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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM HERBAL FRUIT FOR ANTICANCER

S.Krishna Priya [1], S.Nanthini[2], P.Priyadharshini[3], M.Vinotha[4]

Assistant Professor[1], UG Student[2,3,4]

Department of Biomedical Engineering, Dhanalakshmi Srinivasan Engineering College, Perambalur

ABSTRACT: Silver nanoparticles (AgNPs) are getting to be progressively imperative for different vital applications, counting antimicrobial, anticancer, catalytic, and anti-inflammatory. For the most part, nanoparticles are arranged by a assortment of chemical methods which are not naturally inviting. AgNPs biosynthesized from plant extricates have pulled in impressive attention since of their eco-friendliness. The venture includes the amalgamation of AgNPs utilizing the methanol extricate of Selenicereus undatus and the consequent characterization of their physicochemical properties. Explanatory strategies such as UV spectroscopy and infrared spectroscopy (IR), are utilized to analyze the synthesized AgNPs. Breast cancer remains a critical worldwide wellbeing challenge. The ponder explores the potential anticancer impacts of these AgNPs against breast cancer cells, advertising a promising restorative approach.

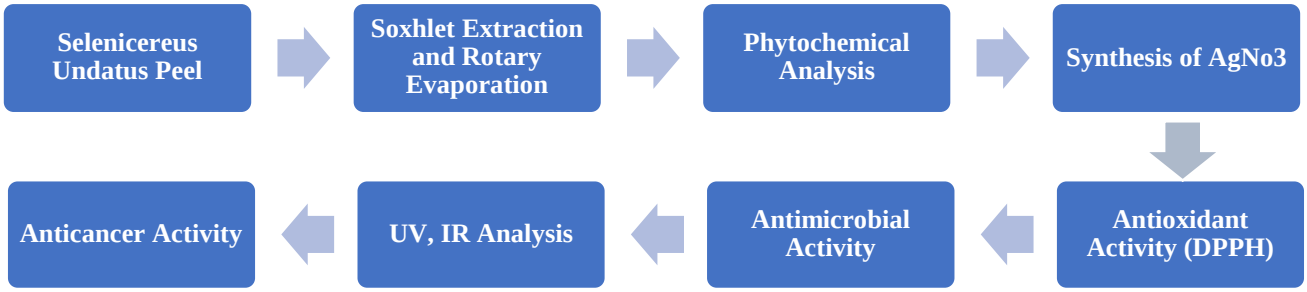
Keywords: Silver Nano-Particles, Selenicereus undatus, Methanol Extricate, Physiochemical action, Characterization, Antimicrobial Movement, UV&IR Spectroscopy, Breast Cancer, Anticancer Activity.

1. INTRODUCTION

Breast cancer remains a significant global health challenge. Breast Cancer is the most common cancer worldwide. Breast cancer is a disease in which cells in the breast cancer grow out of control (Sung *etal*,2020).According to WHO , maligant neo-plasms are the greatest worldwide burden for women estimated at 107.8 million Disability (World Health Organization,2016).The integration of nanotechnology with herbal medicine has emerged as a promising avenue in cancer therapeutics, offering both efficacy and sustainability. Silver nanoparticles (AgNPs), owing to their unique physicochemical properties, have gained considerable attention for their potential anticancer activity. Selenicereus undatus, commonly known as dragon fruit, has garnered attention for its rich phytochemical profile, making it an ideal candidate for nanoparticle synthesis. Methanol is used in extraction for its ability to efficiently extract anticancer compounds like polyphenols and flavonoids from herbal fruits due to its polar nature and ability to penetrate cell walls effectively. The synthesis and characterization of AgNPs from the methanol extract of Selenicereus undatus fruit hold promise for several reasons. Firstly, the eco-friendly nature of herbal extracts aligns with the growing demand for sustainable technologies. Secondly, AgNPs derived from herbal sources may possess enhanced biocompatibility and therapeutic efficacy compared to chemically synthesized counterparts. Thirdly, the inherent anticancer potential of both Selenicereus undatus extracts and AgNPs offers a synergistic approach to combating cancer.

Figure 1: dragon fruit

2. FLOW CHART



3. MATERIALS AND METHODS

3.1 Sample preparation – Selenicerus Undatus

The peel of Dragon fruit (Selenicereus Undatus) is collected and shear dried for 3 days. The dragon fruit peel weighed 50 grams, added to 800 ml of methanol solution. Then it is extracted using hot percolation methods (Soxhlet) and the sample is condensed using rotary evaporation.

Figure 2: dragon fruit sample

3.2 Extraction Process

(a) Soxhlet Extraction

The Soxhlet extraction method will be utilized to extract bioactive compounds from the herbal fruit Selenicereus undatus using methanol as the solvent. This method ensures efficient extraction by continuously cycling the solvent through the plant material, maximizing the extraction yield.

(b) Rotary Evaporation

The rotary evaporation technique will be employed to concentrate the methanol extract obtained from Selenicereus undatus herbal fruit. This method involves the use of gentle heat and vacuum to evaporate the solvent, leaving behind a concentrated extract suitable for further processing in nanoparticle synthesis.

3.3 Phytochemical Test

The phytochemical analysis involves identifying and quantifying the bioactive compounds present in the methanol extract of Selenicereus undatus herbal fruit. This analysis provides valuable information about the composition of the extract, which may contribute to the synthesis and stabilization of silver nanoparticles and their potential anticancer activity. The Phytochemicals present in the sample are Alkaloids, Carbohydrates, Proteins and Phenolic compounds.

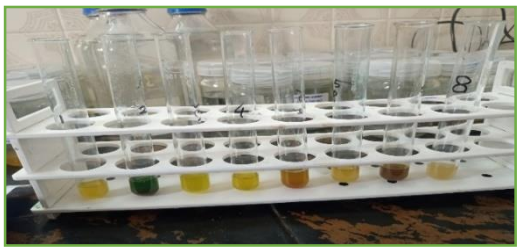


Figure 3: phytochemical test

Table 1: phytochemical analysis

Bioactive Compounds	Solutions	Colour to be Present	Present or Absent
Alkaloids	Dragendorffs Agent + Sample	Yellow Precipitate	Present
Carbohydrates	Fehlings solution I,II + Sample + Kept in Water Bath for 15 min	Red Spot	Present
Glycosides	Ammonia solution + Chloroform + Sample	Pink colour	Absent
Saponins	H2o + Sample	Foam	Absent
Proteins	Sample + Distilled Water + Ethonal + Potassium Hydroxide Pellets	Red or Brown Ring	Present
Amino Acids	Sample + Distilled Water + Ninhydrin Solution	Pink or Red colour	Absent
Phenolic Compounds	Sample + Distilled Water + Feric Chloride Solution	Dark Green colour	Present
Tetpenoids	Sample + Distilled Water +Trichloroaeticacid Solution	Red Precipitate	Absent

3.4 Preparaton of Silver Nanoparticles

A add up to of .0016 mg of AgNo3 powder was broken up in 150 ml of H2O and at that point homogenized.

(a) Synthesis of Silver Nanoparticles

1 mM silver nitrate arrangement in twofold refined water was the source of silver. Silver nitrate and seed extricate were blended together in a proportion of 1:9. The response blend was warmed underneath the bubbling point and ceaselessly mixed at 800 rpm utilizing attractive stirrer. The blend turned ruddy brown in color inside 1 h. The gotten suspension of Ag/T. grandis was centrifuged at 15,000 rpm for 45 min. The pellet containing silver nanoparticles was washed 3–4 times with deionized water to evacuate silver particles and seed extricate buildup. The accelerated nanoparticles were lyophilized. Lyophilized nanoparticles were put away in a cool, dry, and dim put and assist their characterization was carried out.

(b) **Characterization of Silver Nanoparticles**

The compound gotten from the blend of silver nanoparticles utilizing water extricate of mythical beast natural product peel (H. polyrhizus) was at that point characterized on a few parameters, to be specific organoleptic, the surrender was watched outwardly for its color characteristics. The absorbance of the amalgamation arrangement was watched over time to affirm the arrangement of silver nanoparticles.

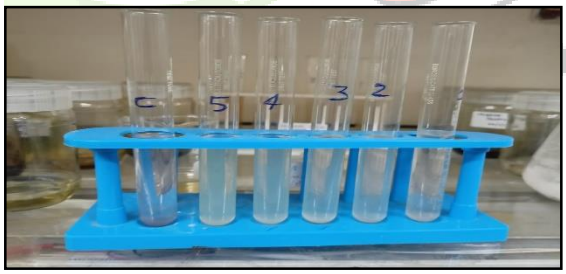


Figure 4: characterization of silver nanoparticles

3.5 Antioxidant Activity – DPPH Assay

DPPH Assay The antioxidant appraisal points to decide the antioxidant potential of the methanol extricate determined from Selenicereus undatus home grown natural product. DPPH test DPPH, or 2,2-diphenyl-1-picrylhydrazyl, is a common reagent utilized to degree antioxidant action. It's a steady free radical that responds with cancer prevention agents by tolerating an electron, turning from purple to yellow. This alter in color is measured spectrophotometrically to decide the antioxidant capacity of a substance. The rate of antioxidant action of each substance was evaluated by DPPH free radical test. The tests were responded with the steady DPPH radical in methanol arrangement. The response blend comprised of including 20 µL of test, 980 µL of H2O and 300 µL of DPPH radical arrangement. Rehash it with diverse estimation. The control arrangement was arranged by blending 1 ml of H2O and 300 µL of DPPH. The scavenging activity percentage was determined according to

% of inhibition = Control O.D – Sample O.D/Control O.D X 100

Control = 1ml H2O + 300µl DPPH
Control value = 0.53

Table 2: antioxidant activity

Sample	Distilled Water	DPPH	Weight	% of inhibition
20µl	980µl	300µl	0.45	15.09
40µl	960µl	300µl	0.65	-22.64
60µl	940µl	300µl	0.68	-28.30
80µl	920µl	300µl	0.69	-30.18
100µl	900µl	300µl	0.35	33.69
120µl	880µl	300µl	0.32	39.62
140µl	860µl	300µl	0.27	49.05
160µl	840µl	300µl	0.80	55.94
180µl	820µl	300µl	0.74	62.39
200µl	800µl	300µl	0.36	33.96

3.6 Antimicrobial Activity

Antimicrobial Activity Antimicrobial movement alludes to the capacity of a substance to hinder the development or slaughter microorganisms, such as microbes, organisms, infections, or protozoa.

Nutrient Broth – 1.95g
Agar – 2.5g
H2O – 150ml

The test life forms were immunized in Supplement broth and hatched overnight at 37°C. MHA plates was refined with standardized microbial culture broth. Each well was filled with shifting concentrations from 100, 125, 150 µg/ml of the tests with positive control as streptomycin 25 mcg and negative/solvent control as DMSO, individually. The plate was permitted to diffuse for almost 30 minutes at room temperature and hatched for 18-24 hours at 37°C. After hatching, plates were watched for the arrangement of a clear zone around the well which compares to the antimicrobial movement of the tried samples.

(a) (b) (c)

Figure 5 : (a) gram-positive - streptococcus aureus, (b) gram-negative - E.coli, (c) fungus – candida albicans

3.7 Analytical Techniques

(a) UV Spectroscopy

UV spectroscopy can be utilized to screen the arrangement of AgNPs amid the blend prepare. The decrease of silver particles (Ag^+) to AgNPs leads to changes in the absorbance range in the UV-Vis locale. Once synthesized, the AgNPs require to be characterized to decide their estimate, shape, and soundness, which are significant for their potential anticancer applications. UV spectroscopy can be utilized for the subjective and quantitative investigation of AgNPs. The UV-Vis retention range of AgNPs ordinarily appears a characteristic surface plasmon reverberation (SPR) crest in the extend of 400-500 nm, depending on the measure and shape of the nanoparticles. The position and concentrated of this top can give data around the estimate dispersion and concentration of the nanoparticles. Once the AgNPs are synthesized and characterized, their potential anticancer action can be assessed through in vitro and in vivo ponders. AgNPs have been detailed to display anticancer properties through different components, counting acceptance of apoptosis, restraint of cell multiplication, and focusing on of particular cancer cell pathways. The AgNPs synthesized from methanol extraction of Selenicereus undatus peel may have extra bioactive compounds that may improve their anticancer activity.

(b) IR Spectroscopy

The blend prepare regularly includes diminishing silver particles (Ag^+) to AgNPs utilizing a lessening specialist show in the methanol extricate of Selenicereus undatus peel. This diminishment prepare can be checked utilizing IR spectroscopy. Utilitarian Bunch Examination: IR spectroscopy permits the distinguishing proof of utilitarian bunches show in the methanol extricate and their intuitive with AgNPs. Crests comparing to particular utilitarian bunches (e.g., hydroxyl bunches, carbonyl bunches) can demonstrate the nearness of bioactive compounds dependable for decreasing and stabilizing AgNPs. Once characterized, the synthesized AgNPs can be assessed for their anticancer action against cancer cell lines. IR spectroscopy can be utilized to survey any changes in the useful bunches of cancer cells upon treatment with AgNPs, giving unthinking experiences into their anticancer impacts. Moreover, IR spectroscopy can be utilized in examining the interaction between AgNPs and biomolecules inside cancer cells, helping in understanding the mode of activity of AgNPs as potential anticancer agents.

3.8 Anticancer activity – MCF7

MTT assay

The MTT measure was utilized to degree cytotoxicity (misfortune of practical cells). This test is based on the metabolic lessening of the dissolvable MTT salt, 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide which reflects the ordinary work of mitochondria dehydrogenase movement and cell reasonability, into an insoluble colored formazan item, which was measured spectrophotometrically (Sadeghi-aliabadiet al; 2010). The assurance of the movement of mitochondrial dehydrogenase in living cells straightforwardly and relatively speaks to the number of reasonable cells (Mosmann, 1983). After treatment with the extricate, the cells were hatched for 72 hours at 37°C, 5% CO2. 20µl of channel sterilized MTT (2mg/ml) in phosphate-buffered saline (PBS) was included to each well and hatched at 37°C for 3 hours. The medium with MTT was evacuated and the shaped formazan precious stones were solubilized by the expansion of 100µl of DMSO and the absorbance perused at 540 nm utilizing a widespread microplate peruser. Treated cells were compared with untreated controls. Tetrazolium salts are cleaved to formazan color by cellular proteins as it were in practical cells. The diminishment of the tetrazolium salt MTT, to colored formazan compounds by cellular proteins as it were happens in metabolically dynamic cells (reasonable cells).

% of inhibition = Control – Sample/Control x 100

Here, (a) speaks to the cancer cell which is considered a control cell. (b) speaks to the cancer Cells treated with extricate at 100 µg/ml which appears the shrinkage at a negligible level and (c) speaks to the cancer Cells treated with extricate at 150 µg/ml which appears the shrinkage at a greatest level. When it's compared with the control, 150 concentration appeared the cancer cells passing. The cancer cells appeared shrinkage of the cell divider and consequently apoptotic cell passing is seen in the study.

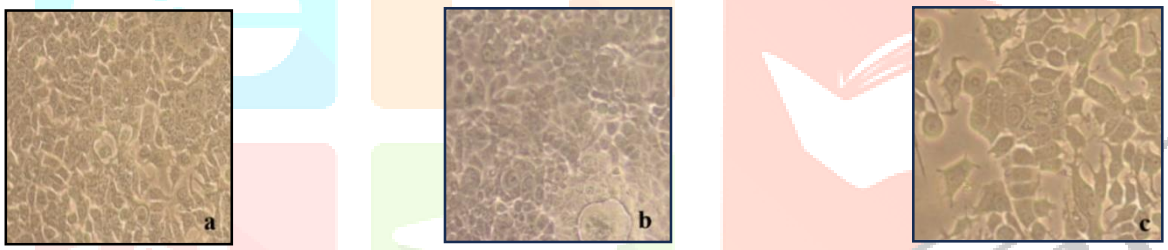


Figure 6: (a) Control cells of MCF-7, (b) Cells treated with extract at 100 µg/ml, (c) Cells treated with extract at 150 µg/ml

4. RESULT AND DISCUSSION

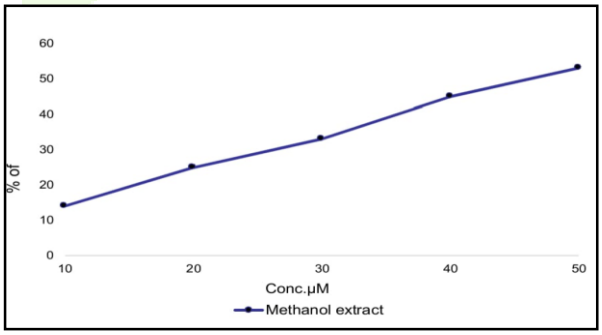


Figure 7: graph

Table 3: % of MCF7 cell death

Conc. µm	% of cell death
100	14
120	25
130	33
140	45
150	53

Therefore, The fruit has the property of curing the cancer at higher concentration of the samples. When its compared with the control, 150 concentration showed the cancer cells death. The cancer cells showed shrinkage of the cell wall, nuclear contents and hence apoptotic cell death is seen in the study

5. CONCLUSION

The cancer cells showed shrinkage of the cell wall, nuclear contents and hence apoptotic cell death is seen in the study. The study of anti-cancer activity was carried out for synthesized silver nanoparticles used in the selenicereus undatus peel. This AgNo3 was screened for its cytotoxicity against the MCF7 Breast cancer cell line at different concentrations with 150 µg/ml showed 53% cancer cell death. Therefore, The fruit has the property of curing the cancer at higher concentrations of the samples. When its compared with the control. The result revealed that *selenicereus undatus peel* extract as a good yield for anti-cancer activity.

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