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Enhanced Bactericidal Activity of Green Synthesized Gold Nanoparticles from *Neptuniatriquetra*

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Abstract: ***Since the last decade, enormous research in developing biocompatible materials for biomedical applications at the Nanoscale level emerged as an excellent essential research factor globally. In this context, in the present study contemplated for developing novel nanomaterials which was synthesized in a green approach way using aqueous extracts of Neptuniatriquetra and assessed for antibacterial activities against both Gram-positive and Gram-negative bacteria. Initially the antibacterial efficiency of Neptuniatriquetra was assessed for different solvent extracts and compared with synthesized gold nanoparticles. A total of 6 bacterial strains viz., Bacillus subtilis, Escherichia coli, Klebsiellapneumoniae, Proteus mirabilis, Pseudomonas, and Staphylococcus aureuswere used in the present study. The results demonstrated that the medicinal plant Neptuniatriquetrais having high antibacterial activity. Interestingly, there is enhanced antibacterial activity of gold nanoparticles.***

Keywords: *Neptuniatriquetra*, antibacterial activity, gold nanoparticles

1. Introduction

India is a sub-continent with diverse topology and climate. It is the land of rich biodiversity. More than 70% of the world plant population is present in India.The traditional treatment systems to cure illnesses are better than conventional antibiotics. The multidrug resistance of pathogenic microorganisms was enhanced in recent years which were developed due to indiscriminate prescription of commercially available synthetic antibiotic drugs which are expensive with adverse side effects. The development of multi drug resistance in bacteria forced the researchers globally for new drug leads which can effectively destroy pathogens. Different systems of Indian traditional treatment systems such as Siddha and Ayurveda are dependent on plants and plant extracts. The herbal remedies served as source for new therapeutic agents especially as drugs and drug leads. As per the WHO estimate, approximately 25% of prescribed drugs are derived from plants and plant extracts. Majority of the synthetic drugs are chemically replicas of natural phytochemicals. Worldwide 3, 50, 000 plant species were identified and most of them are yet to be screenedfor potential capabilities to cure a large number of diseases and disorders (Amoo SO et al., 2011). The efficiency and efficacy of phytochemicals against a large number of microbes are well known (Goc A et al., 2015). The antimicrobial activity of plants is due to the presence of a large variety of phytochemicals (secondary metabolites) (Cowan MM., 1999). These phytochemicals belong to the different chemical classes, most of them include alkaloids, flavonoids, tannins, terpenoids, and polyphenols *etc*., (Abachi S et al., 2016; Wijesundara NM et al., 2018).Because of proven efficacy, effectiveness, with other characteristic features such as availability, ecofriendly, economically feasible, and side effect free nature to eukaryotic cell, the plant based extracts and molecules were represented as alternative to antibiotics (Parimala M et al., 2014).

Even though the plant extracts have bactericidal properties, still there search for developing new, novel drug leads and different methods to effectively enhance the killing capacity against the harmful pathogens. To combat the contamination of bacterial pathogens there is strong need to develop novel materials at Nanoscale level. Developing metal based nanoparticles is one such method which has proven properties of bactericidal and antimicrobial activity. The nanomaterial and composites were even acted as drug delivery systems. For example, the different metals used to reduce at Nanoscale level using plant extracts include silver (Ag) (Jain S., &Mehata MS 2017; Mmola M et al., 2016), gold (Au) (Patra JK &Baek KH (2015; Naraginti S., & Li Y 2017). The other major metals which were synthesized and tested for biomedical and pharmaceutical applications include Palladium (Pd), Platinum (Pt), Zinc (Zn), Iron (Fe), and Copper (Cu) *etc*., (Teow SY et al., 2018). In our past research gold nanoparticles were synthesized using aqueous extract of *Neptuniatriquetra* and evaluated for the anticancer activity *in vitro*. The present study was focused on antibacterial activity of synthesized and structurally characterized gold nanoparticles. The tested bacterial strains include *Bacillus subtilis*which is nonpathogenic to humans but sometimes causes food poisoning (Bandow J.E et al., 2002), extra intestinal pathogenicity was exhibited by *E. coli* (Stromberg ZR et al., 2017), *Klebsiellapneumoniae* which causes a wide range of diseases including pneumonia, urinary tract infections (UTIs), bloodstream infections and sepsis (Bengoechea, JA & Sa Pessoa J 2019).*Proteus mirabilis* causes catheter-associated urinary tract infections (CAUTI) (Armbruster CE et al., 2018). *Pseudomonas* species causes wide range of diseases and a few of them include even suppression of host immunity especially in immune compromised patients (Xin XF et al., 2018).

Staphylococcus aureus is a human pathogen which can infect wide range of tissues including gastrointestinal tract, heart and bones. It is associated with life threatening illnesses (Balasubramanian D et al., 2017). Hence all these six bacterial strains were selected for testing.

Neptuniatriquetra are small herb with yellow flowers. The plant is commonly called as "Yellow Sensitive Plant". The plant has characteristic features of small tiny shrub or herb while the leaves are tiny, small and are sensitive to touch (Thigmonasty). Flowers are yellow and small. This sensitive plant belongs to the *Fabaceae* (Touch-me-not) family. The *Neptuniatriquetra* have potential medicinal properties and a few of them include treatment to the Jaundice as well as to eye infections. As per the available research reports on the *Neptuniatriquetra*, it is revealed that the plant is less explored scientifically. The image of *Neptuniatriquetra* was represented in image-1.

Image 1: Corresponds to the *Neptuniatriquetra*. (a) Represents the whole plant of *Neptuniatriquetra*. (b) Represents the plant with stem and flower

The specific objectives of the present study were to provide the scientific basis for the medicinal properties especially antibacterial activity of medicinal plant *Neptuniatriquetra*. This plant is well known for ameliorating a large number of illnesses.The bactericidal activity against both Gram-Positive and Gram-negative bacteria were assessed and compared with the gold nanoparticles which were synthesized using aqueous extract of *Neptuniatriquetra*.

2. Materials and Methods

Plant collection: The plant *Neptuniatriquetra* was widely distributed in the state of Andhra Pradesh, India. The plant material was collected from in and around the surroundings of Guntur district, Andhra Pradesh, India. After authentication by taxonomist, a voucher specimen was deposited in herbarium. The collected plant material was washed thoroughly and shade dried. After several weeks of drying, the dried leaves were crushed and sieved through No. 22 sized mesh followed by labeling as *Neptunia*, stored in cool and dry place until further use.

Extraction of phytochemicals from *Neptuniatriquetra*:
The *Neptuniatriquetra* leaves powder was taken in a soxhlet apparatus and subjected to successive soxhlet extraction. The various solvents such as Hexane, Chloroform, and Methanol were used for the extraction of phytochemicals. After loading approximately 200gms dry leaves powder in soxhlet apparatus, the successive extraction was carried out with continuous heating and cooling for 40-50 cycles in apparatus and each extract was collected, rotary evaporated and labeled for further use.

Antibacterial activity of crude extracts from *Neptuniatriquetra*:
The anti-bacterial activity of the plant crud extracts was carried out as per the standard procedures and protocols by using agar well diffusion method. For this purpose, the crude extracts were dissolved in DMSO (Dimethyl sulfoxide) and a concentration of ($10\mu g/mL^{-1}$) was used for antibacterial activity studies. The bacterial cultures like *Bacillus subtilis* (MTCC 1034), *Staphylococcus aureus* (MTCC 6908), *Escherichia coli* (MTCC 44), *Klebsiellapneumonia*e (MTCC 9024), Proteus mirabilis (MTCC 1429) and *pseudomonas aeruginosa* (MTCC 11358) were grown in Luria broth for 24 hours at 37°C prior to use (Ginovyan Met al., 2015). Luria agar plates were prepared, sterilized and after solidification bacterial cultures were streaked. Using a cork borer 6mm diameter well was made. The wells were loaded with crude extracts (20µl-50µl) and streptomycin was used as control with a concentration of ($10\mu g/mL^{-1}$). The plates were incubated at 37°C ± 2 for 16-24hours. After the incubation the plates were observed, the zone of inhibition around the wells was measured in mm. All the experiments were carried out in triplicates and mean values were represented in results (Ginovyan M et al., 2017).

Bactericidal activity of gold nanoparticles
Antibacterial activity of Gold Nanoparticles (AuNPs) was tested by disk diffusion method (Bankar AV et al., 2010). For this purpose, nutrient broth media was preparedand inoculatedwith bacterial cultures like *Bacillus subtilis* (MTCC 1034),*Staphylococcus aureus*(MTCC 6908), *Escherichia coli* (MTCC 44), *Klebsiellapneumoniae* (MTCC 9024), *Proteus mirabilis*(MTCC 1429) and *pseudomonas aeruginosa*(MTCC 11358) and incubated at 37°C for 24 hours. The nutrient agar medium was prepared, sterilized and after solidification, streaked with the overnight grown

bacterial cultures. The disks with a diameter of 6mm were placed on the agar plates, followed by loading with synthesized and structurally characterized gold nanoparticles (Au NPs) (10-30µL). Positive control was maintained using Streptomycin with a concentration of 10µg/ mL^{-1}).The plates were kept in refrigerator at 4°C for 5-10minutes to allow diffusion of Au NPs into the disks. The plates were incubated at 37±2°C for 18-24 hours. Then the plates were observed for zone of inhibition, and measured in mm. All the experiments were carried out in triplicates and mean values were represented in results (Ahiwale SS et al., 2107).

3. Results

The bactericidal activities of *Neptuniatriquetra* plant was tested with three different solvent extracts viz., Hexane, Chloroform and Methanol against 6 different bacterial strains such as *Bacillus subtilis*, *Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*, *Pseudomonas*, and *Staphylococcus aureus*. All the crude extracts exhibited the excellent antibacterial activity. The *Bacillus subtilis* was inhibited more by Hexane and Methanol crude extracts with a zone of 21mm. *Escherichia coli*was more inhibited by methanol extract with a zone of 24mm which is almost nearer to control Streptomycin which exhibited the zone of 26mm. The Chloroform fraction effectively inhibited the *Staphylococcus aureus* and *Proteus mirabilis* with a zone of 24mm and 21mm respectively.

The antibacterial activity is associated with secondary metabolites and diverse phytochemicals. These phyto constituents are produced by plants to protect themselves against bacterial, fungal and viral pathogens. As produced naturally by plants for protection, these moieties are effective, efficient components with side effects free nature when used by humans as both plant and humans are eukaryotic system of cells whereas bacteria are prokaryotic cells. Difference in cell system is an added advantage for bactericidal activity against pathogens by plant extracts. The results of antibacterial activity of crude extracts of *Neptuniatriquetra* were represented in table-1 and image-2.

Table 1: Antibacterial activity of crude extracts obtained from *Neptunia triquetra*

S. No.	*Name of bacterial culture*	*VE (Hexane)*	*VC (Chloroform)*	*VM (Methanol)*	*Control (Streptomycin)*
1	*Bacillus subtillis*	21mm	19 *mm*	21 *mm*	24 *mm*
2	*Staphylococcus aureus*	20 *mm*	24 *mm*	20 *mm*	24 *mm*
3	*Escherichia coli*	21 *mm*	18 *mm*	24 *mm*	26 *mm*
4	*Klebsiella pneumonia*	21 *mm*	19 *mm*	21 *mm*	24 *mm*
5	*Proteus mirabilis*	20 *mm*	21 *mm*	18 *mm*	25 *mm*
6	*Pseudomonas aeruginosa*	18 *mm*	20 *mm*	16 *mm*	26 *mm*

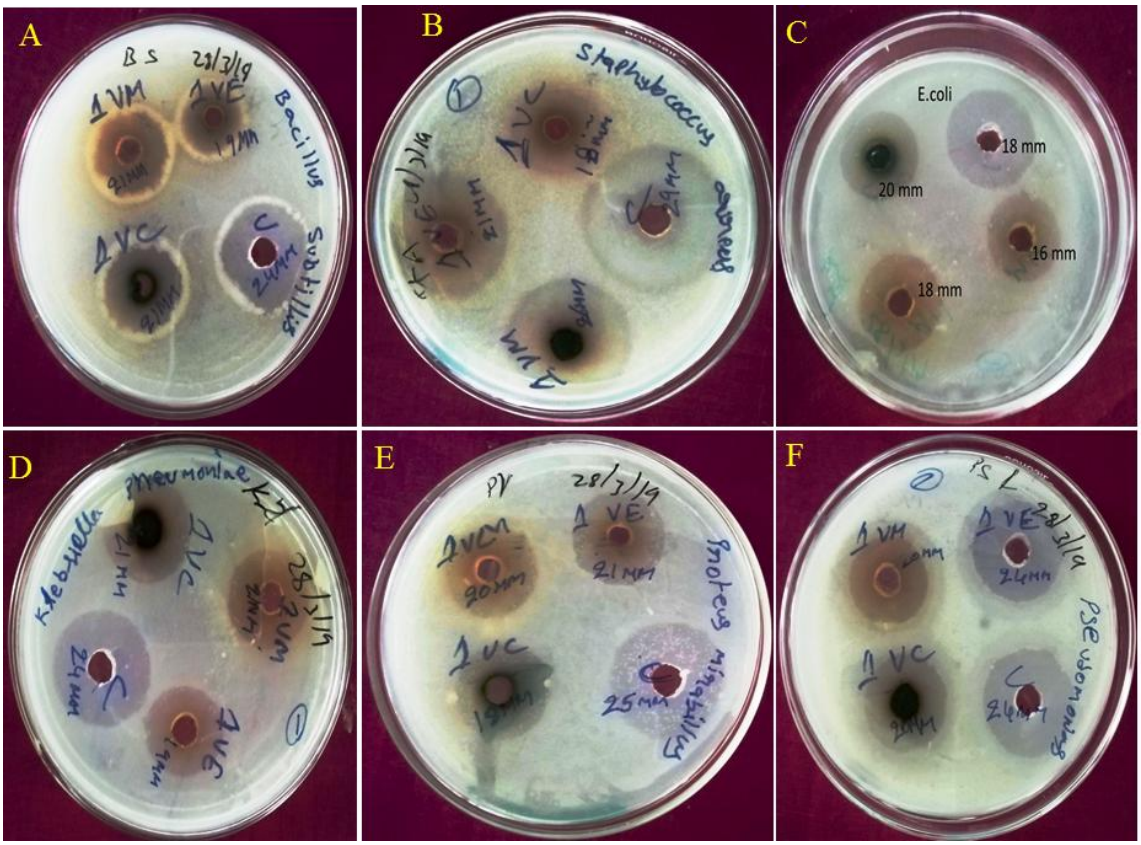

Image 2: The plates corroborates the antibacterial activity of gold nanoparticles as exhibited as zone of inhibition. Image (A) is associated with plates of *Bacillus subtilis*,Image (B) corresponds to the*Staphylococcus aureus*, Image (C) corresponds to *E. coli*, image (D) represents the *K pneumoniae*plate, image (E) represents *Proteus mirabilis*and image (F) shows the *Pseudomonas* plate

Table 2: Antibacterial activity of Gold nano particles synthesized using aqueous extract of *Neptunia triquetra*

S. No.	*Name of bacterial culture*	Gold nano particles replication 1	replication 2	replication 3	*Mean Values*	*Control (Streptomycin)*
1	*Bacillus subtillis*	25mm	29 *mm*	27 *mm*	27*mm*	30 *mm*
2	*Staphylococcus aureus*	28 *mm*	26 *mm*	28 *mm*	27 *mm*	29 *mm*
3	*Escherichia coli*	24 *mm*	22 *mm*	24 *mm*	24 *mm*	29 *mm*
4	*Klebsiella pneumonia*	24 *mm*	19 *mm*	21 *mm*	21 *mm*	28 *mm*
5	*Proteus mirabilis*	22 *mm*	22 *mm*	21 *mm*	22 *mm*	29 *mm*
6	*Pseudomonas aeruginosa*	22 *mm*	25 *mm*	26 *mm*	24 *mm*	28 *mm*

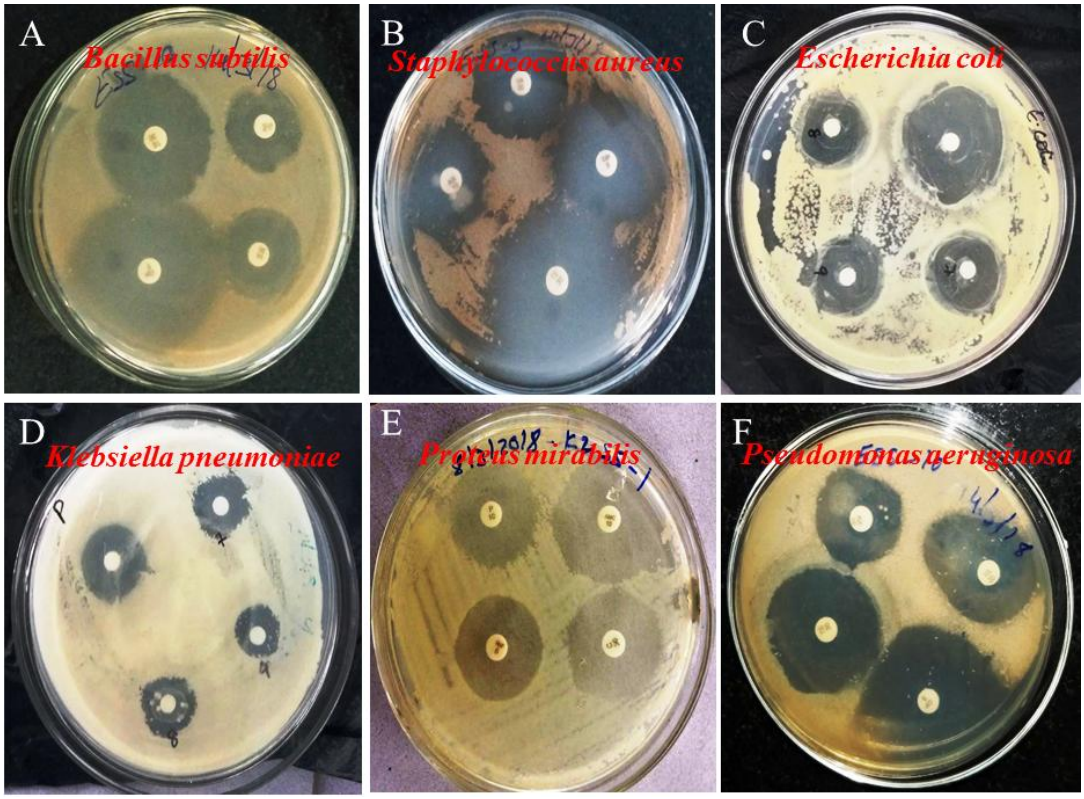

Image 3: The plates corroborates the antibacterial activity of gold nanoparticles as exhibited as zone of inhibition. Image (A) is associated with plates of *Bacillus subtilis*,Image (B) corresponds to the *Staphylococcus aureus*, Image (C) corresponds to *E. coli*, image (D) represents the *K pneumoniae*plate, image (E) represents *Proteus mirabilis* and image (F) shows the *Pseudomonasaeruginosa* plate.

4. Conclusion

The traditional medicinal plant *Neptuniatriquetra* is bestowed with diverse capabilities to cure large number of diseases associated with bacterial, fungal and viral infections. Even though the entire plant is edible and used for treatment, there is less scientific evidence for its capabilities. Hence, in the present study the bactericidal properties of crude extracts of plant *Neptuniatriquetra* were tested and identified that the Methanolic extracts have high potential antibacterial activity when compared with other extracts. Interestingly there is enhanced antibacterial activity for gold nanoparticles when compared with crude extracts. The present experiments were conducted *in vitro*. There is strong need in the future to test the antibacterial activity *in vivo*.

References

[1] Goc, A., Niedzwiecki, A., &Rath, M. (2015).In vitro evaluation of antibacterial activity of phytochemicals and micronutrients against Borreliaburgdorferi and Borreliagarinii. *Journal of applied microbiology*, *119*(6), 1561–1572. doi:10.1111/jam.12970.

[2] Amoo SO, Ndhlala AR, Finnie JF, Van Staden J. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three Barleria species. South African Journal of Botany.2011; 77:435–45.doi: 10.1016/j.sajb.2010.11.002.

[3] Parimala M, Shoba FG. In vitro antimicrobial activity and HPTLC analysis of hydroalcoholic seed extract of NymphaeanouchaliBurm. f'. BMC Complement Alt Med. 2014;14:361. doi: 10.1186/1472-6882-14-361.

[4] Abachi S., Lee S., Rupasinghe H.P.V. Molecular mechanisms of of *streptococcus* species by phytochemicals inhibition. Molecules. 2016;21:215. doi: 10.3390/molecules21020215.

[5] Wijesundara N.M., Rupasinghe H.P.V. Essential oils from *Origanumvulgare* and *Salvia officinalis* exhibit antibacterial and anti-biofilm activities against *Streptococcus pyogenes*. Micro.Pathogenesis. 2018;117:118–127. doi: 10.1016/j.micpath.2018.02.026.

[6] Cowan M.M. Plant products as antimicrobial agents. Clin.Microbiol. Rev. 1999;12:564–582. doi: 10.1128/CMR.12.4.564.

[7] Jain, S., &Mehata, M. S. (2017). Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. Scientific reports, 7(1), 15867.doi:10.1038/s41598-017-15724-8.
[8] Mmola, M., Roes-Hill, M. L., Durrell, K., Bolton, J. J., Sibuyi, N., Meyer, M. E., Antunes, E. (2016). Enhanced Antimicrobial and Anticancer Activity of Silver and Gold Nanoparticles Synthesised Using Sargassumincisifolium Aqueous Extracts.Molecules (Basel, Switzerland), 21(12), 1633.doi:10.3390/molecules21121633.
[9] Patra, J. K., &Baek, K. H. (2015).Novel green synthesis of gold nanoparticles using Citrulluslanatus rind and investigation of proteasome inhibitory activity, antibacterial, and antioxidant potential.International journal of nanomedicine, 10, 7253–7264. doi:10.2147/IJN.S95483.
[10] Naraginti S., Li Y. Preliminary investigation of catalytic, antioxidant, anticancer and bactericidal activity of green synthesized silver and gold nanoparticles using Actinidiadeliciosa. J. Photochem. Photobiol. B Biol. 2017;170:225–234. doi: 10.1016/j.jphotobiol.2017.03.023.
[11] Balasubramanian, D., Harper, L., Shopsin, B., & Torres, V. J. (2017).Staphylococcus aureus pathogenesis in diverse host environments.Pathogens and disease, 75(1), ftx005. doi:10.1093/femspd/ftx005
[12] Teow, S. Y., Wong, M. M., Yap, H. Y., Peh, S. C., &Shameli, K. (2018).Bactericidal Properties of Plants-Derived Metal and Metal Oxide Nanoparticles (NPs).Molecules (Basel, Switzerland), 23(6), 1366.doi:10.3390/molecules23061366.
[13] Bandow, J.E., H. Brtz, M. Hecker. "Bacillus subtilis Tolerance of Moderate Concentrations of Rifampin Involves the B-Dependent General and Multiple Stress Response". Journal of Bacteriology. 2002 January; 184(2): 459-467.
[14] Stromberg, Z. R., Johnson, J. R., Fairbrother, J. M., Kilbourne, J., Van Goor, A., Curtiss, R., Rd, &Mellata, M. (2017). Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. PloS one, 12(7), e0180599. doi:10.1371/journal.pone.0180599.
[15] Bengoechea, J. A., &Sa Pessoa, J. (2019). Klebsiellapneumoniae infection biology: living to counteract host defences. FEMS microbiology reviews, 43(2), 123–144. doi:10.1093/femsre/fuy043.
[16] Armbruster, C. E., Mobley, H., & Pearson, M. M. (2018).Pathogenesis of Proteus mirabilis Infection.EcoSal Plus, 8(1), 10.1128/ecosalplus.ESP-0009-2017. doi:10.1128/ecosalplus.ESP-0009-2017.
[17] Xin, X. F., Kvitko, B., & He, S. Y. (2018). Pseudomonas syringae: what it takes to be a pathogen. Nature reviews. Microbiology, 16(5), 316–328. doi:10.1038/nrmicro.2018.17.