



Enhanced Antibacterial Activity of Silver Nanoparticles Synthesised Using *Symplocos Racemosa*

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ABSTRACT

The green synthesis methods for the nanoparticles are economically feasible. It does not require any adverse effect causing agents such as usage of temperatures, toxic chemicals, energy, high pressure in the scientific and medical applications. Oral pathogens such as *Streptococcus mutans* and *Lactobacillus sp* is the major causes for many oral diseases. The different types of antibiotics are used for the controlling of many oral diseases. In this study we enhance the activity of nanoparticles by adding antibiotics to control the oral pathogen growth in vitro. The silver nanoparticles are prepared from *Symplocos racemosa* plant extract. To study and compare about the enhanced antibacterial activity of silver nanoparticles synthesised using *Symplocos racemosa*. The plant extract was prepared by using double distilled water. The microorganisms such as *Streptococcus mutans*, and *Lactobacillus sp* were chosen and cultured in respective culture media. The silver nanoparticles synthesised is introduced into cultured petri plates to observe the changes. The zone of inhibition of the growth of microorganisms was studied. The effect of the prepared silver nanoparticles is compared using effect with the normal antibiotics. As a result, it is evident that there is an increase in zone of inhibition comparing the normal antibiotic effect. Thus it can be used as drug of the choice in future.

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INTRODUCTION

Symplocos racemosa Roxb, which is otherwise called as lodhra in Sanskrit language belongs to a Symplocaceae family (Acharya *et al.*, 2016). Symplocaceae is a unigeneric family. This plant is found all over the tropical and sub-tropical countries, since

it is evergreen tree and it is small in size. Plants in this family are shrubs and trees with white or yellow flowers. Ethno botanical literature stipulate the medical and commercial uses of *S. racemosa* in carious treatment such as disease related to eye, skin, ear, liver. It is also used in treatment of bowel complaints, uterine disorders, bleeding gums, tumours, spongy gums, fever, snake bite, asthma, arthritis and gonorrhoea (Wang *et al.*, 2004).

According to phytochemical studies, the availability of numerous phenolic glycosidic components such as symplocoside, acetyloleanolic acid, triterpenoids like betulinic acid, oleanolic acid. It also indicates that there is a presence of flavonoids like. The above mentioned components may have put up to the observed protective effects. Numerous ethno botanical profess have been assured by both stematic in-vitro and in-vivo biological and pharmacological regards upon dissimilar and completely different stem bark extracts and isolated constituents (Menon

et al., 2018). Nano biotechnology is considered as one of the interesting and current emerging technical assert which is regarded for the preparation of dependable methodology of materials of Nano scale, which is eco-friendly since it is prepared using biological sources. It is of great exclamation to imagine spherical particles which is of consistent size. A decline or reduction in the size of the particles from 1nm to 10 nm ends in the enhancement of the contact surface area which is measured around 109 times larger. Therefore, surface area of large size is widely expected to enhance and for the enrichment of the extent of bacterial elimination (*Agarwal et al.*, 2018).

Nanoparticles are small sized particles which synthesised by series of different chemical and physical methods. The methods by which nano particles are synthesised include photochemical method, reduction through chemical method, γ -radiation and ablation by laser. Silver nanoparticles of silver of size between 1 nm and 100 nm in size (*Rajeshkumar and Naik*, 2018). Nano particles synthesised using biological pathways are economically feasible. It does not use any chemicals which is toxic in nature, high pressure, temperatures, energy that might possess any adverse effect in the medical and biological applications. silver nanoparticles which are frequently used are spherical in shape. It is also available in shapes such as octagonal, diamond, and thin sheets which are also popular in market. Wet synthesis method is mostly used for the preparation of silver Nanoparticles which includes mainly the usage of reducing sugar, silver mirror reaction, sodium borohydride reduction and citrate reduction, polyol process, growth mediated by seed, growth which is light-mediated (*Rajeshkumar and Naik*, 2018; *Menon et al.*, 2017). The above mentioned method can used separately to synthesis silver Nanoparticles, or collective union above mentioned methods, offers the size distribution control. It also attribute in the distributions of geometric arrangements of the nanoparticle in different degree proportion (*Santhoshkumar et al.*, 2017; *Rajeshkumar and Bharath*, 2017).

New way of synthesis of silver Nano particles through green ultrasonically-assisted synthesis is as upcoming technology. Ultrasound treatment mediated synthesise of silver nanoparticles (AgNP) are done with natural stabilizer such as κ -carrageenan. The above mentioned ultrasonic treatment reaction is done at required temperature. As a result it produce silver nanoparticles with structure of fcc crystal and mainly without impurities. The κ -carrageenan concentration is mainly used to influence the distribution of particle size of the prepared

silver Nanoparticles (*Agarwal et al.*, 2017; *Rajeshkumar*, 2016).

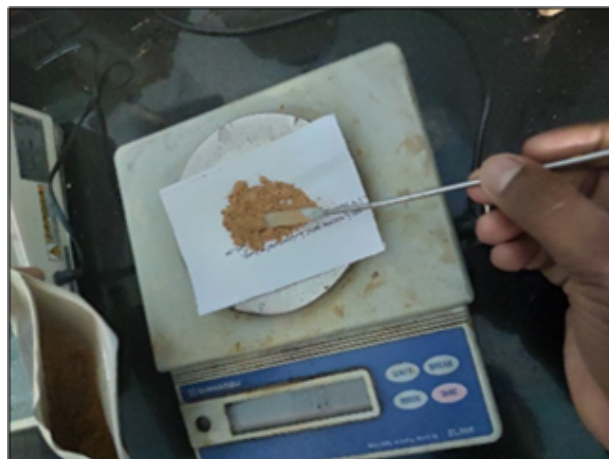


Figure 1: Preparation of Plant Extract

Moreover, synthesis of silver nanoparticle mediated by the plant extracts are considered to be more advantageous comparing the other biological processes. The reason behind this advantage is that it eliminates the long process of culturing the cells and even maintaining the texture, size and characters of the cells. It also can satisfy the production of large scale.

Comparing the convenience of the silver nano particles preparation and its uses, silver nano particles is given great importance which is contributed by its optical property, catalytic nature, electrical and mainly antimicrobial uses (*Annamalai et al.*, 2019; *Sathvika et al.*, 2019). The synthesis of Ag NPs naturally consist of three main steps. Those steps are categorised based on green chemistry nature i.e,

1. selection of the solvent medium,
2. Stability of the silver nano particles are enhanced by choosing nontoxic substances and
3. eco-friendly reducing agent selection. The main objective of the study is to determine the enhanced antimicrobial property the silver nanoparticles obtained from *Symplocos racemosa*.

MATERIALS AND METHOD

Preparation of Plant Extract

The preparation of plant abstract include collection of plant abstract as form of powder (Figure 1). Before using the glassware, it is washed correctly using distilled water and then dried using hot air oven.



Figure 2: Silver Nanoparticles preparation

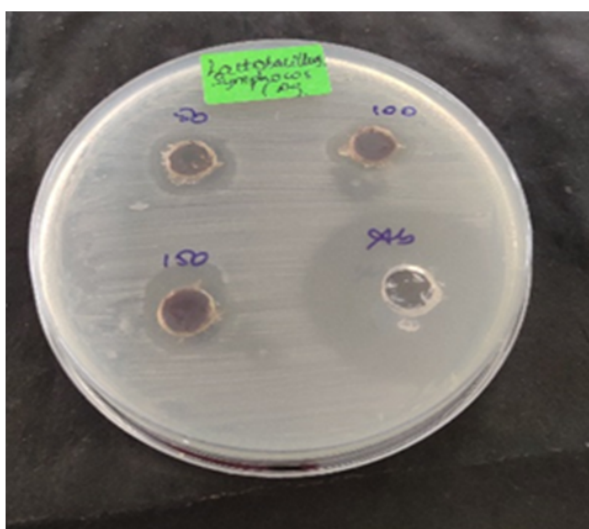
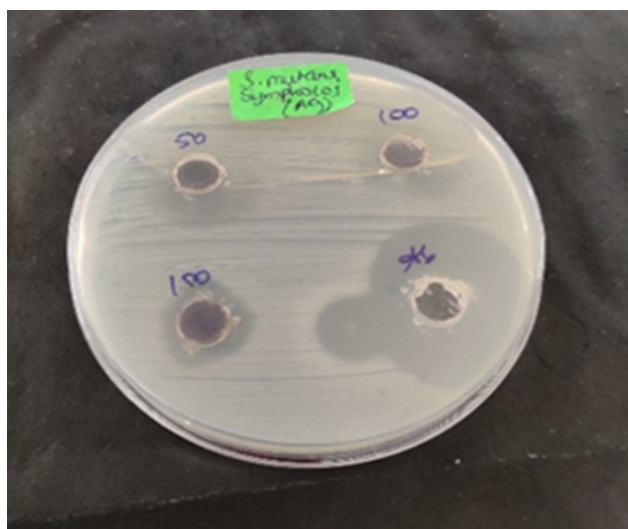


Figure 3: Antibacterial Activity



Figure 4: Enhanced Antibacterial Activity

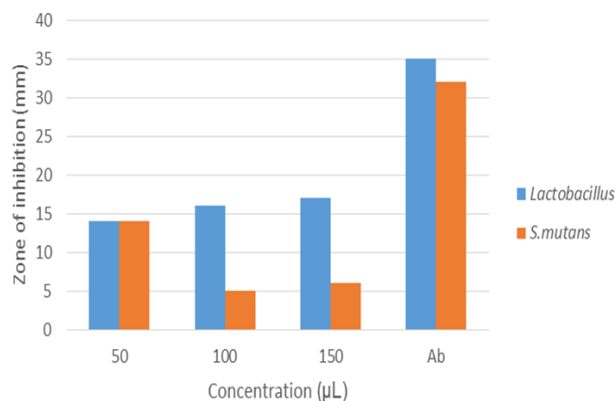


Figure 5: Antibacterial Activity Property

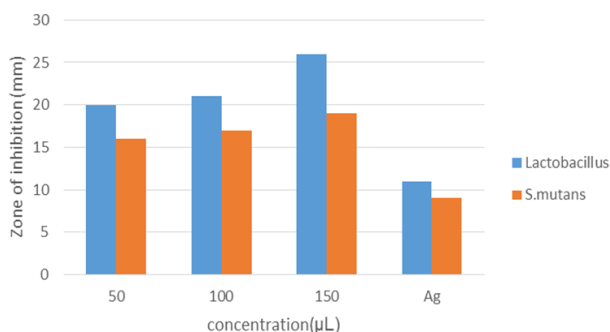


Figure 6: Enhanced antibacterial activity

The plant powder which weighs about 1g were taken and it is mixed with quantity of 100 ml of distilled water in a cleansed conical flask. The solution is now boiled for 5 to 10 minutes and it is filtered through filter paper. The extract thus obtained is filtered and used for further experiment.

Preparation of Silver Nanoparticles

For the silver nanoparticles synthesis, preparation of 0.0169 g of AgNO₃ [silver nitrate] solutions is done. The prepared solution is then mixed along with quantity of 90 ml of distilled water. Now it is mixed with the 10 ml of plant extract solution. Thus total quantity of 100 ml of mixture of silver nitrate and *Symplocos racemosa* solution is obtained. The flask is incubated at 37 degree Celsius in order to resist from the inactivation mediated by light and silver nitrate contamination. The silver Nanoparticles obtained from the above process is purified by means of centrifugation technique at the rate of 10000rpm for 15 minutes.

Test Pathogens Used

Antimicrobial activity of the green synthesised the prepared Nanoparticles is tested against variety of pathogenic microorganisms. The test pathogens were collected from microbiology lab. For observing the antimicrobial activity, species such as gram positive aerobic streptococcus mutans which is com-

monly found in oral cavity and anerobic Lactobacillus were chosen.

Antibacterial Properties of Prepared Silver Nanoparticles

Standard Kirby–Bauer disc diffusion assay is used to determine the bactericidal property of the prepared Ag NP. The main test organisms are kept inside the respective nutrient broth for about 24 hours and preserved for further experimental procedures. The plates made of agar are then sterilized by vaious procedure and solidified later. Following the preparation of nutrient agar, corresponding organism are spreaded consequently on the nutrients of repective test pathogens of the petriplates which is mediated by sterile glass rod. Sterile glass rod is used in order for obtainingperfect lawns of bacteria. After this procedure, silver Nanoparticles are loaded onto corresponding disc which is of required volumes such as 50 micro litre, 100 micro litre and 150 micro litre and incubated at the level of 37 degree Celsius for the time of 24 hours. After the period of incubation, the appearance of zone which is clear around each disc depicts the exact bactericidal property confirmation. Meter ruler is used to measure the exact measurement of the zone of inhibition and mean values for the organisms were clearly recorded. The recorded values are represented in millimeters.

Table 1: Antibacterial Activity of silver Nanoparticles

Concentration	50	100	150	Ab
Species	μL	μL	μL	
Zone of inhibition (mm)				
Lactobacillus ap	14	16	17	35
S. mutans	14	5	6	32

Table 2: Enhance Antibacterial Activity

Concentration	50	100	150	Ab
Species	μL	μL	μL	
Zone of inhibition (mm)				
Lactobacillus	20	21	26	11
S. mutans	16	17	19	9

Enhanced Antibacterial Activity

Current antibiotic NOVOMAX is taken and mixed with the solution of prepared silver Nanoparticles and checked for the antibacterial inhibition zone. Same method of measurement of the zone of inhibition and mean value is done. It is mentioned in milimeters.

RESULTS AND DISCUSSION

Visual Observation

Some of the properties of metal nanoparticles such as physiochemical and optoelectronic properties are observed. It is mainly based on the special characteristics such as distribution, size and morphology of the particle (Agarwal *et al.*, 2018). The colour change indication of the nano particle synthesis (Figure 2). Comparing the nanoparticles which is known to us, Silver is the particle which has been studied for its unique properties such as spectroscopic, antimicrobial, optical, catalytic and SERS properties usually. The reduction in the silver nitrate component from plant extract was done slowly in general, but it has a main advantage of producing uniform size nanoparticles and stable without the involvement of any additional chemical stabilizers (Rajeshkumar *et al.*, 2017; Kumar and Rajeshkumar, 2017).

UV-Vis Spectroscopy

The colour change is observed using UV spectrophotometry. one of the most sensitive methods of spectroscopy is the surface Plasmon resonance based optical sensor for of heavy metals detection. It is considered as one of the simple, sensitive, time consuming, rapid, inexpensive, and selective method for analysis of heavy metal ions. It is more advantageous comparing other techniques used previously because of its simplicity, inexpensive and rapid property (Annamalai *et al.*, 2019).

Antibacterial Activity

The Tables 1 and 2, Figures 3, 4, 5 and 6 clearly indicates the antimicrobial activity and enhanced antibacterial activity of silver nanoparticles synthesised using lodhra bark. Zone of inhibition is referred as area where growth of bacterial growth is inhibited and lysis of bacteria takes place. The area surrounding the wafer where the bacteria growth is inhibited is visible clearly. The size and extent of the inhibitory zone measured is based on the factors like how effective the antibiotic involved in terminating the bacterial growth. The other factor is based on the influence of the size of a zone during the diffusion of the antibiotic within the medium of agar. This criteria varies on the molecular configuration of the antibiotic basis. The zone of inhibition compare the database of existing antibiotics to determine suseptability of the solution and the resistance to the antibiotic.

CONCLUSIONS

Thus with increase in concentration of the silver Nanoparticles, the antibacterial activity increases. The antibacterial activity in relation with normal antibiotic is also evident from the result as shown. Thus from the study, it is evident that drug can be prepared with silver Nanoparticles and can be used as drug of choice.

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Conf lict of Interest

The authors declare that there is no Conflict of Interest.

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