GREEN SYNTHESIS OF NANOPARTICLES USING FRUIT EXTRACTS OF *Murraya koenigii* AGAINST UTI CAUSING *P.aeruginosa*

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Abstract

In the present study, two different extracts of *Murraya Koenigii* fruit were screened for their phytochemicals composition. Among them, methanol extract showed highest phytochemicals (60%) and then acetone extract (40%). Both extracts were utilized for the green synthesis of silver nanoparticles with 2mM of silver nitrate. The synthesized silver nanoparticle was carried out to assess the antimicrobial activity against MBL producing UTI causing *P.aeruginosa*. The highest antimicrobial activity was observed when using methanol extract than acetone extract. The zone of inhibition was ranged between 9mm to mm to 16 mm. Different concentration was used for this susceptibility study when using 10µg of extract, the best inhibition was observed.

Key words : UTI, MBL, *P.aeruginosa*, *Murraya Koenigii*, AgNPs

INTRODUCTION

The plants are a plentiful source of the beneficial substance. A vast range of medicinal plants extracts are used to cured various infections as they have strong antimicrobial activity. Some of these beneficial substances are evaluated and traded in the market as raw material for many herbal industries (Renisheya *et al.,* 2011). From the last decades, experts have selected the medicinal plants for treatments, because no side effects compare then synthetic drugs (Bushra *et al.,* 2012). It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species (Bibi *et al.,* 2011).

In India, more than thousands of plants are used as medicinal plants because of their antimicrobial traits, which are due to beneficial compounds produced in the secondary metabolism of the plants. A number of mechanisms were underlain for that antimicrobial activity of plant extracts, such as plant containing phytochemicals were inhibit the microbial cell wall, denature the bacterial capsule, reduced the level of toxin and biofilm (Mikayel *et al.,* 2017).

Furthermore, Plant metabolites can also act as resistance-modifying agents (RMAs). Nowadays RMAs are recognized as one of the utmost eventual ways to conflict bacterial resistance. Many researchers have developed a keen interest for enhanced antimicrobial activity and their use as anticancer agents. Among the number of technology, nanoparticles and their characterization is a rising field of nanotechnology from the past few years, due to their immense applications in the fields of medical (Song, 2008).

Apart from chemical and physical methods, biological methods have been developed to synthesise nanoparticles with plants. The biological methods are inexpensive, safe, reliable and eco-friendly. A number of research was reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against microbes.

Drug resistance is one of the most serious and widespread problems in all developing countries (Stevanovic *et al.,* 2012). Day by day treating bacterial infection is rising with complicated because of the ability of the pathogens to develop resistance to common antimicrobial agents and existing antibiotics. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics (Shah, 2005). In the present work, an attempt has been made to synthesize silver nanoparticles using solvents fruit extract of *Murraya koenigii*. The synthesized silver nanoparticles were evaluated for their synergistic antimicrobial activity against metallo betalactamase (MBLs) producing isolates.

MATERIALS AND METHODS

Test pathogens

The clinical isolates of *P.aeruginosa* were procured from Microtech, Microbiology Laboratory, Coimbatore and used for the study. All isolates were confirmed with standard biochemical tests and selective media.

Identification of Metallo betalactamase producing isolates

Multiplex PCR amplification for the simultaneous detection of blaIMP and blaVIM metallo betalactamase genes. The composition of the reaction mixture was as follows: Each PCR reaction mixture (25µl) contained 2µl of template DNA (plasmid DNA), 10 µl of 10 X PCR mix, 0.5 µl of (0.5 µM) each of the primers and 12 µl of molecular grade water ().

The PCR program was performed in a Thermal Cycler and it consisted of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of DNA denaturation at 94°C for 1 min, primer annealing at 54°C for 1 min, and extension at 72°C for 1.5 min.

Following PCR, aliquots of the reaction mixtures were analyzed by electrophoresis on a 1.5% Agarose gel, containing ethidium bromide (0.2 mg/ml), in the presence of an appropriate DNA molecular weight marker. Then observe the amplification bands under UV Transilluminater (512nm).

For MBL-IMP gene: IMP-A (5`GAAGGCGTTTATGTTCATAC-3`) and IMP-B (5`-GTACGTTTCAAGAGTGATGC-3`), which give an amplified product of 587-bp. For MBL-VIM gene: VIM2004A (5`-GTT TGG TCGCAT ATC GCA AC-3`) and VIM2004B (5`-AAT GCG CAG CAC CAG GATAG-3`), which give an amplified product of 382-bp (Eman A. Essa and Ibtesam, 2007).

Collection of fruit and prepared the extract

The fruits of *Murraya koenigii* were collected from the Namakkal area, Tamilnadu, India. The collected fruit was dried and prepared the extract with Soxhlet extraction. The extract was taken in a petriplate and kept in a hot air oven and heated at 30-40ºC till the solvent got evaporated. The extract was stored in a sterile bottle and kept under refrigerated condition for further analysis.

Phytochemical analysis

The extract was subjected to various phytochemicals tests to determine the nature of constituents of the extracts (Igara *et al.*, 2016).

Preparation of silver nanoparticles

Five ml of fresh fruit extract was added to a beaker containing 25 ml of 2 mM aqueous $AgNO_3$ solution and heated at 65^0C with continuous stirring. Silver ions were reduced to silver nanoparticles in the extract within 20 mins. The reduced silver nanoparticles can be observed by colour change from light yellow to black.

Determination antibacterial activity of plant extract

This test was carried out according to the method of Selvamani and S.Balamurugan., 2014. The plates were inoculated with freshly prepared over night inoculums which were swabbed over the entire surface of the medium, rotating the plate 60 degrees after each application by using a sterile cotton swab, to ensure the spread of the tested microbes on the surface of the plate completely. Inoculums were 10^8 CFU/ml of bacteria. The 6mm diameter of well was made with borer on the agar plates. Different concentrations of silver nanoparticle synthesized plant extract were filled in well with the help of micropipette and one well filled with plant extract. The Ampicillin (10µg/ml) was added in one well and added 100µl of extract in another well. Incubate the plate at 37ºC for 24hrs, after observed the zone of inhibition.

RESULT AND DISCUSSION

Out of 20 isolates 8 of were showed betalactamase positive isolates, these isolates were utilized for PCR analysis for amplification of MBLs genes (IMP and VIM). Among the 8 isolates, 6 of were harbor any one of the gene, among them, IMP was mostly observed than VIM. Presently two genes were observed from Pa2, Pa12 and Pa13, none of the genes were observed from Pa2 and Pa15 (Plate 1). Among the 8 isolates, 6 positive isolates were subjected to further analysis.

From the past 2 decades, metallo betalactamase encoding genes have been reported all over the world in clinically isolates of *Pseudomonas spp* and *Acinetobacte* spp. The prevalence of metallo betalactamase encoding genes is disquieting; moreover these encoding genes are mobile genetic elements and easily spread in to other species (Xavier *et al.*, 2006). These MBLs isolates were not easily eradicated, because resistance to cephalosprins and betalactamase inhibitors such as clavulanic acid and tazobactam (Kashyap *et al.*, 2017). In this situation urgently need for eradicate the MDR isolates. Therefore, there is a worldwide attempt to find safe antimicrobial compounds against MDR. Although certain natural compounds from plant were investigated for this purpose.

In this present study, the *Murraya koneigii* fruit was collected, dried and prepared the extract from dried fruits. The plant extracts were prepared with solvents from methanol and Acetone, which were subjected to various preliminary phytochemicals analysis. Among the 2 types of extracts, methanol extract showed highest phytochemicals (60%) and followed by acetone (40%). In this study, two phytochemicals were commonly found in all the solvents, namely Flavoniods and Phenols. In methanol extract, Alkaloids, Carbohydrate, Flavoniods, Phenols, Tannin, Sterols and Terpenoids were presents. The Saponins, Quinon, Proteins and Sterols were not observed from both extracts. Alkaloids, Flavoniods and Phenols have anti-microbial properties owing to their ability to intercalate with DNA of the micro-organisms.

In the present study, next part of the investigation was prepare the silver nanoparticles and determine the efficacy of antimicrobial activity of fruit extracts against UTI causing metallo betalactamase producing *P.aeruginosa* by agar well method. Among the 2 solvents extracts, methanol extract of AgNps was more effective than acetone extracts; the zone of inhibition was ranged from 9mm to 16mm. In this study, while using methanol extract, all isolates were suppressed. The maximum zone of inhibition was observed from 10µg of AgNps and while using the non AgNPs, zone of inhibition was lower than AgNPs. Several investigations on medicinal plants indicate that organic solvents such as methanol are extensively used for crude extraction before being re-extracted to obtain purified active compounds using some other organic solvents. Present study also agreed with previous studies, which was inhibited by most of the isolates.

In 2017, Sundaraselvan *et al* were reported that green Synthesis of Zinc Oxide Nanoparticles using seed extract of *Murraya koenigii* and their antimicrobial activity against *P.aeruginosa*. The present study was first investigation, in our literature knowledge, this is the first study, and no one inhibited the metallo betalactamase producing isolates.

The biological methods of nanoparticles synthesis using microorganisms, enzymes, and plant or plant extract have been suggested as possible ecofriendly alternative to chemical and physical methods (Ahmad *et al.*, 2004). Among the all noble metal nanoparticles, silver nanoparticle (AgNPs) are an leading product in the field of nanotechnology because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities (Shakeel *et al.*, 2016).

The present study was concluded that, *Murraya koenigii* had beneficial substance, which showed antimicrobial activity against metallo betalactamase producing *P.aeruginosa*. Hence has a potential to be used as antimicrobial agent against wide range of microbes over conventional antibiotics.ti

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REFERENCE

1. Ahmad A, Senapati S, Khan MI, Kumar R, Ramani R, Srinivas V. 2004. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. Nanotechnology.14:824–828.
2. Bibi Y, Nisa S, Chaudhary F. and Zia M. 2011. Antibacterial activity of some selected medicianal plants of pakistan. BMC Complem Altern Med. 11: 892-897.
3. Bushra I, Fozia Abdul W, Ali R, Ullah Hussain, Iqbal Hamid, Almas, M. and Ahmad, A. 2012. Antimicrobial activity of Malva neglecta and Nasturtium microphyllum. Int J Res Ayurveda Pharm., 3: 808-810.
4. Eman A. Essa and Ibtesam K. Afifi. 2007. Multiplex PCR study of nosocomial *Pseudomonas aeruginosa* for genes of Metallo Betalactamases: could such enzymes bring us to end of antibiotics? Egyptian Journal of Medical Microbiology, 16(1):189-194.
5. Igara DA, Omoboyowa AA, Ahuchaogu NU, Orji and MK Ndukwe. 2016. Phytochemical and nutritional profile of Murraya Koenigii (Linn) Spreng leaf. Journal of Pharmacognosy and Phytochemistry, 5(5): 07-09.
6. Kashyap A, Rani Gupta, Sharma R, Verma VV, Gupta S and Pradeep Goyal. 2017. New Delhi Metallo Beta Lactamase: Menace and its Challenges. J Mol Genet Med, 11(4):3-6.
7. Mikayel Ginovyan, Margarit Petrosyan and Armen Trchounian. 2017. Antimicrobial activity of some plant materials used in Armenian traditional medicine. BMC Complementary and Alternative Medicine, 17(50):2-9.
8. Renisheya JJ, Malar T, Johnson M, Mary UM and Arthy A. 2011. Antibacterial activities of ethanolic extracts of selected medicianal plants against human pathogens. Asian Pac J Trop Biomed., S76-S78.
9. Selvamani and S.Balamurugan. 2014. Evaluation of the antimicrobial potential of various solvent extracts of Murraya koenigii (Linn.) Spreng leaves. Int.J.Curr.Microbiol.App.Sci, 3(9) 74-77.
10. Shah PM. 2005. The need for new therapeutic agents: what is in the pipeline? Clinical Microbiology and Infection, 11:36–42.
11. Shakeel Ahmed, Saiqa Ikram. 2016. Chitosan Based Scaffolds and Their Applications in Wound Healin. Achievements in the Life Sciences, 10:27–37.
12. Song JY, Kim BS. 2008. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng, 32:79–84.
13. Stevanovic MM, Skapin SD, Bracko I, Milenkovic M, Petkovic J, Filipic M. 2012. Poly (lactide-co-glycolide)/silver nanoparticles: synthesis, characterization, antimicrobial activity, cytotoxicity assessment and ROS-inducing potential. Polymer, 53: 2818–2828.
14. Sundaraselvan S. Darlin Quine. 2017. Green Synthesis of Zinc Oxide Nanoparticles using Seed Extract of Murraya koenigii and Their Antimicrobial Activity against Some Human Pathogens. J. Nanosci. Tech, 3 (4):289–292.
15. Xavier DE, AC, Gales RE, Mendes AC, Pignatari LF, Cirilo L, Santos Filho and M. Castanheira.2006. IMP-18-producing *Pseudomonas aeruginosa (PSA):* Increasing diversity of mobile metallo-β-lactamases (MBL) in Brazil, abstr. C2-420:110-112.