "Antibacterial principles from *Myristica fragrans* seeds," Journal of Medicinal Food 9(3):395–399, 2006.

33. Juven BJ, Kanner J, Schved F, Weisslowiez H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. Journal of Applied Bacteriology 76(6): 626-631, 1994.

34. Kim J, Marshall MR, Wei C. Antibacterial activity of some essential oil components against five food-borne pathogens. Journal of Agricultural and Food Chemistry43(11): 2839-2845, 1995.

35. Dalirsani Z, Abidpour M, Aghazadeh, Amirchaghmaghi M, Falaki F, Mozafari PM, et al. In vitro comparison of inhibitory activity of 10 plant extracts against *Candida albicans.* Australian Journal of Basic and Applied Sciences 5(5):930-5, 2011.

36. Amin M, Kazemi M, Rasaie N. *In Vitro* Comparison of the Effects of Garlic Juice and Chlorhexidine Mouthwash on Oral Pathogens. Jundishapur J Microbiology 5(2):398-400, 2012.

37. Dalirsani Z, Aghazadeh M, Amirchaghmaghi M, Pakfetrat A. In vitro comparison of the antimicrobial activity of ten herbal extracts against *Streptococcus mutans* with chlorhexidine. Journal of Applied Sciences 11(5):878-82, 2011.