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Antibacterial activity of plant extracts and silver mediated nano particles of *Ipomoea pes caprae* and *Spinifex littoreus*

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Abstract: Preparation of plant extracts using methanol and silver mediated nanoparticles using 1 mM $AgNO_3$ for both the plants *Ipomoea pes caprae* and *Spinifex littoreus.* Isolation and identification of bacteria from the fish sample. Testing the antibacterial activity of both the plant extracts and nanoparticles against *Enterococcus faecalis* and *Bacillus subtilis* using agar well diffusion method. The antibacterial activity of both the plant extracts and nanoparticles is confirmed. The silver mediated nanoparticles showed remarkable resistance against the above-mentioned bacteria. These plants can be a potential alternative to therapeutic useful antibiotics, chemical preservatives to reduce food spoilage since they show antibacterial activity.

Key words: *Ipomoea pes caprae, Spinifex littoreus,* 1mM $AgNO_3$, Methanol, Nano particles

Introduction

Plants are not only source of food but also source for commercially valuable products such as wood, timber, rubber, latex etc.., Plants contain active substances such as oils, waxes, flavonoids, alkaloids etc., which have great importance in the preparation of oils, perfumes and medicines. Plants exhibit several properties which will vary from place to place due to their surrounding environment. Plants that grew in harsh conditions are known to exhibit a variety of properties that are different from those that grow in tropical or temperate regions. Plants that grow in the coastal area can exhibit special properties due to the harsh conditions they grow in.

Human beings exploit the nature around them for their benefit. Among them, Plants are one of the most important ones. These are source for food, wood, timber, medicines etc. Plants usage in the field of Medicine was mentioned in the ancient Sanskrit text "Sushruta Samhita". [Petrovska, 2012] This shows that plants possess certain chemical compounds that are useful in treating diseases. These compounds are produced in the plants to cope up with several conditions such as defence mechanism, pollination, growth and metabolism etc. Apart from these some compounds produced by the plants exhibit antimicrobial activity.

Plants produce several chemical compounds as secondary metabolites which possess antimicrobial activity. These include Alkaloids, Flavonoids, Polyphenols, Quinones, Terpenes and Resins etc. All these compounds are of great value to the pharma industry in the preparation of the drugs. [Compean and Ynalvez, 2014] Due to biological activity of the above-mentioned compounds, research is being conducted to identify alternative source for antibiotics from these plant extracts.

The concept of nanotechnology was first highlighted by Nobel Laureate, Richard Feynman (1959) in his lecture "There are plenty of room at the bottom". The term 'Nanotechnology' was first identified by Norio Taniguchi in 1974. Nanoparticles were used in several industries such as electrical, biological

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and chemical and textile industry. Depending upon their size, nanoparticles play important role in the electronic device, antimicrobial gene expression and catalytic and electromagnetic properties. [Appenzeller, 1991] Metallic Nano particles are synthesised by using various functional groups which allow them to form conjugate with antibodies, ligand and drugs of interest to enhance their activity. These include Iron oxide Nanoparticles, Gold Nanoparticles, and Silver Nanoparticles which are beneficial for the human beings in various ways. [Mody, *et al.*, 2010]

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity and biological properties Silver Nano particles range between 1 and 100 nm in size. These are synthesised by using Silver Nitrate or Silver oxide. Silver when used in right quantities is suitable in treating wounds. Silver Nano particles are now replacing Silver sulfadiazine as an effective agent in the treatment of wounds. [Qing *et al.*, 2018]

Silver Nano particles are more effective against Gram negative bacteria compared to Gram positive bacteria. These Nano particles bind to the cell wall and infiltrate into the bacterial cell which results in physical alterations in the bacterial membrane leading to cell leakage and eventually cell death. [Atiyeh and Costagliola, 2007]

The effect of Silver Nano particles on the bacterial cell depend on the size of the particles. Smaller Nano particles reach the cytoplasm more easily than the larger ones due to their large surface area contact with the bacterial cell. The Silver Nano particles on entering into the bacterial cell combine with biomolecules such as Proteins, Lipids, DNA and also Carboxyl and thiol groups of β – galactosidase inhibiting cellular functions leading to cell death. [Khalandi, 2017] It has been found that the Silver Nano particles can modulate the Signal transduction in bacteria. They alter the Phospotyrosine profile of the bacteria by dephosphorylating the peptide substrates on Tyrosine residues. This leads to the inhibition of Signal transduction which results in stoppage of bacterial growth. [Dakal, 2017]

Spinifex littoreus also called as Ravan´s Moustache which is a sand dune found along the coasts of India, Africa, Middle East, Asia, New Zealand, Australia [Wikipedia contributors, 2019] and *Ipomoea pes caprae* also known as Beach Morning Glory or Goat´s Foot which is a salt tolerant plant belonging to the family Convolvulaceae. [Satyavani, 2013] In our studies we have evaluated their antimicrobial activity using their plant extracts aswellas silver mediated nanoparticles.

Materials and Methods

Spinifex littoreus and *Ipomoea pes caprae* were collected from the Rushikonda Beach of Visakhapatnam on the North Eastern coastal districts of Andhra Pradesh, India during December to January of 2018-19. The water used to clean the fish while cutting was the sample collected from the Visakhapatnam Harbour. The work was carried out at Adhya Biosciences Pvt. Ltd. Research Institute in Visakhapatnam, Andhra Pradesh, India.

Bacterial isolates of *Enterococcus faecalis*, Gram positive cocci and *Bacillus subtilis,* Gram-positive rod-shaped bacteria were used for the study.

Preparation of Plant extracts

The collected plant material (leaves) was washed in the salt water first and then washed thoroughly with the fresh water. The leaves were than air dried in a shady place. The dried plant material was then made into a coarse powder using an electrical grinder. [Nagababu and Umamaheswara Rao, 2015] The powder is then weighed 50-100 grams and then packed in the soxhlet extractor. Methanol is used for this extraction process. This is a cyclic process that is carried out for 6-8 cycles. In this the extract from the plants was collect in the round bottomed flask containing methanol. Then the process is further continued to separate methanol from the plant extract. The same process was followed

for extraction for the two plants. Formulations were made from these plant extracts by using Dimethyl sulfoxide (DMSO) as 500 mg, 250 mg and 125 mg. These formulations were used to test the antimicrobial activity.

Preparation of Silver Nano particles

The leaves of the both the plants were taken, 1 gm each and are grinded in a mortar to make it into a paste by adding little amount of water in regular intervals. These were than mixed with 100 ml of water. To this 1 mM Silver Nitrate ($AgNo_3$) was added mixed well and allowed to incubate at room temperature for a week. [Satyavani, 2013] This process is called as Cold Percolation in which heat sensitive chemicals were also retained. After the formation of the Silver Nano particles, they are separated by using a cooling Centrifuge at 3000 rpm for 10 mins. Later the supernatant is discarded. The pellet containing Nano particles is collected and stored in append off tubes. Formulations were made from these by using Dimethyl sulfoxide (DMSO). 500 mg formulation was used to test the antimicrobial activity.

Antimicrobial activity was tested by pour plate method and agar well diffusion method. Nutrient agar was autoclaved at 121^0 C at 15 lbs pressure for 15 minutes in an autoclave. Later it was cooled down, the inoculation was done for both bacteria separately and the conical flask was rotated between the fingers to mix the contents. Two Petri dishes were taken and Nutrient agar along with the inoculation was poured on to the petri dish and let it solidify. Five wells were made on the agar with the help of a metal borer. The wells were numbered 1, 2,3,4,5 for plan extract formulations of 500 mg, 250 mg, 125 mg, Antibiotic (Ciprofloxacin) and Control respectively. Using a micropipette, transfer the plant extracts and antibiotic to their respective wells. Incubate the plate at 37^0 C in an Incubator for 24 hrs. Repeat the process for both the plant extracts using both the bacteria. The above same process was repeated with the Silver mediated nanoparticles synthesised using the plant extracts. The formulations used in this step are 500, 250 and 125 mg. Three wells are made on the petri plates using metal borer. The wells were named 1, 2, 3 and Plant extract (Sliver mediated), Antibiotic (Ciprofloxacin) and Control were added respectively. The plates were incubated at 37^0 C for 24 hrs in an incubator.

Results and Discussion

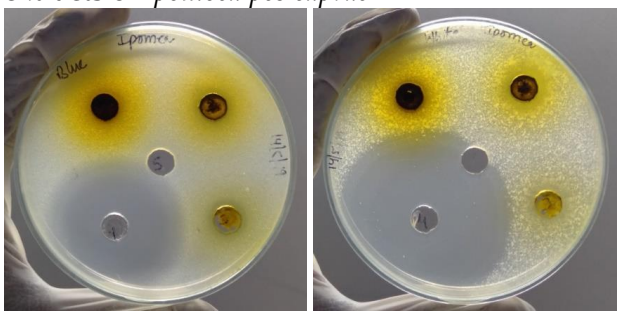
The present study reveals that the methanol extracts and the Silver mediated Nano particles of both the coastal plants, *Spinifex littoreus* and *Ipomoea pes caprae* showed antibacterial activity against the bacterial species (*E. faecalis* and *B. subtilis*). The latter showed greater antibacterial activity when compared to the former. The Nano particles showed good antibacterial activity against *Bacillus subtilis*. Further observations over a week showed that the Nano particles exhibited resistance superior to the antibiotics even the zone formed is small when compared with the antibiotics.

Table: Antibacterial activity using Methanol extracts of Plants

Plant Species	Bacterial Species	Methanol extracts			Silver mediated Nano particles 500 mg	Ciprofloxacin (Diameter in mm)
		500 mg	250 mg	125 mg		
	E. faecalis	19 mm	17 mm	16 mm	21 mm	40 mm
I. pes caprae	*B. subtilis*	12 mm	13 mm	11 mm	20 mm	>40 mm
S. littoreus	*E. faecalis*	11 mm	12 mm	10 mm	23 mm	40 mm
	B. subtilis	12 mm	13 mm	13 mm	18 mm	>40 mm

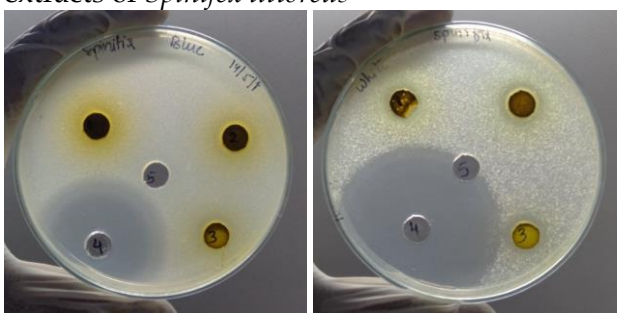
Zone of inhibition represented in mm

Figure 1: Antibacterial activity of Methanol extracts of *Ipomoea pes caprae*



Enterococcus faecalis ***Bacillus subtilis***

Figure 2: Antibacterial activity of Methanol extracts of *Spinifex littoreus*

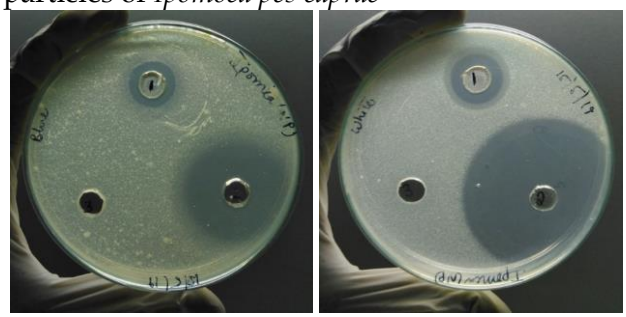


Enterococcus faecalis ***Bacillus subtilis***

The usage of Silver Nano particles showed tremendous results compared to plant extracts. The plate contains three wells for 500 mg Nano particles, Ciprofloxacin (Antibiotic) and Control respectively.

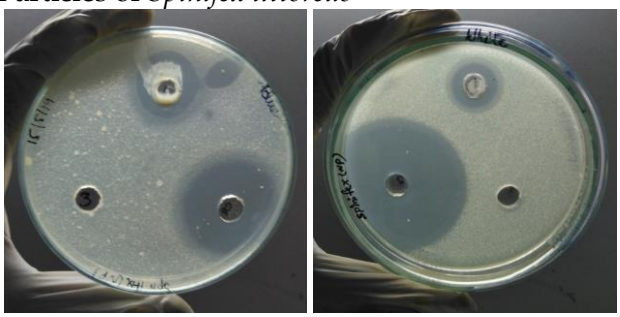
The Plates were observed over a week and the Nano particle mediated plant extract showed remarkable resistance over antibiotic against the identified bacterial species. The Silver mediated nano particles synthesised from both the plant extracts show enhanced antibacterial activity against both the bacterial species compared to the plant extracts. 500 mg concentration of silver Nanoparticles is used. The antibiotic used is Ciprofloxacin. The Figures 7, 8,9,10 represent the Day 1 activity of the Silver Nanoparticles. The Figures 1, 2, 3 and 4 represent activity of the Silver Nanoparticles. Resistance against the Antibiotic (Ciprofloxacin) showed resistant colonies whereas the Silver Nanoparticles show resistance against the bacterial growth in all four cases. Three days after study silver nanoparticles still shows a great deal of resistance against the bacterial growth in all four cases. By the end of the week, more number of resistant colonies are observed in the antibiotic (Ciprofloxacin) whereas the Silver Nanoparticles show no signs of resistant bacterial colonies in their vicinity from their well in all four cases.

Figure 3: Antibacterial activity of Siver Nano particles of *Ipomoea pes caprae*



Enterococcus faecalis ***Bacillus subtilis***

Figure 4: Antibacterial activity of Silver Nano Particles of *Spinifex littoreus*



Enterococcus faecalis ***Bacillus subtilis***

Conclusion

The study revealed the antibacterial activity of the methanol extracts and silver mediated Nano particles of both *Ipomoea pes caprae* and *Spinifex littoreus*. The Nano particles of both the plants show remarkable resistance against *Enterococcus faecalis* and *Bacillus subtilis*. These plants can be potential alternative source for the antibiotics that are used to treat diseases and chemical preservatives that are used to prevent food spoilage. These plant extracts are from natural sources which can have minimal side effects and can also use as a preservative which plays a major role in the exports and imports of the food material. Further work on these plants might pave to the development of a natural drug which is much needed for the society.

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