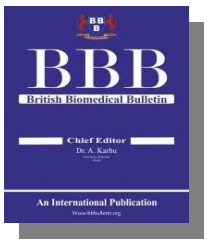
Journal Home Page www.bbbulletin.org

BRITISH BIOMEDICAL BULLETIN



Original

Synthesis of Silver Nanoparticles from Plant Chewing Sticks and their Antibacterial Activity on Dental Pathogen

M.N. Abubacker*[1] and C. Sathya[2]

[1]*Department of Biotechnology, National College, Tiruchirappalli 620001, India*
[2]*Department of Botany, Seethalakshmi Ramasamy College, Tiruchirappalli 620002, India*

A R T I C L E I N F O

Received 23 Jan. 2015
Received in revised form 13 Feb. 2015
Accepted 18 Feb. 2015

Keywords:
Antibacterial activity,
Chewing sticks,
Oral pathogens,
Silver nanoparticle.

Corresponding author: Department of Biotechnology, National College, Tiruchirappalli 620001, India.
E-mail address:
abubacker_nct@yahoo.com

A B S T R A C T

Objective: Synthesis of silver nanoparticles from plant chewing sticks of *Azadirachta indica*, *Ficus bengalensis* and *Salvadora persica* and their antibacterial activity on dental pathogens were conducted.
Methods: Oral pathogens were isolated from the oral cavities of student community and antibacterial assay was conducted by disc diffusion method.
Results: *Lactobacillus acidophilus* NCBT-060, *L. lactis* NCBT-063, *Micrococcus luteus* NCBT-036, *Proteus vulgaris* NCBT-020, *Staphylococcus aureus* NCBT-052 and *Streptococcus mutans* (NCBT-058) which cause gingivitis, periodontitis and plaque were tested for antibacterial activity. The antibacterial effect of chewing stick extract as well as silver nanoparticles from chewing sticks revealed that *S. persica* is highly effective against oral pathogens followed by *A. indica* and *F. bengalensis* when compared with control ($AgNO_3$) at 1 mM concentration, ethyl alcohol and standard antimicrobial disc used as positive controls.
Conclusions: The different response of each microbe to the various chewing stick extracts indicated that the natural chewing stick posses different phytochemical compounds which inhibited the growth of oral pathogens. The silver nanoparticles prepared from *S. persica* proved scientifically about the epidemiological and clinical beneficial effects on oral hygine.

Introduction

The use of plant materials for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness, and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols. Chemical synthesis methods lead to the presence of some toxic chemicals absorbed and bring about adverse effects in medical applications. Bioinspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals[1]. Silver nanoparticles are the metal of choice as they hold the promise to kill microbe's effectively[2]. The silver nanoparticles act on a broad range of target sites both extracellularly as well intracellularly. In fact, microbes generally have a harder time developing resistance to silver than they do to antibiotics[3]. The oligodynamic effect that silver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation.

Many bacteria and fungi produce diseases which are manifested in or about the oral cavity. Some of these diseases or lesions are of a specific nature and are produced by a specific contribution to the problem of caries etiology[4]. The highest caries susceptibility is in the age group of 20-40 years, also the females are more susceptible to dental caries as compared to males[5].

Dental decay is a chemical parasitic process consisting of two stages, the decalcification of enamel or its total destruction and the decalcification of dentine (dissolution of the softened residue)[6]. The carcinogenic Streptococci is critical to the development of pathogenic plaque. A large number of *Streptococcus*, *Actinomyces* and *Lactobacillus* species are involved in root caries and periodontal diseases[7]. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem[8,9]. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants[10].

Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens[11,12]. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens[13,14]. Medicinal plants have been used as traditional treatment for numerous human diseases for thousands of years and in many parts of the world[15,16]. The search of biologically active component of plants has always been much interest to scientists looking for new sources of practical alternative against diseases[17,18]. In rural areas of the developing countries, they continue to be used as the primary source of medicine[19]. About 80% of the people in developing countries use traditional medicines for their health care[20].

The World Health Organisation has recommended and encouraged the use of chewing sticks. Recently, chewing sticks have been comprehensively reviewed and examined for their effectiveness in oral hygiene[21,22].

There is a long history of the use of plants to improve dental health and promote oral hygiene. In various parts of the world where tooth brushing by modern method is uncommon, the practice of tooth cleaning by chewing sticks is very commonly observed. This practice was recorded by the Babylonians in 5000 BC. This natural tooth brushing is very common today among many African and Southern Asian communities as well as in isolated areas of tropical America and the southern United States**.** In many African homes teeth are cleaned in the morning by chewing the root

or slim stem of certain plants until they acquire brush like ends. The fibrous end is then used to brush the teeth thoroughly[23].

Green synthesis and application of silver nanoparticles against antimicrobial activities were made by many workers and well documented, for example, synthesis of silver nanoparticle from *Argemone mexicana* leaf silver nanoparticles[24], geranium leaf[25], *Capsicum annum*[26], *Papaya* fruit[27], *Phoma glomerata*[28], black tea leaf[29], *Clerodendron*[30], *Nicotiana tobaccum*[31], *Catharanthus roseus*[32,33], *Baliospermum*[34], *Cissus quadrangularis*[35], *Elettaria cardamomom*[36], *Wrightia tinctoria*[37], *Terminalia chebula*[38], *Nitraria schoberi*[1]. But the green synthesis and application of silver nanoparticles using chewing sticks against dental pathogen information's and literatures are not documented so far. Hence the present work was focused on this area of research selecting three major chewing sticks commonly used by most of the village population in India and their antibacterial activities against dental pathogens was concentrated.

The aim of this study was to compare the antimicrobial effects of three major chewing sticks commonly used by Indian population using crude water extract as well as its silver nanoparticles effect against the dental pathogens.

Materials and Methods

The plant chewing stick used for the present work were *Azadirachta indica* A. Juss (Meliaceae), *Ficus bengalensis* L. (Moraceae) and *Salvadora persica* L. (Salvadoraceae) (Fig. 1).

A. indica A. Juss (Meliaceae)

The neem tree, in Sanskrit, Nimba and Arishta, is a native of India, and is cultivated in all parts of the subcontinent on account of its medicinal properties. The leaf bark and other products of Neem have been articles of the Indian materia medica since antiquity and are mentioned in the Ayurveda of Sushruta. The active principles of the plant were brought to the attention of natural products scientists in 1942, named as nimbin, nimbinin, and nimbidin. Azadirachtin is a chemical compound belonging to the limonoids was later identified. Neem mouth rinse is very effective in the treatment of infections, tooth decay, bleeding and sore gums. A mouthwash, using Neem oil, has been manufactured and used for the treatment of mouth ulcers.

F. bengalensis L. (Moraceae)

The Banyan, a large tree throwing out numerous large aerial roots from the main trunk and large branches, which descend to the soil and form supports, and capable of separate existence when severed from the parent tree. Bark is grayish-white, wood grayish-white and moderately hard is used for various purposes. The stem stick is used as chewing stick for oral hygine.

S. persica L. (Salvadoraceae)

The Miswak (*miswak, siwak*) is a natural toothbrush made from the twigs of the *Salvadora persica* tree (Arak). Miswak was used by the Babylonians some 7000 years ago; they were later used throughout the Greek and Roman empires and have been used by Jews, Egyptians and in the Islamic empires. It is believed that this precursor to the modern day toothbrush was used in Europe until about 300 years ago**.** Today, Miswak is being used in Africa, South America, Asia, the Middle East including Saudi Arabia, and throughout the Islamic countries. In the Middle East, the most common source of chewing sticks is the Arak (*S. persica*) tree. Chemically, the air dried stem bark of *S. persica* through chemical studies showed that it is composed of trimethyl amine, salvadorine, chlorides,

high amounts of fluoride and silica, sulphur, vitamin-C, small amounts of tannins, saponins, flavonoids and sterols. Its fibrous branches and roots have been used as toothbrushes.

Collection of chewing sticks

Samples of the most commonly used chewing sticks in India, namely Neem (*A. indica*), Banyan (*F. bengalensis*), and Miswak (*S. persica*) (Fig. 1). Out of these three chewing sticks *S. persica* was obtained from the commercial source and other chewing sticks were collected from the respective trees grown in the campus of National College, Tiruchirappalli.

Preparation of chewing stick extracts

Hundred gram of each of the chewing sticks were used in the experiment. The chewing sticks were cut into small pieces and ground to powder in a ball mill. The powder was kept separately in sterile, dry and screw-capped bottles, which were stored in a dry cool place before aqueous extraction. Each successive 10 gm quantity was put into a sterile screw-capped bottle to which 100 ml of sterile deionized distilled water was added. The extracts were allowed to soak for 48 hours at room temperature before the mixtures were centrifuged at 2,000 rpm for 10 minutes. The supernatants were used for antimicrobial activity.

Isolation of Pathogens from Oral Cavity

The pathogens isolated from oral cavities from 25 students from the Department of Biotechnology, National College, Tiruchirappalli revealed the presence of oral pathogens such as *Lactobacillus acidophilus* NCBT-060, *L. lactis* NCBT-063, *Micrococcus luteus* NCBT-036, *Proteus vulgaris* NCBT-020, *Staphylococcus aureus* NCBT-052 and *Streptococcus mutans* NCBT-058. From the heterogenous microbial colonies, pure colonies were isolated and grown in Nutrient Glucose Agar Medium[39] for all the bacteria.

Synthesis and characterization of silver nanoparticles

Silver nitrate ($AgNO_3$) (Merck India Ltd.) was used for the study. One gram of dried powder material of chewing stick was added into the aqueous solution of 1 mM silver nitrate and kept in a water bath with temperature 50-70°C for 10-20 minutes. Change of colour (dark brown) was noticed. It was then observed in UV-vis spectroscopy at 280-580 nm w/l. The bioreduction of silver ions in solution was noticed at the absorption peak at 420 nm w/l. The spectra were recorded at 2, 10 and 20 minutes intervals for the conformation of silver nanoparticles. The solution was then centrifuged at 12,000 rpm for 15 minutes, the supernatant was discarded, the pellet was collected, it was then dried in hot air oven and the dried samples were used for Scanning Electron Microscopy - Energy Dispersive X-ray (SEM-EDX) pictures (Fig. 2 and 4).

Antimicrobial Assay by Disc Diffusion Method

The prepared chewing stick extracts and silver nanoparticles of chewing stick were tested for antimicrobial activity for dental pathogen by the disc diffusion method[40]. The pure cultures of the dental pathogens were individually streaked uniformly all over the Nutrient Glucose Agar media in 9 cm Petri dishes. The aqueous chewing stick extracts and silver nanoparticles of chewing stick were loaded on plain sterile disc (Himedia SD 067) and placed on the medium in the Petri dish under sterile condition. Positive control discs with $AgNO_3$ 1 mM, ethyl alcohol 70% and standard antibiotic (Streptomycin 10 µg) were placed as positive controls. The Petri plates were incubated at 37 ± 1° C for 48

hours. The experiment was carried out, in triplicate for each extract.

Results and Discussion

Antimicrobial effect of natural chewing stick extracts and silver nanoparticles of chewing sticks

The natural chewing stick extracts of three traditional Indian plants *viz. A. indica, F. bengalensis,* and *S. persica* were selected and their antimicrobial susceptiblity test was conducted against six oral pathogens, *viz., L. acidophilus, L. lactis, M. luteus, P. vulgaris, S. aureus* and *S. mutans* which cause dental caries, gingivitis and plaque. The results are given in Table-1.

Antimicrobial effect of chewing sticks against dental pathogens

The chewing stick extract of *S. persica* has shown the maximum inhibition zone of 10.0 mm for *L. acidophilus,* whereas *S. persica* silver nanoparticle have shown 20.0 mm inhibition zone, *A. indica* has shown the maximum inhibiton zone of 9.0 mm for *S. aureus,* whereas *A. indica* silver nanoparticle has shown 13.0 mm inhibition zone. *F. bengalensis* has shown the maximum inhibition zone of 9.0 mm for *L. lactis* and *S. aureus,* whereas *F. bengalensis* silver nanoparticle has shown the maximum inhibition zone of 12.0 mm for *L. acidophilus* and *S. mutans*. The positive control antibiotic disc Streptomycin 10 µg have shown 12.0 to 16.0 mm of inhibition zone for the six dental pathogen tested for this work (Table 1, Fig. 3).

The selection of chewing sticks in Asian countries was based on a number of factors. The use of chewing sticks is most common in Asian countries especially in the Indian subcontinent and the Middle East region; further more chewing sticks are cheap, readily available in urban and rural areas of the countries. Their taste is agreeable and not unpleasant and reported to have anti-plaque and many other pharmacological properties[41].

A recent survey in Asia showed that more than half of the rural population used chewing sticks as an oral hygiene tool[42]. So it was important to find out the antimicrobial properties of those chewing sticks as they are so commonly used in Asian countries. It is claimed that the mechanical plaque-removing properties of chewing sticks may be similar to that of a conventional toothbrush[43,44].

Most studies on chewing sticks have been carried out, where more than 90% of the population uses different types of sticks from trees that grow there such as *Fagara zanthoxyloides, Serindei werneckei, Azadirachta indica, Accacia arabica*[45,46]. Certain chewing sticks including those derived from *Salvadora persica, A. indica* and *Accacia arabica* are active against several types of carcinogenic bacteria frequently found in the human oral cavity[45]. Results from the present study showed that *S. persica,* the maximum followed by *A. indica* and *F. bengalensis* showed antibacterial activity against all the six tested microbes *viz., L. acidophilus* NCBT-060, *L. lactis* NCBT-063, *M. lutens* NCBT-036, *P. vulgaris* NCBT-020, *S. aureus* NCBT-052 and *S. mutans* NCBT-058 for both chewing stick extracts as well as chewing stick nanoparticles. Antimicrobial activities of silver nanoparticles by several previous experiments by Landsdown (2002)[47], Castellano *et al.* (2007)[48], Singh *et al.* (2008)[49], Marambio-Jones and Hock (2010)[50], Kim *et al.* (2011)[51], Guzman *et al.* (2012)[52] and Justin Packia Jacob (2013)[53] are the supporting evidence of antimicrobial activities of nanoparticle. It is to be stated that the present work is first of its kind with reference to the antibacterial activities of silver nanoparticle prepared from plant chewing sticks on dental pathogens. The use

of the chewing stick conforms to the notion of Primary Health Care Approach (PHCA) and the well established associations with certain cultural and religious beliefs. The chewing sticks have been proven effective as an oral hygiene tool and its use should be promoted with scientific rational and proper method of its preparation and usage. The use of chewing sticks will be a great help in developing countries with financial constraints and limited oral health care facilities for their population.

Conclusion

It has been scientifically proved to be very useful in the prevention of tooth decay even when used without any other tooth cleaning means. The users of Miswak have shown a remarkable lack of tooth decay as compared to other chewing sticks. From this study, it could be concluded that *S. persica* chewing stick as well as the nanoparticle are effective antimicrobial agent for oral pathogens when utilized clinically as an irritant in the endodentic treatment of teeth with necrotic pulps.

Acknowledgements

The authors expresses thanks to Padmavibhushan Dr. V. Krishnamurthy, President; Sri K Ragunathan, Secretary and Dr K Anbarasu, Principal, National College, Tiruchirappalli for all the supports and encouragement given to PG and Research Department of Biotechnology to carry out the research work.

References

1. Rad MS, Rad JS, Heshmati GA, Miri A, Sen DJ. Biological Synthesis of Gold and Silver Nanoparticles by *Nitraria schoberi* Fruits. *Am J Advan Drug Deliv*. 2013;1(2): 174-179.
2. Sarkar R, Kumbhakar P, Mitra AK. Green synthesis of silver nanoparticles and its optical properties. *Digest Journal of Nanomaterials and Biostructure*. 2010; 5: 491.
3. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobial. *Biotechnol Adv*. 2009; 27: 76.
4. Williams S, Williams K. *Clinical practice of dental hygienist*, 7th ed, Esther W Willikins, London, 1994.
5. Nigam P, Srivastava A B. Betel chewing and dental decay, *Fed Oper Dent*, 1990; 1: 36-44.
6. Marsh PD. Microbiological aspects of the chemical control of plaque and gingivitis, *Journal of Dental Resistance*, 1992; 71: 1431-1438.
7. Sachipach P, Osterkwalder V, Guggenheim B. Human root caries microbiota in plaque covering sound carious and arrested carious root surfaces, *Caries Res*, 1995; 29: 382-395.
8. Rad JS, Alfatemi SMH, Rad MS, Iriti M. *In-vitro* antioxidant and antibacterial activities of *Xanthium strumarium* L. extracts on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Ancient Sci Life*. 2013;33: 107-11.
9. Sharifi-Rad J, Hoseini Alfatemi SM, Sharifi Rad M, Iriti M. Antimicrobial synergic effect of allicin and silver nanoparticles on skin infection caused by methicillin-resistant *Staphylococcus aureus* spp. *Ann Med Health Sci Res* 2014;4: 863-8.
10. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Setzer. WN Chemical composition, antifungal and antibacterial activities of essential oil from *Lallemantia royleana* (Benth. In Wall.) Benth. *J Food Safety*. 2014; doi:10.1111/jfs.12139.
11. Alfatemi SMH, Rad JS, Rad MS, Mohsenzadeh S, Teixeira da Silva JA. Chemical composition, antioxidant activity and in vitro antibacterial activity of *Achillea wilhelmsii* C. Koch essential oil on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* spp. *3 Biotech*. 2014; doi: 10.1007/s13205-014-0197-x.
12. Rad JS, Alfatemi MH, Rad MS, Sen DJ. Phytochemical and Antimicrobial Evaluation of the Essential Oils and Antioxidant Activity of Aqueous Extracts from Flower

and Stem of *Sinapis arvensis* L. A *Am J Advan Drug Deliv*. 2013;1(1): 001-010.
13. Rad JS, Alfatemi SMH, Rad MS. *In vitro* assessment of antibacterial activity of *Salicornia herbacea* L. seed extracts against multidrug resistant gram-positive and gram-negative bacteria. *Int J Biosci*. 2014;4(6): 217-222.
14. Sharifi-Rad J, Miri A, Sharifi-Rad M, Sharifi-Rad M, Hoseini-Alfatemi SM, Yazdanpanah E. Antifungal and Antibacterial Properties of Grapevine (*Vitis vinifera* L.) Leaf Methanolic Extract from Iran- *In vitro* Study. *American-Eurasian J Agric & Environ Sci*. 2014; 14(11): 1312-1316.
15. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Miri A. Phytochemical screening and antibacterial activity of different parts of the *Prosopis farcta* extracts against methicillin-resistant *Staphylococcus aureus* (MRSA). *Minerva Biotec*. 2014; 26(4): 287-93.
16. Sharifi-Rad J, Miri A, Hoseini-Alfatemi SM, Sharifi-Rad M, Setzer WN, Hadjiakhoondi A. Chemical Composition and Biological Activity of Pulicaria vulgaris Essential Oil from Iran. *Nat Prod Commun*. 2014; 9(11): 1633-1636.
17. Sharifi-Rad J, Hoseini-Alfatemi SM, harifi-Rad MS, da Silva JAT. Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from *Nitraria schoberi* fruits. *3 Biotech*. 2014; doi 10.1007/s13205-014-0266-1.
18. Rad JS, Hoseini Alfatemi SM, Rad MS, Iriti M. Free radical scavenging and antioxidant activities of different parts of *Nitraria schoberi*. *TBAP*. 2014; 4(1): 44-51.
19. Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrheal activity of *Calotropis gigantea* R. Br in experimental animals. *J Phar Pharmacent Sci*. 2003; 7: 70-75.
20. Kim HS. Do not put too much value on conventional medicines. *Journal of Ethnopharmacology*. 2005; 100: 37-39.
21. WHO (World Health Organisation, *Prevention of Diseases*). Geneva, 1987.
22. Wu CD, Darout IA, Skang N. Chewing Sticks: Timeless natural toothbrushes for oral cleansing. *J Periodont Res.* 2001; 36: 275-284.
23. William Charles Evans. *Trease and Evans Pharmacognosy*, 15th ed. W B Saunders, London, 2002.
24. Singh AD, Jain, Upadhyay MK, Khandeiwal N, Verma HN. Green synthesis of silver nanoparticles using *Argimone Mexicana* leaf extract and evaluation of their antimicrobial activities. *Dig J Nanomater Bios*. 2001; 5: 483-489.
25. Shankar SS, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles. *Biotechnol Programmes.* 2003; 19: 1627-1631.
26. Li S, Shen Y, Xie A, Yu X, Qiu Z, Zhang Q. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract. *Green Chemical.* 2007; 9: 852-858.
27. Jain D, Kumar Daima H, Kachhwahas S, Kothari L. Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *J Nanomater Bios*. 2009; 4: 557-563.
28. Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Raj MK. Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus. Lett Application Microbiol*. 2009; 48: 173-179.
29. Begum NA, Mondal S, Basu S, Laskar RA, Mandal D. Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of black tea leaf extracts. *Colloids Surf B.* 2009; 71: 113-118.
30. Arshad F, Prakash Singh Chantan MD, Praveen K, Jameelshaik. Extraction of silver nanoparticles from the leaf extracts of *Clerodendron inerme. J Appl Biomed.* 2010; 1: 43-49.
31. Kumar P, Suranjit P, Darshit P, Patel A, Dalwadi P, Prasad R, Patet P, Selvaraj K, Prasad S. Biogenic synthesis of silver nanoparticles using *Nicotcana tobaccum* leaf extract and study of their antibacterial effect. *Afr J Biotechnol*. 2011; 10: 8122-8130.
32. Mukunthan KS, Elumalai EK, Trupti NP, Murti VR. *Catharanthu roseus*: A natural source for the synthesis of silver

nanoparticles. *Asian Pac J Trop Biomed*. 2011; 1: 207-274.
33. Ponarulselvam S, Panneerselvam C, Mumpan K, Aarthi N, Kalimuthu K, Thangamani S. Synthesis of silver nanoparticles using leaf of *Catharanthus roseus* Linn G Don and their antiplasmodial activities. *Asian Pac J Trop Biomed*. 2012; 2: 574-580.
34. Renugadevi K, Aswini V, Raji P. Microwave irradiation assisted synthesis of silver nanoparticles using leaf extract of *Baliospermum montanum* and evaluation of its antimicrobial, anticancer potential acvtivity. *Asian J Pharm Clin Res*. 2012; 5: 283-287.
35. Renugadevi K, Inbakandan D, Bavanilatha M, Poornima V. *Cissus quadrangularis* assisted biosynthesis of silver nanoparticles with antimicrobial and anticancer potentials. *Int J Pharm Bio Sci*. 2012; 3: 437-445.
36. Gnanadhas G, Annadurai G and Kannan C. Green synthesis of silver nanoparticles using *Elettaria cardamomom* and assessment of its antimicrobial activity. *Inter J Pharma Sci and Research*. 2012; 3: 975-992.
37. Rajathi K, Sridhar S. Green synthesised silver nanoparticles from the medicinal plant Wrightia tinctoria and its antimicrobial potential. *Int J Chem Tech Res*. 2013; 5: 1707-1713.
38. Nandagopal S, Ganesh Kumar A, Dhanalakshmi DP, Prakash P. Bio-prospecting the antibacterial and anticancer activities of silver nanoparticles synthesized using *Terminalia chebula* seed extract. *Int J Pharm Pharm Sci*. 2014; 2: 368-373.
39. Allen ON, Experiments in Soil Bacteriology. Burgess Publishing Co, Minneapolis, Minn, USA, 1953; pp 65-70.
40. Dulger B, Gonuz A. Antimicrobial activity of certain plants used in Turkish Traditional Medicine. *Asian J Plant Sci.* 2004; 3: 104-107.
41. Lewis ME. Plants and Dental Health. *J Prevent Dent.* 1990; 6: 75-78.
42. Asadi SG, Asadi ZG. Chewing sticks and the oral hygiene habits of the adult Pakistani Population. *Int Dent J*. 1997; 47: 275-278.
43. Hardie J, Ahmed K. The Miswak as an aid in oral hygiene. *J Phillip Dent Assoc.* 1995; 47: 33-38.
44. Hattab FN. Miswak: The Natural Tooth Brush. *J Clin Dent.* 1997; 8: 125-129.
45. Akpata ES, Akinremisi EO. Antimicrobial activity of extract from some African Chewing Sticks. *Oral Surg Oral Med Oral Path.* 1997; 44: 717-725.
46. Rotimi VO, Mosadomi HA. The effect of crude extracts of some African chewing sticks on oral anaerobes. *J Med Microbiol.* 1987; 23: 55-60.
47. Landsdown ABG. Silver: Its antibacterial properties and mechanism of action. *J Wound Care*. 2002; 11: 125-138.
48. Castellano JJ, Shajii SM, Ko F, Donate G, Wright TE, Mannari RJ, Smith DJ, Robson MC. Comparative evaluation of silver containing antimicrobial dressing and drugs. *Int Wound J*. 2007; 4: 114-122.
49. Singh M, Singh S, Prasad S, Gambhir IS. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. *Dig J Nanomater Bios.* 2008; 3: 115-122.
50. Marambio-Jones C, Hoek EMV. A review of the antibacterial effects of silver nanoparticles and potential implications for human health and the environment. *J Nanopart Res.* 2010; 12: 1531.
51. Kim HS, Lee SH, Ryu DS, Choi SJ, Lee DS. Antibacterial activity of silver nanoparticles against *Staphylococcus aureus* and *Escherichia coli*. *Korean J Microbiol Biotechnol*. 2011; 39: 77-85.
52. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. *Nanomedicine*. 2012; 8: 37-45.
53. Justin Packia Jacob S, Anand Narayanan PR, Finub JS. Green synthesis of silver nanoparticles using piper nigram leaf extract and its cytotoxic activity against Lep-2 cell line. *World Res J Pharmaceut Res*. 2013; 2: 1607-1616.

 ISSN-2347-5447

Table 1. Antimicrobial effect of chewing stick against dental pathogen

Dental Pathogen	C (mm)	C_1 (mm)	S (mm)	*A.i* (mm)	*F. b* (mm)	*S. p* (mm)	*A. i* Ag (n) (mm)	*F. b* Ag (n) (mm)	*S. p* Ag (n) (mm)
L. acidophilus	6.0	6.0	12.0	7.0	8.0	10.0	14.0	12.0	20.0
L. lactis	6.0	0.0	16.0	8.0	9.0	8.0	11.0	9.0	15.0
M. luteus	0.0	0.0	14.0	7.0	7.0	8.0	13.0	11.0	16.0
P. vulgaris	6.0	6.0	14.0	8.0	8.0	8.0	11.0	8.0	13.0
S. aureus	6.0	6.0	15.0	9.0	9.0	9.0	13.0	9.0	16.0
S. mutans	0.0	6.0	15.0	7.0	7.0	8.0	9.0	12.0	17.0

C (Control): Silver nitrate 1 mM solution, C_1 (Control): Ethyl alcohol 70%, S (Control): Streptomycin 10 µg, *A. i*: *Azadirachta indica* (crude water extract), *F. b*: *Ficus bengalensis* (crude water extract), *S. p*: *Salvadora persica* (crude water extract), *A. i* Ag(n): *A. indica* silver nanoparticle, *F. b* Ag(n): *F. bengalensis* silver nanoparticle, and *S. p* Ag(n): *S. pertica* silver nanoparticle.

 ISSN-2347-5447

Figure - 1

Plant and its chewing sticks

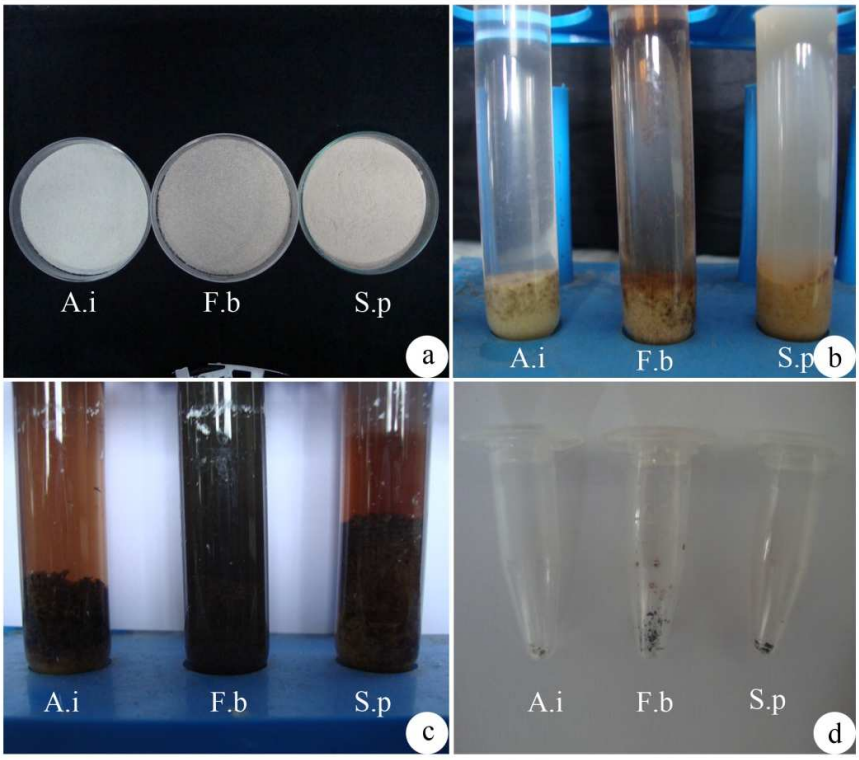
a) *Azadirachta indica*
b) *Ficus bengalensis*
c) *Salvadora persica*

a1) *A. indica* chewing stick
b1) *F. bengalensis* chewing stick
c1) *S. persica* chewing stick

 ISSN-2347-5447

Figure - 2

Synthesis of silver nano partical using chewing sticks



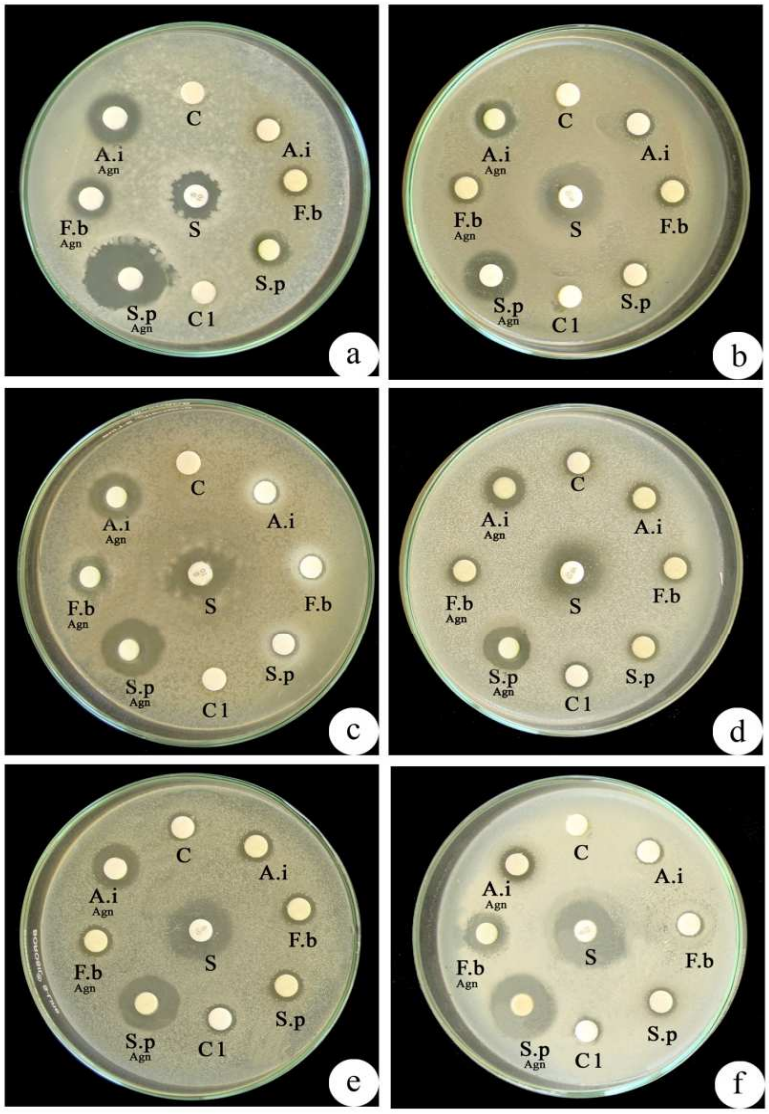
a) chewing stick powder
b,c & d) prepration of nano partical

A.i) A. indica
F.b) *F. bengalensis*
S.p) *S. persica*

 ISSN-2347-5447

Figure - 3

Antibacterial activity of silver nano partical on Dental pathogens



a) *Lactobacillus acidophilus*
b) *L. lactis*
c) *Micrococcus luteus*
d) *Proteus vulgaris*
e) *Staphylococcus aureus*
f) *Streptococcus mutans*

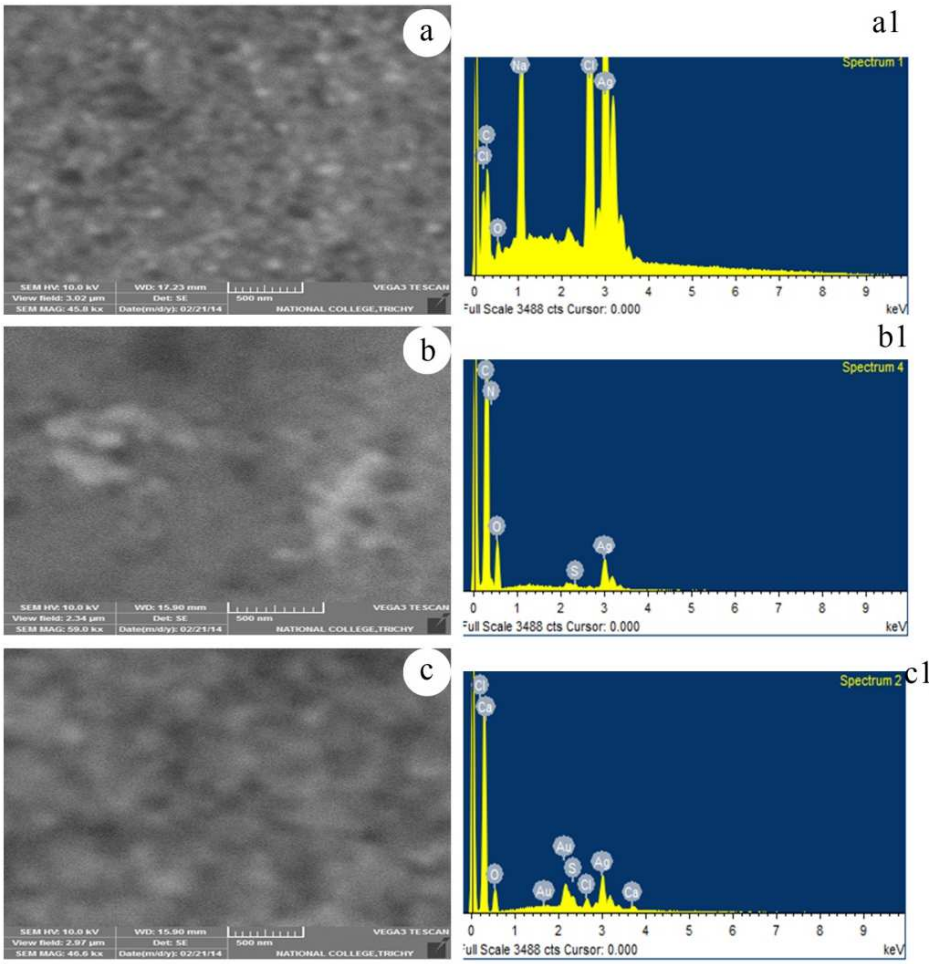
c) Control AgNo3 1mM solution
c1) Control Ethyl alcohol 70%
s) Streptomycin 10µg
A.i) A. indica
F.b) *F. bengalensis*
S.p) *S. persica*
A.i$_{Agn}$) *A. indica* silver nano partical
F.b$_{Agn}$) *F. bengalensis* silver nano partical
S.p$_{Agn}$) *S. persica* silver nano partical

British Biomedical Bulletin

 ISSN-2347-5447

Figure - 4

SEM-EDX picture of silver nano particals of plant chewing sticks



a) Silver nano partical of *Azadirachta indica*
a1) Elements in chewing stick of *A. indica* Na, Cl, O, and C
b) Silver nano partical of *Ficus bengalensis*
b1) Elements in chewing stick of *F. bengalensis* C, N, O, and S
c) Silver nano partical of *Salvadora persica*
b1) Elements in chewing stick of *S. persica* Cl, Ca, O, and S