ORIGINAL RESEARCH PAPER

Pharmacy

KEY WORDS: Antimicrobial activity, herbal nanoparticles.

SCREENING OF ANTIMICROBIAL ACTIVITY OF HERBAL NANOPARTICLES OF *CITRUS SINENSIS.*

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ABSTRACT

The aim of present study was to determine antimicrobial activity of herbal nanoparticles. This research tries to put formulation of herbal nanoparticles as antimicrobial, consideration with toxicity or resistance of gentamicin in health. This opens door to a completely new dimension in medicinal plant research combing the nanotechnology with herbs that is herbonanoceuticals. An increasing awareness towards green chemistry and use of green route for synthesis of metal nanoparticles lead a desire to develop environment-friendly techniques.

INTRODUCTION:

Nowadays, green chemistry is an emphasized area of research and requires some additional efforts for the implementation of sustainable methods in order to achieve the desired products as well as minimize and further eliminate the waste materials produced. Metal nanoparticles (NPs) have received significant interest in the area of scientific research and industrial applications [1] Silver (Ag) NPs have generated substantial demand not only in fundamental research and development but also at the industrial scale due to their excellent properties [2]. Different traditional methods have been employed in the production of nanosized metallic silver particles with different morphologies and sizes, for example, chemical reduction, electrochemical, photochemical, microwave-assisted, hydrothermal, laser ablation, and sol-gel methods [3–6].

Orange is one of the world's largest fruit crop with a global production of 48.8 million tons. A large portion of this production is used for the industrial extraction of citrus juice, which leads to vast amounts of residues, including peel and segment membranes. Peels represent between 50 and 65% of the total weight of the fruits and remain as the primary byproduct is rich in bioflavonoid, insoluble and soluble fibers, as well as proteins, all of which have potential applications in nanobiotechnology such as in the synthesis of nanoparticles [7,8].

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites and over 60% of people in Nigeria rural areas depend on the traditional medicine for the treatment of their ailments. Many plant species have been evaluated for their antimicrobial activity in the past 20 years. Since then efficacy of many medicinal plants in the treatment of many diseases have been put to test in many laboratories [9,10].

Taking into account that the physicochemical properties of silver nanoparticles of Citrus sinensis (Orange peel) with inhibitory capacity against microbes have led to increase in the research on herbal nanoparticles and their potential application as antimicrobials.

MATERIALS AND METHODS:

Collection of plant material: Citrus sinensis in this study were obtained from local market in kodoli (Maharashtra). The plant material was authenticated from Shivaji University, Kolhapur.
Extraction: The successive Soxhlet extraction method was used with powder: solvent ration as (1:10) and extraction period as 24 hours for each of the solvent. The methanol extract fraction was collected and concentrated to get semisolid consistency.

Formulation: For the plant broth preparation, around 10 g of the dried powder is boiled with 100 ml of distilled water. To 10 ml AgNO3 solution, on addition of 100 ml of plant extract and separately 3 % w/v (20 mL) gentamicin as standard prepared by using probe sonicator.

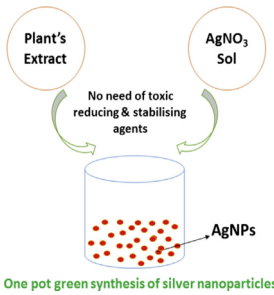

Fig. 1. Green synthesis of herbal nanoparticles

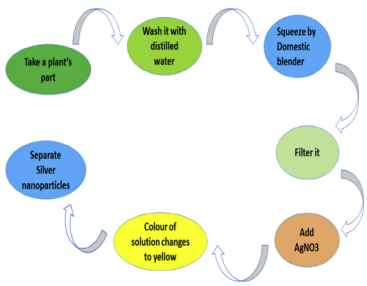

Fig.2 Protocol for synthesis of silver nanoparticles using plant extract.

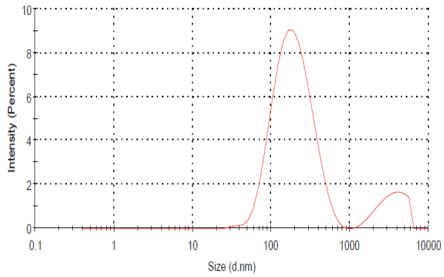

Fig. 3 Particle size distribution

Table No.1 Particle size analysis

Particle Size (nm)	Zeta Potential(mV)
180.4	17.9

Antimicrobial activity: Standard bacterial test organisms were sub cultured on freshly prepared nutrient agar and incubated at 370C in incubator for 24h. Original cultures were further stored at 40C in the refrigerator to maintain stock culture. Fresh cultures were used for testing antimicrobial activity using cup plate method.

Preparation of media: Nutrient agar: Accurately weighed 24 gm of Nutrient agar was dissolved in the 1000 ml of distilled water by heating with frequent agitation. The media was finally sterilized in autoclave at 1210C for 15 min.

Preparation of test and standard drug solution: Preparation of test extract: The test extract were prepared by dissolving 50 mg of dried extract in the 10 ml of methanol. This gives the 5 mg ml-1 of stock solution, from which 100 µl was used for test.

Preparation of standard drug solutions: Weighed accurately 100 mg gentamicin and dissolved in 100 ml of 0.1M hydrochloric acid to get 1000 g/ml stock solution. This was then diluted further with distilled water to get 10 µg/ml. of working standard.

Sterilization of Equipments and Media: Dry Heat Sterilization: All the glass wares previously washed were sterilized in hot air oven. Petri-dishes, pipettes, test tubes were wrapped separately in the paper and kept in the hot air oven for sterilization at 1800C for 1h.

Moist Heat Sterilization: Normal saline solution and nutrient Medias were sterilized in autoclave at 1210C for 15 min.

Preparation of microbial suspension: Microbial suspensions were prepared by transferring one loop full of stock culture to the 10 ml of normal saline solution.

Antimicrobial study: The antimicrobial efficiency of silver nanoparticles was determined on Staphylococcus aureus .The agar plate method was used to determine the inhibitory effect of silver nanoparticles. Also, the prepared petri dishes were incubated for 24 hours to examine any microbial growth. Microbial cultures were inoculated to prepare subcultures in agar slant. From this subculture two loops of microbial culture were seeded on prepared nutrient agar petri dish. Three wells were punched into the nutrient agar previously seeded with test organism under aseptic conditions. The wells were filled with 100 µl of test sample, standard solution and blank respectively. Gentamicin (3%) was taken as standard solution. The plates kept at 4o C and incubated for 24 hrs at 37o C and the diameters of inhibition zones were measured. The optimized test solution was compared with standard gentamicin solution. Bacterial suspensions (3 ml) were then poured in the plates. As soon as nutrient agar attained 500C temperature, 20 ml of media was poured in to the petriplates containing bacterial suspension and plates were rotated to mix the suspension with media. The diameter of zone of inhibition was accurately measured by zone reader in each treated plate as and was compared with standard at tested concentrations shown in Fig. no. 4.

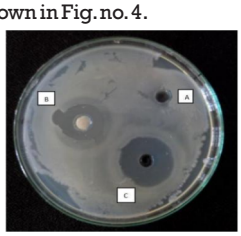

Fig. 4. Antimicrobial efficiency where A) Blank B) optimized herbal nanoparticle C) Standard

Determination of MIC: The antimicrobial activity of silver nanoparticles was checked by determination of Minimum Inhibitory Concentration. Suspension of bacterial culture was prepared and swabbed on agar plates then wells were made on the plates. Different concentrations of silver nanoparticles that are 10, 20, 30, and 40 µg/ml were added to the plates. The plates were incubated at 37°C for 24h in order to determine inhibitory growth of silver nanoparticles on Staphylococcus aureus and then observed for the MIC. The results are as given in table no 2.

Table No. 2 Antibacterial activity of silver nanoparticles:

Sr. No.	Name of organism	Zone of inhibitions		
		Silver Nanoparticles	Blank	Gentamicin (Std,)
1.	Staphylococcus aureus	9 ± 2.12 mm at 24 hour	--	12 ± 2.12 mm at 24 hour

RESULT:

The zone of inhibition in gentamicin eye solution against staphylococcus aureus was obtained 12 ± 2.12 mm at 24 hour; however the zone of inhibition of herbal formulation was observed 9 ± 1.41 at 24 hour. It was found that formulation is active against staphylococcus aureus. The silver nanoparticles had MIC of 30 µl on Staphylococcus aureus which was shown in table no.3. Further study is necessary to isolate the constituent responsible for activity from ethyl acetate and methanol extracts.

Table No. 3 Minimum Inhibitory Concentration of silver nanoparticles:

Sr. No.	Name of organism	Zone of Inhibition (mm in diameter)			
		10	20	30	40
1.	Staphylococcus aureus	15	13	9	14

CONCLUSION:

The result of this study by using agar plates suggested that herbal nanoparticle effective for antimicrobial therapy, to prevent resistance developed by gentamicin as available marketed formulation.

REFERENCES:

1. Hess O, Pendry JB, Maier SA, Oulton RF, Hamm JM, Active nanoplasmonic metamaterials, Nature Materials, 2012; 11(7):573–584.
2. Bhattarai B, Zaker Y, Bigioni TP, Green synthesis of gold and silver nanoparticles: challenges and opportunities, Current Opinion in Green and Sustainable Chemistry, 2018; 12: 91–100.
3. Gudikandula K, Charya Maringanti S, Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties, Journal of Experimental Nanoscience, 2016; 11(9) :714–721,.
4. Zhang T, Song YJ, Zhang XY, Wu JY, Synthesis of silver nanostructures by multistep methods, Sensors, 2014 ; 14 (4): 5860–5889.
5. Nowack B, Krug HF, Height M, 120 years of nanosilver history: implications for policy makers, Environmental Science & Technology, 2011; 45(4): 1177–1183.
6. Gopinatha V, Ali MD, Priyadarshini S, Thajuddinb N, Velusamy P. Biosynthesis of silver nanoparticles from tribulus terrestris and its antimicrobial activity: a novel biological approach. Colloid Surf B: Biointerface 2012; 96:69–74.
7. Tomoko N, Takashi A, Hiromu T, Yuka I, Hiroko M, Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. J. Health Sci. 2002; 48; 273–276.
8. Sieradzki K, Roberts RB, Haber SW, Tomasz A. The development of vancomycin resistance in a patient with methicillin-resistant Staphylococcus aureus infection. N. Engl. J. Med. 1999: 340: 517–523.
9. Ghani A, Abdulrahman EM, Onaolapo JA. Chemical and microbiological evaluation of some Nigerian traditional preparations. The Rep. Oct. 7. 1989.
10. B. Yang, F. Qi, J. Tan, T. Yu, and C. Qu, Study of green synthesis of ultrasmall gold nanoparticles using citrus sinensis peel, Applied Sciences, 2019; 12(9): 2423.