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ORGANIC SYNTHESIS OF SILVER NANOPARTICLES AND THEIR POTENTIAL APPLICATION IN THE TREATMENT OF DENTAL CARIES

Aayesha G Siddiqui*, Rajendra prasad S. Awasthi[1], Prasanna T. Dhake[2], Yogita Deshmukh
[1]Assistant professor, Department of Microbiology and Biotechnology, Dayanand Science College, Latur, Maharashtra, India. Email-aayesha.siddiqi@yahoo.com,
[2]Principal, Shivaji College, Renapur, District Latur, Maharashtra, India Email-rsawasthi114@gmail.com.
[3]Associate Professor, Department of Pedodontics, MIDSR Dental College, Latur. mail: Prasannadahake@gmail.com
[4]CSIR, CIMAP, Lucknow, Uttar Pradesh, India: Email: yogita.deshmukh2@gmail.com

ABSTRACT— Taking in consideration potential applications in alternative medicine silver nanoparticles are being synthesized by various methods based on physical, chemical and biological techniques. Biological techniques are comparatively cheaper and environmentally friendly, these techniques employ the use of microbial cells and organic compounds such as lipids, proteins, plant materials, plant extracts, etc. In the present study we preferred an organic method using aqueous extract of *Psoralia corylifolia* leaves, as these leaves are used in Ayurveda for treatment of dental caries. These organically synthesized SNPs were characterized by UV-Visible absorption spectra, FTIR, XRD and TEM techniques. Average size of SNPs was found to be about 26 nm. Antimicrobial activity of confirmed SNPs was checked against some oral pathogens *viz* MIC was determined by microtiter broth dilution method. Biocompatibility of SNPs was checked by MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay. Results of our study showed that *Psoralia corylifolia* aqueous leaf extract is a good reducing agent for synthesis of SNPs from Silver nitrate, and these SNPs have significant antimicrobial activity against oral pathogens under study as well as they are highly biocompatible.

Key words: Organic synthesis, silver nanoparticles, Dental caries, *Psoralea corylifolia*, antimicrobial activity, Biocompatibility

I. INTRODUCTION

Noble metals, such as gold, silver, and platinum, semiconductors, such as CdS, ZnS, TiO_2, PbS, magnetic compounds, such as Fe_2O_3, Fe_3O_4, FePt, CoPt, and their combinations, such as core-shell and composite nanostructures, are used to make nanoparticles. Physical, chemical, and biological methods have been used to create noble metal nanoparticles (Gold, Silver, and Platinum) [1]. Silver nanoparticles are widely utilized in drug administration, pathology, bioscience, pathogen detection, catalysis, tumor detection, diagnostics, wound healing, antimicrobials, and other fields due to their exceptional optical, microbicidal, electrical, and chemical properties [2-4].Silver nanoparticles have been used in the treatment of a variety of illnesses, including wound infections and serious burn injuries. They have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and themethicillin-resistant strain of *Staphylococcus aureus* (MRSA) [5].

Chemical photochemical reactions, reduction in solutions, heating, electrochemical processes, biological reduction, and other methods have all been used to make silver nanoparticles [6-8]. Biological approaches are environmentally friendly because they do not require poisonous chemicals; these methods utilize microbial cells, biomolecules, and plant materials, among other things [9].

Psoralea corylifolia is a medicinal herb that is used in a variety of medicinal compositions to treat a variety of ailments [10]. Many skin problems, such as eczema, psoriasis, vitiligo, and hair loss, are treated with psoralea in Indian and Chinese traditional medicine. Psoralea roots have been used to treat dental caries and leprosy, while the leaves have been used to cure diarrhoea and dental caries [11-12].

Carbohydrates, glycosides, flavonoids, tannins, saponins, and phenolic compounds, gums and mucilages, fixed oils, and fats were found in *Psoralea corylifolia* leaf extracts made in solvents such as water, ethanol, and methanol. Because of their broad-spectrum antibacterial action, *P. corylifolia* leaves are prospective indigenous pharmaceuticals [13].

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As a result, in this study, the aqueous leaf extract of *Psoralea corylifolia* was used as an organic source for the biosynthesis of silver nanoparticles of silver nitrate, and these silver nanoparticles were characterised by UV-Vis absorption spectrum, FTIR, XRD, and TEM and used to study their antibacterial activity against oral pathogens *Lactobacillus fermentum, Streptococcus mutans, Streptococcus oralis,* and *Candida albicans*

II. METHODOLOGY

2.1 Preparation of leaf extract.

In a 250 ml beaker, 10 gm leaf powder was placed, 100 ml distilled water was added, and the mixture was heated for 15 minutes at 1000 C, cooled, and filtered using a Whatman filter paper number 1 before being collected in a sterilized, weighted conical flask. The water was dried in a water bath at 80-90^0 C after the whole extract was collected. The extract flask was weighed once the water had completely evaporated. The weight of the empty flask was subtracted from the weight of the flask containing dried extract to determine the amount of extract [14, 15].

2.2 Synthesis of silver nanoparticles.

To get a 100 mg/ml concentration of leaf extract, it was dissolved in dimethyl sulfoxide (DMSO). In a 250 ml flask containing 100 ml of 0.1M silver nitrate, 3 ml of this leaf extract was added and labeled as 'E' [16]. The control was obtained by adding 3 ml DMSO in 100 ml 0.01M silver nitrate and labeled it as 'Ec'. Both flasks were shaken for 5 hours on a rotary shaker at room temperature and 120 rpm.

2.3 Characterization of silver nanoparticles

The color change was observed as an indication of SNPs synthesis. Preliminary confirmation of SNPs synthesis was done by UV-Vis spectral analysis using Systronics double beam UV-Vis absorption spectrophotometer 2202 with a resolution of 2 nm between 200 to 800 nm at room temperature. After confirmation, the sample was centrifuged at 12000 rpm for 10 min at room temperature and washed with deionized water to remove unreacted silver nitrate and plant material. Centrifugation and resuspending were repeated thrice to ensure better separation and dried in the microwave oven. FTIR spectrum was checked at transmission from 400 to 4000 cm^{-1} on Perkin Elmer Spectrum version 10.5.3. XRD analysis was done at wavelength 1.5406 Å using X-ray Diffractometer Ultima IV, Rigaku Corporation, Japan. TEM analysis was carried out on transmission electron microscope model: Jeol JEM1400 (Jeol Ltd,Tokyo, Japan) with resolution point 0.38 nm / lattice 0.2.

2.4 Determination of minimum inhibitory concentration of silver nanoparticles.

The Clinical and Laboratory Standards Institute (CLSI) standards M07-A8 [17] were used to determine the MIC of described silver nanoparticles using a microdilution quantitative technique. In all wells of a 96 well microtiter plate, 100 ml of sterile brain heart infusion broth was filled with a sterile micropipette, and then 100 ml of silver nanoparticle solution (E) (1000 g/ml) was added and thoroughly mixed. By removing 100 ml from the first well and adding it to the second well, and so on until the tenth well was reached, 100 ml from the last tenth well in each row was discarded, resulting in a 100 ml solution in each well. Only BHI medium was used in the last two wells of each row as a positive and negative control. Dilutions of the conventional antibiotic Chlorhexidine(C) and silver nitrate (A) were made in the same way. 24 hours old microbial cultures, i.e. *Streptococcus mutants, Streptococcus oralis, Lactobacillus fermentum, Candida albicans* were suspended in sterile distilled water, and the suspension was adjusted to get 10^{8}CFU/ml culture by comparing with McFarland standard 0.5(CLSI M7-A10). 10 µl of this suspension was inoculated up to the 11th well in each respective row. 12th well in each row was kept un-inoculated, plates were incubated at 37^{0}C for 24 hours, At the end of incubation,20 µl of 0.002 % resazurin (Alamar blue) was added to each well and observed for reduction of resazurin by a color change from blue to pink and results were noted down [18].

2.5 Biocompatibility of silver nanoparticles

The effect of silver nanoparticles on in vitro growth suppression was determined by spectrophotometric determination of MTT [3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] conversion into "Formazan blue" by live cells (L929cell line). In a 96 well microtiter plate, 50 µl of cell suspension containing 10^5 cells/ml was added into

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each well, and the final volume was increased to 150 µl by adding DMEM (Dulbecco's Modified Eagle's Medium) medium. In the DMEM medium. 1000 µg/ml stock solution of silver nanoparticles was prepared in sterile deionized water and dilutions from 500 µg/ml to 12.5 µg/ml were generated. 100 µl of the silver nanoparticles at various concentrations were added to the wells and incubated for 24 hours at 37^0C in a CO2 incubator in the presence of 5% CO2. After 24 hours, the wells were filled with 20µl of 5 mg/ml MTT reagent. The plate was kept in a dark place at room temperature for 4 hours of incubation. (Because the MTT reagent is photosensitive, the plate was covered with aluminum foil.) The supernatant was carefully removed without disturbing the Formazan crystals which had formed, and 100m1 of DMSO was added to dissolve the crystals. Optical density (OD) was measured at a wavelength of 492 nm. The study was performed in triplicates. The result represents the mean ± SD of three readings.

Microbial biofilms form on oral surfaces, such as the surfaces of teeth. The oral biofilm is adapted to the oral environment, maintains oral tissue integrity, and maintains a balance with host defenses. When this equilibrium is tipped in favor of the biofilm, illness can result. According to the World Health Organization, biofilm infections cause 65-80% of bacterial illnesses in people. Dental caries and periodontal disorders account for 60 to 90 percent of all oral diseases worldwide [19]. The silver solution, specifically silver diamine fluoride (Ag [NH3] 2F), has been used to prevent dental cavities for this reason [20-22]. *Streptococcus mutans, Streptococcus oralis, Lactobacillus fermentum,* and *Candida albicans* were adopted to examine the potential use of silver nanoparticles to eliminate several oral pathogens linked with oral illnesses and dental caries.

III. RESULTS AND DISCUSSION.

3.1 Biosynthesis of silver nanoparticles

The synthesis of Silver nanoparticles is indicated by the dark brown hue in flask 'E' as illustrated in photo plate 2. Variations in color from golden to dark brown or ruby red have previously been attributed to plant extracts reducing silver nitrate and producing silver nanoparticles [23-24].

0 hours **5 hours**

Figure 1. Organic synthesis of silver nanoparticles
E - 50 ml 0.01M Silver nitrate + 1.5 ml aqueous leaf extract.
Ec - 50 ml deionised water + 1.5 ml aqueous leaf extract.

3.2 Characterization of organically synthesized silver nanoparticles:

3.2.1 UV-Vis absorption spectrum of Silver nanoparticles.

The peak of the absorption spectrum was found to be at 418 nm (Figure2). According to previous studies, UV-Vis absorption peaks of silver nanoparticles have been obtained between 400 to 500 nm [23-24].

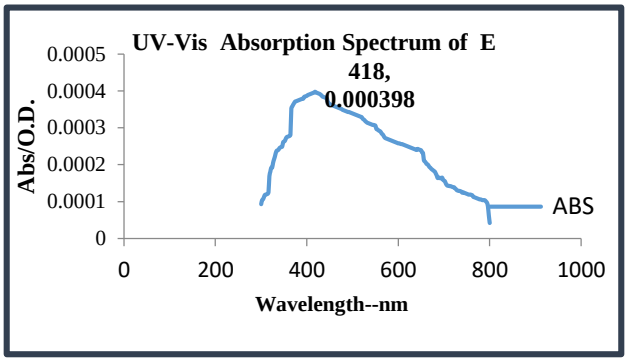

Figure 2. UV-Vis. absorption spectrum of silver nanoparticles.Wavelength 300-800.
Note: E= Silver nanoparicles of AgNO3 synthesized by using aqueous leaf extract of *Psoralea corylifolia L*

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR) Study of Silver Nanoparticles.

The current study's FTIR analysis revealed sharp peaks at 3307, 2115, 1635, and 487.5 cm-1 (Figure 3 and Table no 1). The availability of several functional groups in biomolecules responsible for the organic reduction of Ag+ and the capping/stabilization of silver nanoparticles was determined using FTIR analysis. Organic biomolecules have functional groups that play a crucial role in the formation of silver nanoparticles, contributing to silver nanoparticle reduction and capping, which may be measured using FTIR. If the

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functional groups remain in their original places, it means the nanoparticles have been reduced and synthesized. Flavonoids with functional groups as –OH, -CHO, C-C, -COOH, CH3 help to reduce and synthesize Silver nanoparticles [25].

To identify the functional groups, observed intense bands were compared with standard values. The following Table 1 shows the presence of chemical groups in the sample [26].

The presence of-OH, alkyne amide, and sulphide groups in this study [26-27] indicate the presence of phenolics, Saponins, and flavonoids, as well as sulphides and proteins. Absorption at 487.05 cm-1 suggests Ag-O stretching, but absorption below 1000 cm-1 indicates metal interatomic vibrations [28]. Bonded -OH group indicates phenol group may be involved in the capping of Silver nanoparticles. Flavonoids, phenolics, carbohydrates, and proteins have all been discovered in Psoralea corylifolia aqueous leaf extracts [29]. Based on prior research and our findings, silver nanoparticles have been synthesized and aqueous leaf extract of *Psoralea corylifolia (Linn)* has been used.

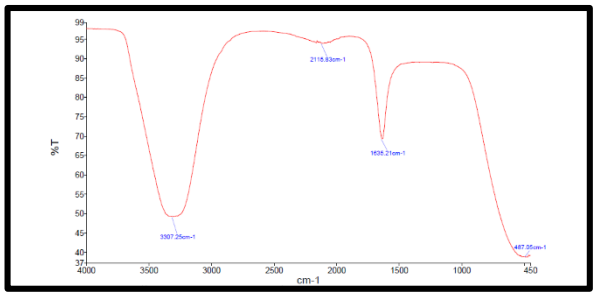

Figure 3. FTIR spectrum of Silver Nanoparticles (E)

Table 1 Functional groups obtained FTIR spectra of SNP

Wavelength cm^{-1}	Bond	Functional group	Group band cm^{-1}
3307.25	H-bonded -OH	Alcohol or phenol	3600-3200
2115.83	C≡C-C	Alkyne	2260-2110
1635.21	N-H bending	Amide	1660-1560
487.05	S-S	Sulfide	500-470

3.2.3 X-ray diffraction study of silver nanoparticles.

The particle size of silver nanoparticles was calculated using the formula,

$$D = \frac{K\lambda}{\beta cos\theta} \quad (1)$$ -- [30].

D = Crystal size

K = Scherer constant/ crystalline-shape factor.

Λ = Wavelength of X-radiation in Å.

B = the half-high width of the diffraction peak of the sample

θ (Degree) = Angle between an incident beam of X-Ray and crystallographic plane(diffraction angle).,

The chart in Figure 4 shows the XRD pattern of silver nanoparticles, We obtained the average size of Silver nanoparticles equal to 28.73757 nm.

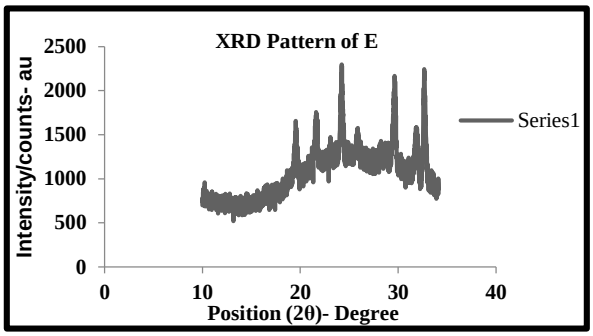

Figure 4. X-Ray diffraction Pattern of Silver nanoparticles

***Note: au- Arbitrary unit.**

Previous studies also demonstrated that silver nanoparticles synthesized using plant extracts have sizes ranging between 20 to 32 nm [25].

2.2.4. Transmission electron microscopy (TEM) study of silver nanoparticles:

The size and shape of silver nanoparticles have been confirmed by TEM examination. Silver nanoparticles have an average size of roughly 25 nanometers. Nanoparticles, on the other hand, have an oval to a spherical form, as shown in Figure 5.

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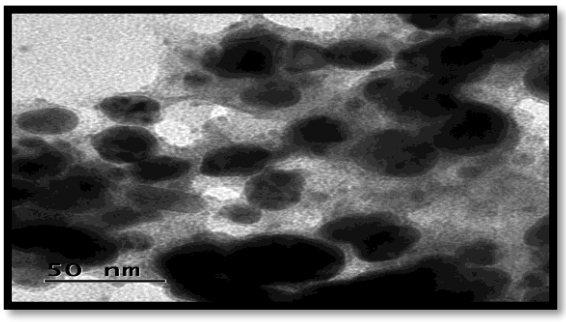

Figure 5. TEM micrograph of silver nanoparticles

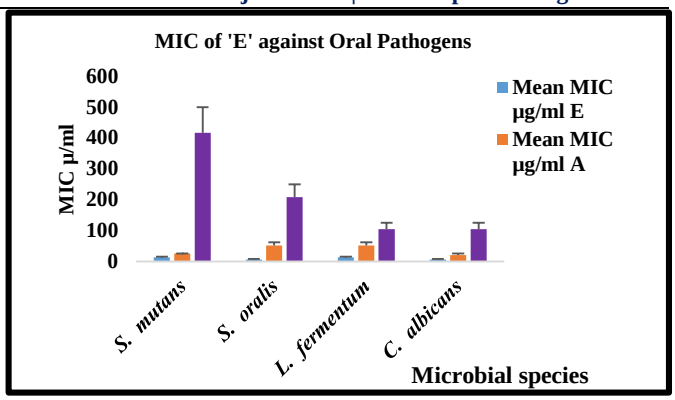

Figure 6: MIC of Silver nanoparticles against Oral Pathogens. Note: E - Silver Nanoparticles synthesized by using aqueous leaf extract of *Psoralea corylifolia* Linn. A =Silver Nitrate ($AgNO_3$), C = Chlorhexidine (Antibiotic used in Mouth wash for control of Oral Pathogens).

3.3 Determination of MIC of silver nanoparticles against oral pathogens.

Null hypothesis: No significant difference was found when minimum inhibitory concentration (MIC) of silver nanoparticles (E) and silver nitrate (A) and antibiotic chlorhexidine(C). MIC of organically synthesized silver nanoparticles against oral pathogens is shown in table 2, figure 6,

Table 2: MIC of Silver Nanoparticles against Oral Pathogens

Microbial species	Mean MIC µg/ml ±SD			*P* value
	E	A	C	
S. mutans	13.08 ± 4.51	26.04 ±0.02	416.66 ±144.33	P1,P2 = 0.01<
S. oralis	6.51 ±2.25	52.08 ±18.04	208.33 ±72.16	
L. fermentum	13.08 ± 4.51	52.08 ±18.04	104.16 ± 36.08	
C. albicans	6.51 ± 2,25	20.83 ± 9.02	104.16 ± 36.08	

***Note = P1 = P-value for comparison of MIC of silver nanoparticles with Silver nitrate and**

P2- P-value for comparison of MIC of silver nanoparticles with MIC of antibiotic chlorhexidine.

The MIC of Silver nanoparticles is significantly lesser than that of Silver nitrate and chlorhexidine.

3.4 Biocompatibility of silver nanoparticles.

The viability of cells diminishes as the concentration of Silver nanoparticles increases, as seen in Table 3 and figure 7. The maximum cell viability i.e. 98.1% could be seen in cells treated with SNPs at a concentration of 15.625 µg/ml, followed by 89.6 % at 31.25 µg/ml, 75.7 % at 62.5 µg/ml, and 72.7 % at 125 µg/ml. These results indicated that up to a concentration of 62.5 µg/ml, silver nanoparticles aren't very hazardous. There have been no previous studies on the cytotoxic activity and biocompatibility of silver nanoparticles synthesized using *Psoralea corylifolia.*

Table 3. Cytotoxic activity of silver nanoparticles (E)

Concentration µg/ml of E	% cell viability – Mean ± SD
500	61.1 ± 0.2
250	67.5 ± 0.264
125	72.7 ± 0.264
62.5	75.7 ± 0.2
31.25	89.6 ± 0.556
15.625	98.1 ± 0.5

*** Note- Biocompatibility of Silver nanoparticles (E) has been expressed in terms of mean % cell viability ± SD (n=3).**

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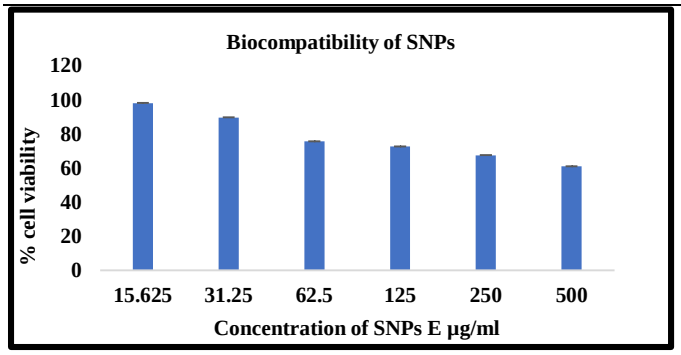

Figure 7. Cytotoxicity of silver nanoparticles E

IV. CONCLUSION

Using aqueous leaf extract of *Psoralea corylifolia* Linn, we attempted a simple one-step procedure for the chemical synthesis of silver nanoparticles. UV-Vis absorption spectrum analysis revealed maximal absorption at 418 nm, enabling for detailed characterization of these silver nanoparticles. The average size of these nanoparticles was 28.737 nm, according to XRD analysis. The formation of nanoparticles and the involvement of *Psoralea corylifolia* aqueous extract in their synthesis were confirmed by FTIR analysis. The size and form of nanoparticles were confirmed by TEM analysis; the size was found to be between 20 and 30 nm, and the shape was oval to spherical.

As a result of all of these findings, we can conclude that silver nanoparticles were generated organically from Silver nitrate utilizing *P.corylifolia* aqueous extract.

Against four oral pathogens, *Streptococcus mutans*, *Streptococcus oralis*, *Lactobacillus fermentum*, and *Candida albicans*, the minimum inhibitory concentration of organically synthesized silver nanoparticles was determined. The MIC of silver nanoparticles was found to be significantly lower than that of silver nitrate and chlorhexidine. Chlorhexidine is an antibiotic that is found in mouthwashes and is used to treat oral infections.

We put up a null hypothesis that there is no significant difference between the activity of silver nanoparticles and that of silver nitrate and antibiotic chlorhexidine, and calculated P-value using Microsoft Excel, and obtained P = 0.01 0.05 significance threshold. As a result, the null hypothesis was rejected.

Hence, the silver nanoparticles have been found significant antimicrobial activity at very low concentrations, such as 13.08 µg/ml and 6.51 µg/ml, and that they are highly biocompatible at these concentrations. All of these findings suggest that silver nanoparticles have a promising future in preparation of formulations for the treatment of dental caries..

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Conflict of interest statement: Authors confirmed that no potential conflicts of interest are involved in the current study.

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APPENDICES

Appendix1.

Materials

Psoralea corylifolia leaves, Silver Nitrate (AR 290, Himedia Laboratories Pvt Ltd), De-ionized water, Dimethyl sulfoxide (DMSO 23125-Fischer Scientific), Mercuric Chloride extra pure (GRM 1076, Himedia Pvt Ltd), Brain Heart Infusion Broth (M210-Himedia,Pvt,Ltd,India), Agar Agar (GRM 666-Himedia), Mitis Salivarius Agar Base (M259- (M096-Himedia). Resazurin (Alamar blue) A.R.,

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Chlorhexidine (0.2 percent W/V (Dr Reddy's laboratory,India), Chlorhexidine (0.2 percent W/V Dr (Reddy'laboratory,India), Resazurine (0.2 percent W/V (Dr Reddy's laboratory,India), Resazurine (RM124 Himedia Pvt Ltd)

Microbial species *Streptococcus mutans* MTCC 497, *Streptococcus oralis* MTCC 2696 *lactobacillus fermentum*, MTCC 903, *Candiad albicans* NCIM347 were procured from Microbial Type Culture Collection Chandigarh, India, and National Collection of Industrial Microorganisms, Pune, Maharashtra, India.

Appendix 2

Collection of sample

Psoralea corylifola leaves were collected from wayside plants on the outskirts of Latur city in December, identified, and confirmed by the botanist (Head, Department of Botany, Dayanand Science College, Latur, Maharashtra, India). Leaves were brought into the lab, cleaned under running water to remove dust and grime from the surface, drained entirely through a clean sieve, and sun-dried for four days. In a sterilized big Petri plate, dried leaves were disinfected with ethanol for five minutes, then the surplus ethanol was drained and allowed to evaporate while the Petri plate was kept under laminar airflow. When all of the ethanol had been evaporated and the leaves had beendried, they are pulverized in a disinfectant.

BIOGRAPHY

Author 1. Corresponding author.

Dr Ayesha Giasuddin Siddiqui.
Email: aaysha.siddiqi@yahoo.com
MSc, PhD Microbiology, NET (Life Sciences)
Assistant professor in Microbiology. Dayanand Science College, Latur, Maharashtra, India.
25 years' experience of teaching in Microbiology
Research area: Microbiology, Biotechnology, Bioferilizers, Phytomedicin, Nanobiotechnology. Authored 12 research articles in national international journals.

Author 2.

Author Prof Dr Rajendraprasad S. Awasthi
Email: rsawasthi114@gmail.com.
The author is the Principal at Shivaji College, Renapur, District, Latur, and Maharashtra, India. He has 34 years of experience in teaching and research. He is recognized PhD Supervisor in Microbiology at SRTMU, Nanded and BAMU, Aurangabad, Maharashtra, India. His research areas are, single cell oils, biofrtilizers, endophytes, effluent treatment, Medical Microbiology, Nanobiotechnology. He has authored 9 books and many research articles in reputed journals.

Author 3.

Dr Prasanna T. Dahake:
Email: prasannadahake@gmail.com.

The author is an Associate Professor, Department of Pedodontics and Preventive Dentistry MIDSR Dental College, Latur, Maharashtra, India. His research is focussed on Endodontic Microbiology of primary and young permanent teeth - Formulation of obturating material and intracanal medicament for permanent - Effect of Biomaterials in Dental pulp stem cells - Oral pathological lesions and conditions in children. He has published many research articles in reputed national and international journals.

Author 4.

Dr Yogita Deshmukh
Email: yogita.deshmukh2@gmail.com