FRUIT MEDIATED SYNTHESIS OF SILVER NANOPARTICLE USING ACAI BERRY AND ITS CYTOTOXIC EFFECT

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ABSTRACT
Acai berry /Euterpe oleracea mart. has a high antioxidant, anti inflammatory, antiproliferative,cardioprotective activities. The use of silver nanoparticles have great antibacterial and antimicrobial properties,nowadays it has been used in medical devices,wound dressing which releases silver in lower amounts to provide a microbe free environment. Cytotoxicity tests are very important in nanoparticles as they help in the determination of the proposed biomedical use to cause any cell death due to leaching of toxic substances or from direct contact of the nanoparticle.The aim of the study is to find the cytotoxic effect of silver nanoparticles using Acai berry fruit extract.All the materials used are under laboratory conditions.The brain shrimp lethality assay results show a low toxicity profile under the concentration of 80µL where there are 80%of viable napoli.

Key words: Silver Nanoparticles, Acai berry, cytotoxicity , brine shrimp lethality assay

INTRODUCTION

The acai berry, which comes from the Euterpe oleracea Mart. tree, is a fruit that is popularly consumed in Brazil and comes from the Amazon region. The Arecaceae family includes Euterpe oleracea Mart. It grows from 6 to 14 meters tall and is the most well-known huge palm tree from the floodplain region of the Amazon.(Carvalho *et al.*, 2017). Due to its high concentration of phenolic compounds, including anthocyanins, flavonoids quercetin, vitexin, luteolin, chrysoeriol, and dihydrocaempferol, as well as the anthocyanins cyanidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-sambioside, and peonidine-3-rutoside, acai berries exhibit antioxidant(Yamaguchi *et al.*, 2015)

In addition to it acai berry has antitumorigenic activity which produces polyphenolic, glycoside, and aglycone compounds to induce apoptosis in leukemia cells (Del Pozo-Insfran, Percival and Talcott, 2006). Investigative study reported that the locals consuming acai berry has induced colon cancer.(Fragoso *et al.*, 2013).Because of their distinctive characteristics, silver nanoparticles (AgNPs) have the potential to be employed therapeutically. They have also been used as potent, broad-spectrum antibacterial agents in bandages, catheters, food storage containers, antiseptic sprays, and clothing.(Qasim *et al.*, 2014)

Fig1: Acai berry Fruit.

By causing cellular apoptosis through mitochondrial-dependent and mitochondria-independent routes against a variety of cancer cells, such as human breast cancer cells, lung cancer cells, liver, skin, and oral carcinoma cell lines, via the leakage of lactate dehydrogenase, reactive oxygen species (ROS) formation, and the impairing of DNA synthesis, it has been demonstrated that silver nanoparticles have anti-cancer effects and exhibit cytotoxicity on cancer cells.(Gurunathan *et al.*, 2013)

Additionally, silver nanoparticles have good prospects as catalysts in a number of fields, including photodetectors, biosensors, antimicrobials, and drug delivery systems. Both chemical and biological processes are used to create these nanoparticles.(Anderson and Moskovits, 2006).

Silver nanoparticles are more readily prepared biologically, more economically, and environmentally acceptable. The chemical process, on the other hand, is more involved, has adverse side effects, and has an influence on the ecosystems. Likewise, silver nanoparticles are often used in biomedical devices that release silver in smaller amounts to create an antibacterial environment, as well as molecular diagnostics, photonic devices, antimicrobial films, and optoelectronic devices.The release rate of the Ag^{+} ions depends on a number of factors including the size and shape of nanoparticles, capping agent, and colloidal state. Smaller or anisotropic silver nanoparticles with a larger surface area showed more toxicity and exhibited a faster ion release rate due to high surface energy originating from highly curved or strained shapes of nanoparticles. The small-sized silver nanoparticles also exhibited a superior release rate of silver ions particularly into the Gram-negative bacteria(Sun *et al.*, 2003)

Our team has extensive knowledge and research experience that has translated into high quality publications (Sathivel *et al.*, 2008; Neelakantan *et al.*, 2011; Felicita, Chandrasekar and Shanthasundari, 2012; Jain, Kumar and Manjula, 2014; Lakshmi *et al.*, 2015; Keerthana and Thenmozhi, 2016; Sekar *et al.*, 2019; Johnson *et al.*, 2020)(Sahu, Kannan and Vijayaraghavan, 2014; Jose, P. and Subbaiyan, 2020; Sivasamy, Venugopal and Mosquera, 2020; Vikneshan *et al.*, 2020; Ezhilarasan, 2021; Marofi *et al.*, 2021; Preethi and Sekar, 2021)

MATERIALS AND METHODS:

PREPARATION OF FRUIT EXTRACT:

Acai berry powder was measured and dissolved in 100 ml distilled water and the solution was heated in the heating mantle for about 15-20 min for 60°C-70°C.

SOLVENT EXTRACTION:

Synthesis of silver nanoparticles was done by mixing 30ml of acai berry solution with 1mmolar aqueous silver nitrate solution and kept the Acai berry silver nanoparticle solution in the shaker for 2-3 days .

SYNTHESIS OF SILVER NANOPARTICLES :

After 2-3 days the solution was added to six 15cc centrifugal tubes for centrifugation at 8000 rpm for 10min to separate pellets and the supernatant.The pellet solution was collected for assay analysis.

CYTOTOXICITY OF SILVER NANOPARTICLES

The cytotoxicity of the synthesized silver nanoparticles was measured usingBrine Shrimp Lethality Assay.

BRINE SHRIMP LETHALITY ASSAY.

2g of iodine free salt was taken and dissolved in 200 ml of distilled water . Then in six well ELISA plates were taken and add 10-12 ml of saline water was added into the wells .The water filled wells were added with 10 nauplii each.Then acai berry silver nanoparticle pellet solution of concentration ranging from 5µL,10µL,20µL,40µL,80µL, were added to to the wells respectively and labelled one of the well as a control group and incubated the plates for 24 hours .After 24 hours of incubation the ELISA plates were observed and noted for number of nauplius live and dead and calculated by the formula

Number of dead nauplius / number of dead +live nauplius)*100

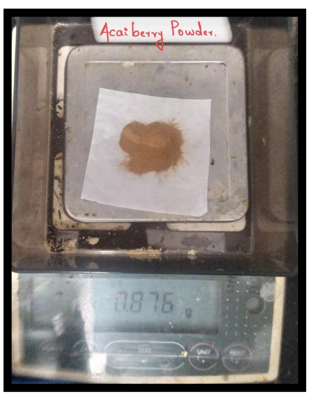


Fig2: Açai berry powder

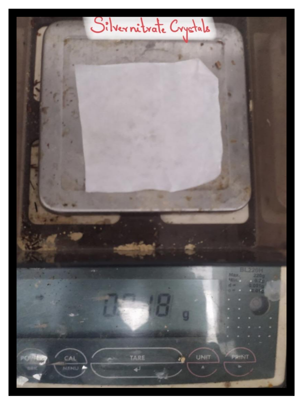


Fig3: Silver nitrate crystals

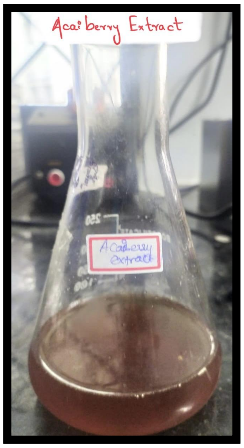


Fig4: Açai berry extract.

Fig5 : Silver nitrate solution

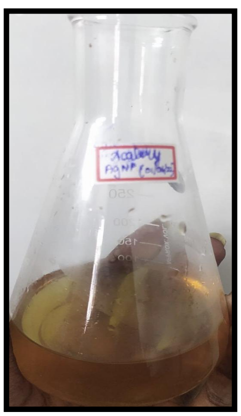


Fig6: Açai berry extract and
Silver nitrate solution mixture

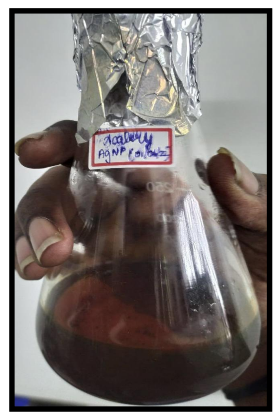


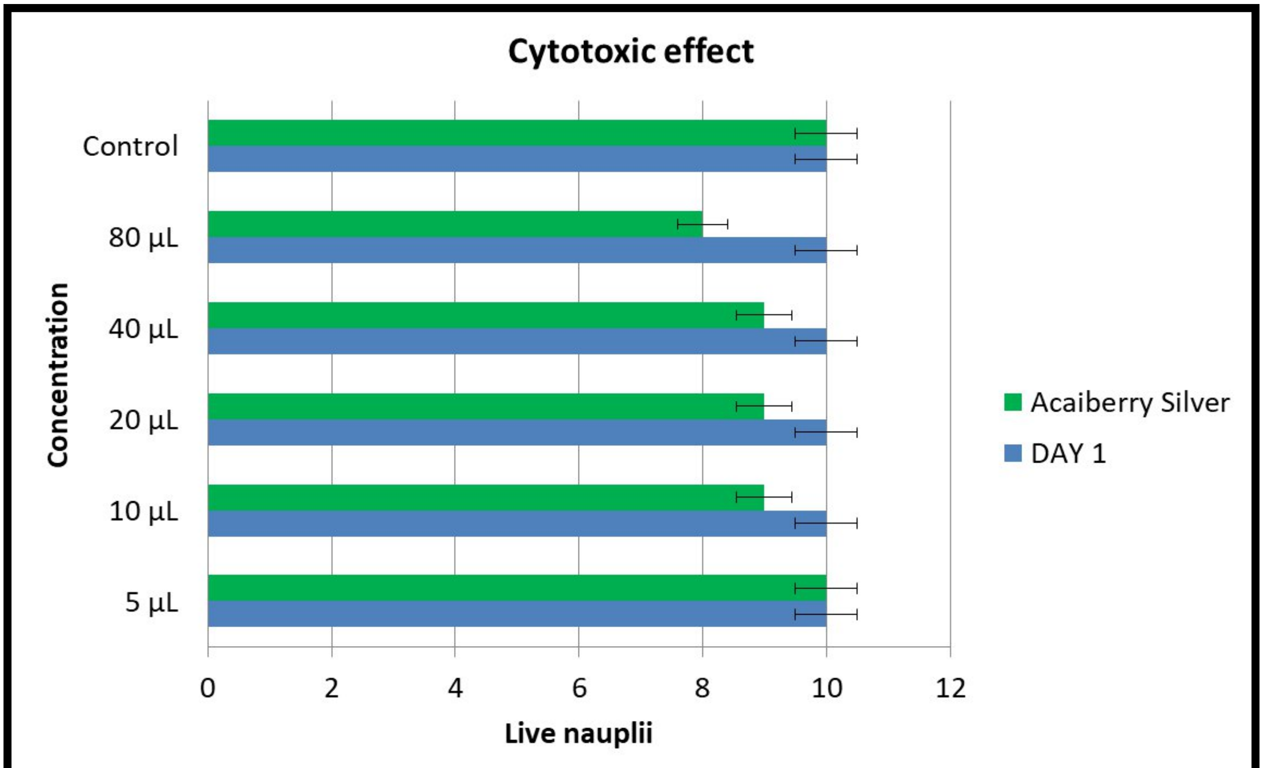
Fig7: Açai berry AgNP after 2-3days

RESULT

The cytotoxic profile of Acai berry was formulated by Brine shrimp lethality assay where the nauplies are exposed to different concentrations of the fruit extract .The effect was inferred from the graph that the x axis indicates the number of live nauplius and the y axis indicates the concentration of acai berry ranging from 5µL,10µL,20µL,40µL,80µL. After observation of each well at different concentrations , at 5µL concentration there were no dead nauplius. From 10µL,20µL,40µL concentration there were the same number of nauplius dead in each well. In the well with a concentration of 80µL there were only 8 living nauplius. As a result there were more than 50% of nauplius where live in the highest concentration; the cytotoxicity profile of Acai berry silver nanoparticles is at the minimal level .

CONCENTRATION	DAY1	DAY2
5µL	10	10
10 µL	10	9
20 µL	10	9
40 µL	10	9
80 µL	10	8
Control	10	10

Table 1; Shows the number of live naupli alive in the medium in different concentrations of the extract.



Graph 1: This graph depicts the cytotoxic effect of Açais berry and silver nanoparticle mixture. The x axis shows the number of living nauplii and the Y axis represents the concentration of the Acai berry AgNP mixture in micro litters(µL).There is dose dependent increase in the cytotoxic effect at the highest concentration.

DISCUSSION

Exudate secretion and inflammation are the initial steps in wound healing. After that, the inflammation is minimized, which enables the wound to constrict and re-epithelialize.According to a few research, exudate secretion and inflammation are the initial steps in wound healing. After that, the inflammation is minimized, which enables the wound to constrict and re-epithelialize.(Clark, 1993) In this trial, groups treated with acai berry aqueous extracts recovered more quickly than groups treated with a vehicle control. Based on this finding, Acai berry water extract application is projected to accelerate quick wound healing. That could be because acai berries contain potent anti-inflammatory properties like anthocyanin and antioxidant compounds.(Kang, Choi and Kim, 2017)

The composition of açai pulp has also been studied, and it contains a number of phytochemicals that have physiological activity. Considerable research has confirmed the neuroprotective potential of açai berries. Numerous of these diseases have various root causes, which include oxidative stress, chronic neuroinflammation, excitotoxicity, mitochondrial dysfunction, abnormal protein accumulation in brain tissues, and other cellular aetiologies. They are the result of a combination of aging, genetic disorders, and exposure to one or more environmental factors. Açai berry extracts protect against neurodegenerative diseases through, among so many other things, maintaining calcium homeostasis, mitochondrial function, and anti-inflammatory and antioxidant capabilities. Likewise, açai fruit exhibits antidepressant and anticonvulsant characteristics that may be advantageous for those with various neurological conditions.(Xie *et al.*, 2012; ALNasser and Mellor, 2022)

In the medical and healthcare fields, silver has made a remarkable comeback as a possible antibacterial molecule, either in the form of AgNPs or in conjunction with traditional antibiotics. Numerous studies have indicated that AgNPs are highly effective against mycobacteria in both bacterial cultures and macrophages.(Mohanty *et al.*, 2013; Montelongo-Peralta *et al.*, 2019)

Combining traditional anti-TB therapies with silver nanoparticles, which together strengthen the antimycotic activity both extra and intracellularly. The ongoing research efforts for the production of novel anti-TB nanoparticles should emphasize on approaches to enhance the local availability of antibacterial nanoparticles due to the peculiar shape, visceral distribution, and TB-induced lesions. A significant antimycobacterial action, good intra-macrophagic availability, increased local availability, and minimal immunosuppressive and toxic effects should all be present in a candidate for nanoparticle therapy for tuberculosis in the future.(Tăbăran *et al.*, 2020)

Toxicokinetic studies of NPs including absorption, distribution, metabolism and elimination have provided important data related to their in vivo behavior and risk assessment. In this context, there is very limited data on AgNPs' toxicokinetic properties, particularly metabolism and clearance. Though several other studies discussed in this review have demonstrated organ distribution following the translocation of AgNPs, it is not yet clear whether the distribution and effects observed were due to their particulate forms, ionic forms, or a combination of both forms.(Lee *et al.*, 2018)

Silver nanoparticles would affect vulnerable members of society, such as those with pulmonary conditions, obesity, hypertension, and diabetes. It is commonly known that people with pre-existing cardiorespiratory disorders such as asthma and chronic respiratory illnesses like cystic fibrosis, obstructive pulmonary disease , pneumonia, and ischemic cardiovascular issues are much more susceptible to the threat of air pollution..(Hooper and Kaufman, 2018)

CONCLUSION

The present study highlights the cytotoxic effect of Acai berry fruit extract using silver nanoparticles which was determined using Brine shrimp lethality assay. As a result there were more than 50% of living nauplies in the highest concentration of 80 µL, so toxicity levels should be analyzed further to determine the future use in the medical field.

CONFLICT OF INTEREST

There is no conflict of interest.

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