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PRODUCTION AND BIOACTIVE SCREENING OF NANO-PARTICLES PRODUCED FROM PLANT EXTARCTS

Nair Sreecha Chandran*, C. Prabha Kumari,

KS. Arya Prasad and Reshma RK. Pillai

Department of Biotechnology, CEPCI Laboratory and Research Institute, Kollam, Kerala, Thiruvananthapuram, India.

ABSTRACT

Nano-particles are those substances which have a minutesute size mostly ranging in nm scale. Due to this their surface are by volume ratio is high. And because of this property they are widely used in industries like food, clothes, biomedical, optical, electronic fields etc. Nano-particles are uniform throughout their properties and they are conducting too. They have the amazing property of linking bulk substances with smallest atomic or molecular structures. So many researches are going on these nano-particles to make it much more effective and to use it up to it's fullest.. In the present study ZnO nano-particles were synthesized from leaves extracts of *Lilium candidum* and *Ocimum tenuiflorum*. Further production of nano-particles from plant extracts was performed. The bacterial and fungal activities were tested and it was found out that nanoparticle enhanced the prohibition of growth of bacteria- *Salmonella typhi, Pseudomonas aeruginosa, Listeria monocytogenous, Escherichia coli.* and it also prevented the growth of fungi- *Aspergillusniger,Aspergillusflavus, Aspergillusfumigatus, Penicilliumchrysogenum, Rhizopusoryzae.* The results evaluated that, In case of bacteria, *Lilum candidum* (LT) nanoparticles seem to be more effective and for fungi, *Ocimum tenuiflorum* (TT) was observed to be more effective.

Keywords: Nano-particles, ZnO, plant extract, *Lilum candidum and Ocimum tenuiflorum.*

1. INTRODUCTION

Nanoparticles are those substances which has a size lower than 100nm at least in 1 dimension. (Batista *et al.* 2015). Nanoparticles are uniform through out their properties and they are conducting too. At some cases they have special optical properties.(Hewakuruupu Y. L. *et al.* 2013). Due to their property of having high value of surface area by volume they can be widely used in so many industries, like biomedical, optical, electronic fields etc for applications like fluorescent biological labels, d rug and gene delivery, bio detection of pathogen, detection of proteins, probing of DNA structure, tissue engineering, tumour destruction via heating, separation and purification of biological molecules and cells, MRI contrast enhancement, phagokinetic studies. Also nano-particles are used in the production of food products, cosmetics, medicine, clothing, disinfectants, households, stain-resistant materials, fuel catalysts etc. The nano-particles can be synthesized in so many techniques including chemical as well as using natural raw materials. Like always, the green synthesis of a substance will be highly advanced because of its ore favoured properties. *Lilium candidum* aka Madonna lily, is a species cominutesg under the true lily family. It is now at the verge of extinction and recorded by IUCN in the list of near threatened species. (Chadbum H 2014). It is a native to Middle East as well as Asia. is used in medical field against carcinogenic factors, ulcers, tumors, external inflammations etc and it is also used in the production of tincture and ointments. And the pollen grain of *Lilium* candidum is used in the treatment against epilepsy. *Ocimum tenuiflorum* aka basil, is a perennial plant cominutesg under the family Lamiaceae. It is a native through out Indain sub-continent. (Staples *et al.* 1999) (Warrier P

K 1995)It covers a wide range of uses, and mostly in medical field and in anti-infectious preparations. Phytochemical constituents responsible for the medicinal properties are mainly rosmarinic acid ,oleanolic acid, beta-caryophyllene, ursolic acid, carvacrol , eugenol, and amounts of linalool and.(Sundaram R *et al.* 2012). The genome of *Ocimum* has beem reported with having 612 megabases. (Upadhayy *et al.* 2015). So, production of nano-particles from these two plants will be useful such that both have antimicrobial as well as antifungal properties. And also threatened species like *Lilum candidum* could get more attention and protection when it is use commercially.

2. MATERIALS AND METHODS

2.1 Collection of samples

Leaves of Lilum are collected from Mavelikara, near Kayamkulam of Alleppey district in Kerala. And the leaves of *Ocimum* are collected from Ramapuram, nr.KayamkulamofAlleppey district in Kerala. First these leaves were rinsed, dried and powdered. And 20g of the powdered leaves are weighed and taken in a conical flask and then mixed with 300 ml water which was sterilized once and then the mixtures are again undergone sterilization at 70 ⁿC. After 45 minutes, the solution appeared to be colored with plant extract. And then the solutions containing powdered leaves are filtered using whatman filter paper.

2.2 Preparation of Nano-particles

100ml of each solutions were taken and were individually and were separately mixed with 100 ml of solution containing 50 ml 0.1M Zinc Sulphate and 50 ml 0.4M Sodium Hydroxide. And those were put in a shaker for 24 hours, and after 24 hours, pellets were appeared. Then these two solutions were heated on a rectangular hot plate for 1 hr and then cooled and centrifuged for 5 minutes at 5000 rpm. Then more pellets are appeared to be present and these were extracted out and put in a china dish and put in a hot air oven for 24 hours to dry.

2.3 Preparation of broths

2.3.1 Nutrient broth for bacterial medium.

0.5g of peptone along with 0.3g beef extract and 0.5g NaCl were mixed with 100 ml of distilled water and was undergone sterilization for 45 minutes. And then this broth was equally divided in 5 test tubes.

2.3.2 Potato dextrose broth for fungal medium.

2.4g of potato dextrose broth was mixed with 100 ml of distilled water and was undergone sterilization. This solution was further divided equally in 5 test tubes.

2.4 Preparation of cultures

2.4.1 Bacterial culture

A little of pre-made 5 kinds of bacteria were transferred into their respective test tubes using swabs, as an inoculum into equal amounts of distilled water. And kept in an incubator for 24 hours.

2.4.2 Fungal culture

A little of pre-made bacteria of 5 types were transferred to individual test tubes using a swab. And it was left in incubator for 48 hours.

2.5 Preparation of agar medium

2.5.1 Agar medium for bacterial culture

0.75g of peptone, 0.45g of beef extract, 2.25g agar and 0.75g of NaCl were mixed with 150ml of distilled water and was undergone distillation for 45 minutes.

2.5.2 Agar medium for fungal culture

3.6g of dextrose nutrient broth and 2.25g agar were mixed in 150ml of distilled water and was undergone sterilization for 45 minutes.

2.6 Agar well diffusion technique

2.6.1 Agar well diffusion technique by bacterial medium

The nutrient agar medium prepared was poured equally into 4petriplates and was left to solidify and after solidification using a swab in LAF, 5 kinds of bacteria were transferred individually. And after that using a borer, 5 wells were bored. And in one well antibiotic(A) was poured completely, in other one the extract of *Lilum* leaves(L –ve) in another the extract of *Ocimum* leaves(T – ve) , in other one antibiotic along with the nano-particles of *Lilum*(LT) and finally in the remaining one the *Ocimum* nano-particles along with antibiotic(TT) is poured using a pipette. And they were left it for 24 hours in an incubator. The zones of inhibition were measured.

2.6.2 Agar well diffusion technique with fungi medium

Potato dextrose agar medium was poured equally into 5 sterilized petri plates followed by solidification. And after solidification the 5 fungal cultures were transferred into 5 petriplates individually and then 5 wells are bored in each. One well was filled with antibiotic(A, another with the leaf extract of *Lilium*{-ve L), other with the extract of *Ocimum*(-ve T), one with nanoparticles of *Lilium* and antibiotic in equal amounts(LT) and

the final one with antibiotic and *Ocimum* nano-particles(TT) . And those 5 were left in incubator for 48 hours. Then the zone of inhibition was measured.

3. RESULT AND DISCUSSION

3.1 Extraction of plant leaves

Extraction of *Lilium and Ocimum* was done by heating the dried and powdered leaves with distilled water for 45 minutes at 60 to 80° degree Celsius. And after 45 minutes a light Green colored liquid was formed from the solution containing *Lilum* and a dark Brown color was observed in *Ocimum* extraction (refer fig; 7.1)

3.2 Synthesis of nano-particles

Along with the extractions, 50 ml 0.1M Zinc Sulphate and 50 ml 0.4M Sodium Hydroxide were added into each of the solutions. And these solutions were put in a shaker for 24 hours. And after 24 hours pellets were formed. And they were centrifuged later for 5 minutes at 5000 rpm and was able to get more pellets formed. And these pellets were separated and dried in a hot air oven for 24 hours. And finally nano-particles were formed (refer fig; 7.2).

In order to find out the inhibition of nano-particles on anti-bacterial and anti-fungal activity, agar well diffusion method was used. And at the end it was seen that *Lilum* more than *Ocimum,* was able to inhibit the growth of bacteria like *E.coli, Listeria, P.aeruginosa and S. typhi (refer figs; 7.3.1 to 7.3.4).* And in the case of fungal growth inhibition, *Ocimum* was seen to be much more effective against the fungi- *A. flavus, A. fumigates, A.niger, P.chrysogenum* and *R.oryzae* (refer figs; 7.4.1 to 7.4.5).

4. CONCLUSION

Nano-particles could be made from plant leaf extracts and also it could be used to prevent the microbial growth. For bacterial inhibition, *Lilium* nano-particles seemed to be much more effective. And for fungal growth inhibition, *Ocimum* was seemed to be effective.

5. ACKNOWLEDGEMENT

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3.3 Agar well diffusion method

6.1 Tables

6.1.1: Table containing the diameter of zones of inhibition of bacteria

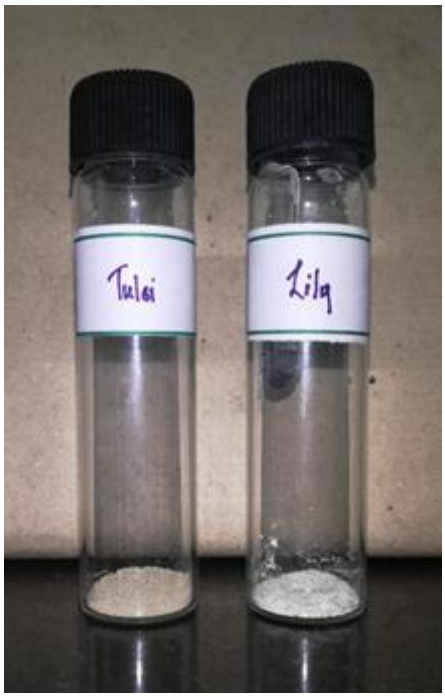
NAME	A(cm)	LT(cm)	TT(cm)	L-ve(cm)	T-ve(cm)
Escherichia coli	2	2.5	1	1.2	-
Listeria monocytogenous	3	0.7	1.3	0.7	-
Pseudomonas aeruginosa	2.3	0.7	0.6	0.9	-
Salmonella typhi	2	1	1.3	0.2	0.7

6.1.2: Table containing the diameter of zones of inhibition of fungi

NAME	A(cm)	LT(cm)	TT(cm)	L-ve(cm)	T-ve(cm)
Aspergillus flavus	0.7cm	0.7cm	1.4cm	0.6	0.7
Aspergillus fumigates	0.6	1.1	1.4	0.6	-
Aspergillus niger	0.6	1.4	1.1	0.5	0.6
Penicillium chrysogenum	0.7	1.5	1	0.5	0.6
Rhizopus oryzae	1.5	1	0.8	0.7	0.6

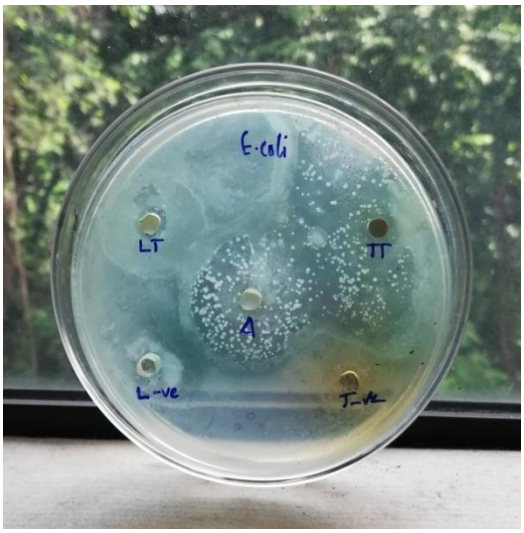
7.1 Figures

7.1: Extract of leaves

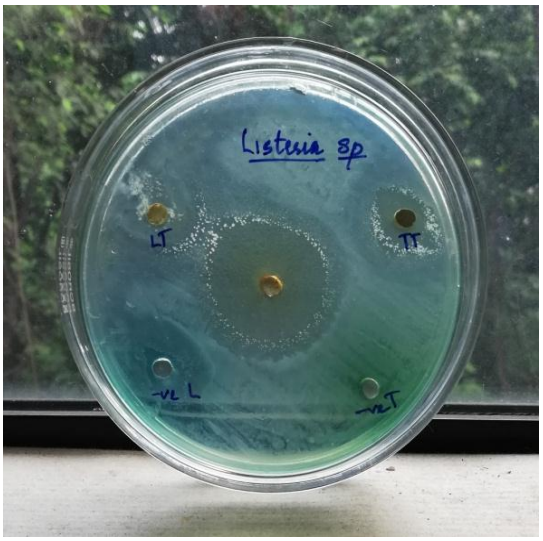


7.2 : Nanoparticles synthesised

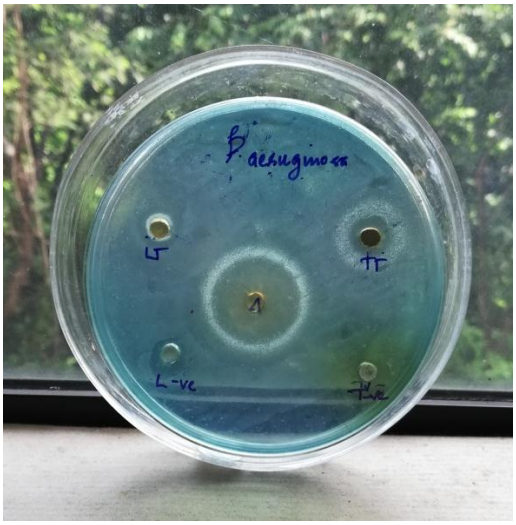
7.3: Agar well diffusion of bacteria



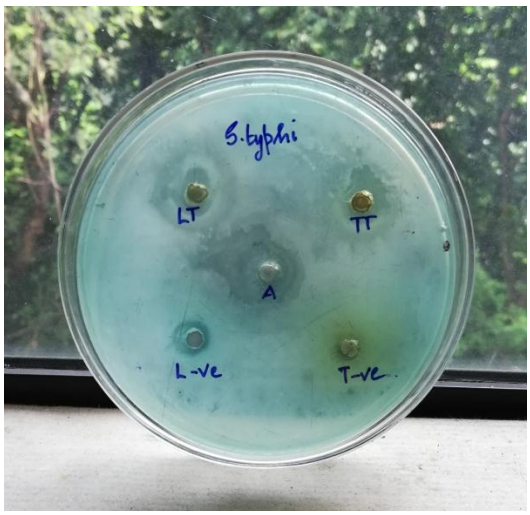
7.3.1: *Escherichia coli*



7.3.2: Listeria *monocytogenous*

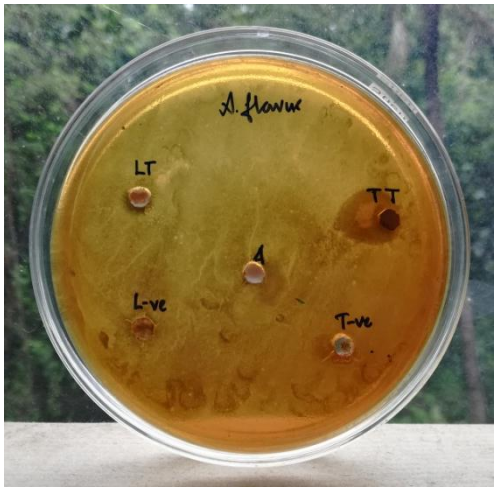


7.3.3: *Pseudomonas aeruginosa*

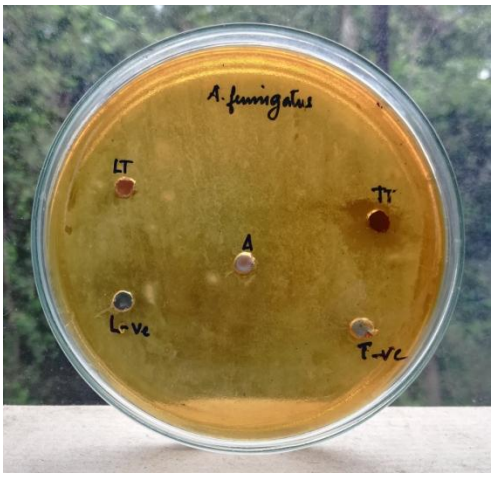


7.3.4: Salmonella typhi

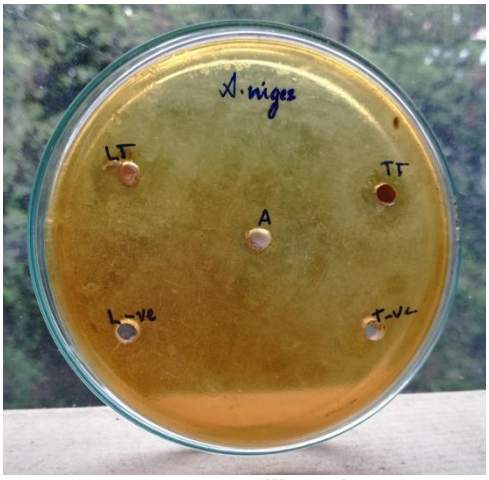
7.4 Agar well diffusion of fungi



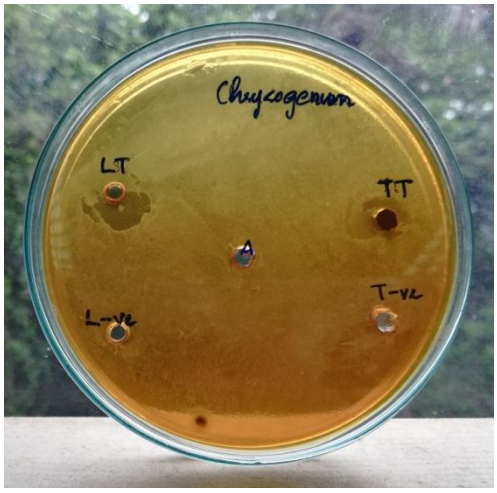
7.4.1: Aspergillus flavus



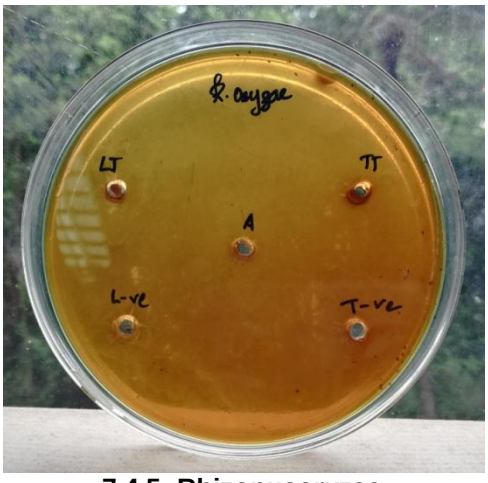
7.4.2: Aspergillus fumigates



7.4.3: Aspergillus niger



7.4.4: Penicilliumchrysogenum



7.4.5: Rhizopusoryzae

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