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A Awareness And Knowledge On Comparative Cytotoxic Effect Of Hibiscus- Marigold Herbal Formulation And Its Mediated Silver Nanoparticles

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ABSTRACT
Introduction: In recent years, there has been a resurgence in the use of traditional medicinal herbs, and as a result, pharmaceutical companies are investing heavily in developing natural medications derived from plants. Nanoparticles act in a number of potential ways and fields. Chemical synthesis of nanoparticles is no longer advantageous compared to plant-based synthesis. The current study uses a green method to create silver nanoparticles (AgNPs) utilizing flower extract.
Materials and methods: The cytotoxic activity for gel was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations. 0.05 mL of hibiscus and marigold of various fixations.
Result: Using rosa and jasminum extract in the manufacture of silver nanoparticles, the nanoparticles showed remarkable antioxidant and anti-inflammatory activity. It was discovered that the cytotoxic effect was less harmful, demonstrating biocompatibility. Rosa jasminum extract of AgNps showed good anti-inflammatory efficacy in the EA and BSA assays.
Conclusion: From our study we concluded that Silver nanoparticles were created using rosa and jasminum extract, and the nanoparticles displayed impressive cytotoxicity.

Keywords: Silver Nanoparticle, Hibiscus, Cytotoxic Effects

INTRODUCTION

Nanotechnology is the branch of technology that deals with dimensions and tolerances of less than 100 nanometres,the manipulation of individual atoms and molecules. The utilization of various plant parts for the synthesis of nanoparticles is considered as a green technology. Silver nanoparticles (AgNPs) have exceptional properties for applications like mosquitocidal, antimicrobial, larvicidal, metastatic tumor, anticancer and wound healing.(1) The synthesis and characterization of silver nanoparticles was confirmed by UV-Visible spectrophotometer,Scanning Electron Microscopy (SEM), Fourier remodel Infrared spectroscopy (FTIR)(2)The use of plants for the assembly of silver nanoparticles has received lots of attention due to its rapid, eco-friendly, non- pathogenic, economical protocol and providing a single step technique for the green synthesis processes.(3). Since Ag NPs are smaller than the microorganisms, they diffuse into cell and rupture the cell wall which has been shown from SEM and TEM images of the suspension containing nanoparticles and pathogens(4) Cytotoxicity of nanomaterials depends on their size, shape, coating/capping agent and the type of pathogens against which their toxicity is investigated. Nanoparticles synthesized from the green method are generally more toxic than those obtained from the non-green method. Some pathogens are more prone to nanomaterials, especially Ag NPs than others due to the presence of both the Ag ions released and Ag NPs. They slowly envelop the microbes and enter into the cell, inhibiting their vital functions.(5)
Hibiscus rosa sinensis belongs to the family Malvaceae. They are not only grown for decorative purposes, but also have some nutritive and biological properties and the extract that can induce the cytotoxic action against cancer cells and initiate antiproliferative effects leading to cancer cell death.The genus Hibiscus from the Malvaceae family is widely grown in tropical regions(6).phytochemical investigation reported the presence of alkaloids, flavonoids, phenols, tannins and terpenoids in leaf, stem and root extracts of H. rosa-sinensis.
Genus Tagetes (Asteraceae) is native to Americas but some of its members (in particular T. erecta and T. patula) commonly known as marigolds.Phytochemicals of marigold, particularly lutein and flavonoids are reported to

have anti-inflammatory, free radical-scavenging and cytotoxic effects against various cancerous cells.(7) The plant species have been used for the treatment of skin diseases.

MATERIALS AND METHODS

Plant Material

The *Hibiscus rosa sinensis* is a small tree or shrub having glossy leaves.
Family: Malvaceae
Subfamily: Malvoideae
Genus: Hibiscus
Species: rosa sinensis
Botanical name: *Hibiscus rosa sinensis*

Brine Shrimp Lethality Assay

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200 ml of distilled water. 6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10 µL,20 µL,40 µL,80 µL and control). Then the nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for number of live nauplii present and calculated by using following formula, number of dead nauplii/number of dead nauplii+number of live nauplii×100

RESULTS

In the present study, the results show that figure 1, shows the dry marigold and dry hibiscus is heated under conventional heating at 80 C in an aqueous solution. Figure 2, shows the Flower extract of marigold and hibiscus in aqueous solution.Figure 3, shows the Silver nanoparticles formulations of hibiscus and marigold flower extract. Figure 4, Brine Shrimp Lethality assay of hibiscus and marigold AgNps, shows the comparison of flower extract, hibiscus and marigold AgNps the cytotoxic effects of this flower extract compared with the standard concentrations of live nauplii. The concentrations are 5µl, 10µl, 20µl, 40µl, 80µl and control. There are no changes in the live nauplii in 5µl,10µl, 20µl, 40µl concentrations. In 80µl, the live nauplii is decreased and the concentration is decreased therefore, this shows cytotoxic effects of Hibiscus marigold silver nanoparticles.

Fig 1: This figure shows Dry marigold and Dry hibiscus is heated under conventional heating at 80°C in an aqueous solution.

Figure 2: This figure shows the Flower extract of marigold and hibiscus in aqueous solution.

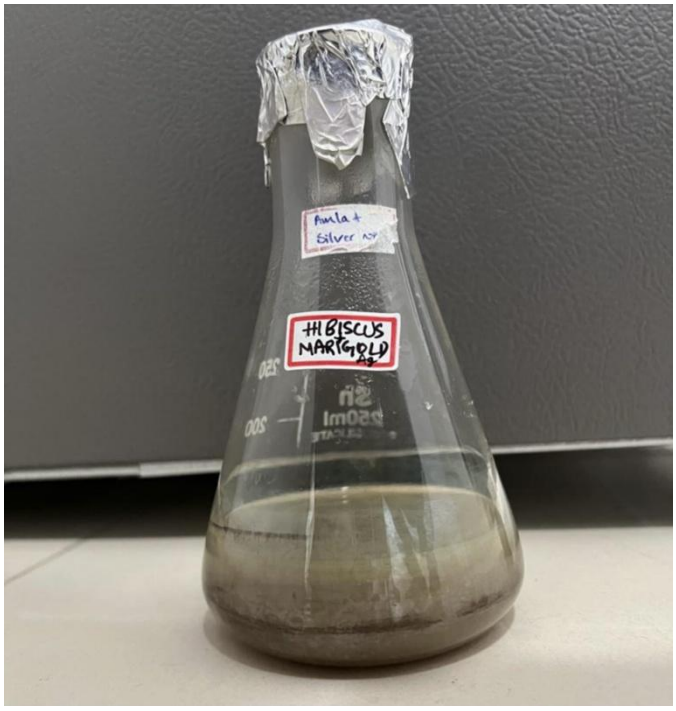

Fig 3: This figure shows the Silver nanoparticles formulations of hibiscus and marigold flower extract.

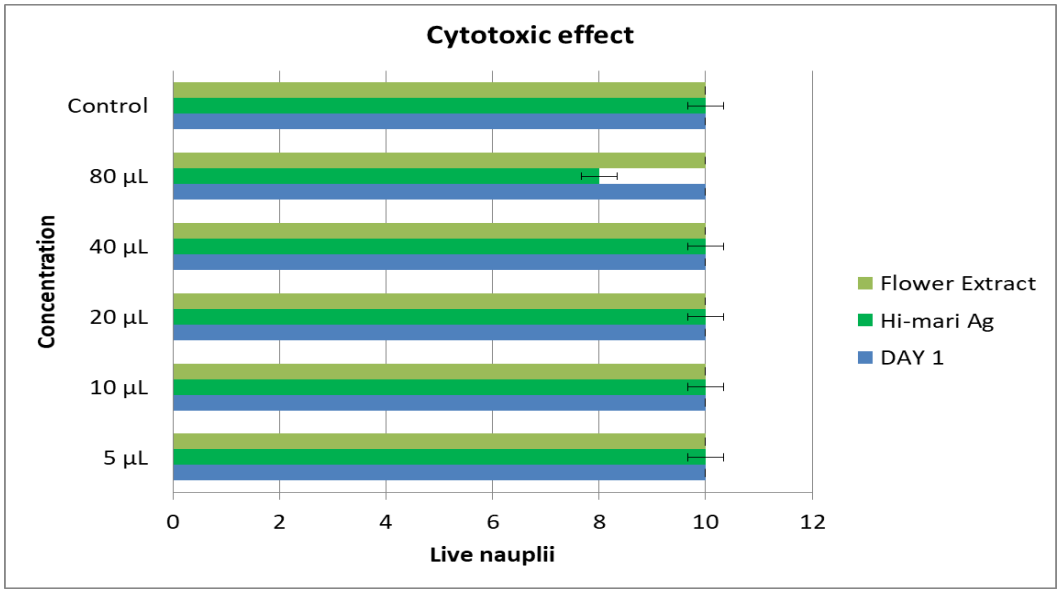

Figure 4: Brine Shrimp Lethality assay of hibiscus and marigold AgNps, shows the comparison of flower extract, hibiscus marigold silver nanoparticles and the live nauplii with standard concentrations. Compared with different concentrations, In 80µl concentrations live nauplii counts were decreased and it shows the cytotoxic effects.

DISCUSSION

The majority of the world's population is using herbal medicines for many diseases nowadays. From the ancient days, the herbs were used as the medicinal drugs for number of diseases.(8) Many herbs are having pharmacological actions, which are abundant in nature.(9) The medicinal herbs are having compounds like secondary plant metabolites like flavonoids and tannins which have been used for thousands of years for healthcare.

The *Hibiscus rosa sinensis* is a medicinal herb which is rich in secondary plant metabolites, and these compounds are acting as reductant and the capping agent in the formation of silver nanoparticles(10). In this green chemistry synthesis method of silver nanoparticles, the metal salts are reduced into the aqueous metal ion precursors. As a result of this reduction, the colour changes in the mixture.(11) This colour change of the solution indicates the formation of the silver nanoparticles.

In a previous study by Avinash etal., 2020. The silver ions from silver nanoparticles are believed to become attached to the negatively charged particles which leads to denaturation of protein and finally cell death.(12) In another study AgNPs were created using(13) A. esculentus flower extracts as a moderating agent. All of the examined gram-positive and gram-negative microbial pathogens were resistant to the NPs' antibacterial activities. The synthesised NPs' IC50 values for the examined cell lines were comparable to those of a common medication.

In another study done by(14) Against this ESKAPE pathogen, the biogenic NPs demonstrated substantial cytotoxic activity. Furthermore, compared to bigger AgNPs, smaller AgNPs with a higher surface area would show a more notable microbicidal activity. In another study, H.rosasinesis produces silver nanoparticles in this way. (15)demonstrated strong cytotoxic effects against the MCF-7 and 5637 cell lines and may be used as a new cytotoxic drug(16).(17)

In the present study, the cytotoxic activities of normal and silver nanoparticles synthesized H.rosa-sinensis extract were carried out by agar well diffusion method. Synthesized silver nanoparticles were tested against selected fish bacterial pathogen A.hydrophila.

CONCLUSION

From our study, It is evident that the activity of cytotoxic effects of hibiscus- marigold herbal formulation are significantly inhibited by its mediated silver nanoparticles. Silver nanoparticles were created using hibiscus and marigold extract, and the nanoparticles displayed impressive cytotoxic effects and the prepared silver nanoparticles showed good antimicrobial activity against Escherichia coli, Proteus mirabilis. Also this extract showed antibacterial properties. The fact that the anti-inflammatory effect was less detrimental was found to be a sign of biocompatibility. Thereby, this study may encourage the low-cost production of green-AgNPs and their safe usages.

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Conflict of Interest
The authors reported no conflict of interest while performing this study.

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