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# In Vitro Anticancer Activity of Methanolic Leaf and Stem Extracts of *Ipomoea parasitica* Against Human Cervical (HeLa) and Lung Carcinoma (A-549) Cell Lines

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**Abstract:** Ipomoea is an important genus that involves about 600 species of the Convolvulaceae family. The morning glory family, Convolvulaceae, is extensively distributed in temperate, tropical, and subtropical areas. Many Ipomoea species have considerable importance such as medicinal, ornamental, industrial, and food crops. Various species of the genus Ipomoea have been reported for anticancer activity as well. This study represents the green synthesis of silver nanoparticles (AgNPS) using Ipomoea parasitica (''Yellow- Throated Morning glory'')leaf and stem extracts, and investigate the cytotoxicity.The silver nanoparticles of leaves (IPL-AgNPS) and stem (IPS-AgNPS) of I. parasitica showed more cytotoxicity to cancer cell lines, Helawith $IC_{50}$ values of 64.32±1.672µg/ml, 43.45±1.159µg/ml, and A-549 with $IC_{50}$ values of 78.67±2.146µg/ml, 70.12±1.823 µg/mlrespectively. These findings conclude that I. parasitica is a promising source of active compounds effective against cancer cell lines and hence we can further use for the development of new anticancer agents especially for the Hela and A-549.

**Keywords:** I. parasitica, silver nanoparticles, cytotoxicity, Hela, A-549 cell lines.

## 1.0 Introduction

Cancer is deliberated as one of the life-threatening ailments, which include the unusual cell development with the capacity to unceasingly multiply and attack from one tissue to the other in the body (Abida *et al.,*2016). Natural products preserve vast pharmacological significance and have been considered as a key source of potential chemotherapeutic treatments (Bhatnagar and Satija 2017). Plants considered as rich sources for secondary metabolites and also several drugs are presently used from plant basis for the cure of numerous human ailments including cancer.

Searching for the role of medicinal plant in cancer prevention has become a trend in the current decades. Plants are considered as one of the best sources of chemically diverse compounds, several with valuable assets to human health. Approximately 50 % of the anticancer beneficial agents recognized are isolated from plant origin

(Balunas and Kinghorn 2005). Several plant-based molecules that consist of vinblastine, vincristine, taxol, and camptothec in derivatives are used clinically to treat various types of cancers (Greenwell and Rahman, 2015). The National Cancer Institute selected around 35,000 plants from 20 nations and has confirmed 114,000 isolated compounds among them for anticancer action (Mohammad, 2006). More than 3000 types of plants with antitumor properties have been reported as well (Reveal and Hartwell, 1984).

Metallic nanoparticles were recently synthesized via green synthesis to develop environmentally friendly processes with less usage of chemicals that led to the synthesis of metallic nanoparticles by bio reduction mechanism. Biologicals such as, enzymes, fungus and plant extracts are few possible ecofriendly and less expensive compared to that of others previous methods of AgNPS(Subha*et al*., 2018).

The AgNPS synthesized by green approach is nontoxic and effective antibacterial agent that can function against about 650 types of diseases caused by microorganisms (*Min Cho,et al*.,2005). Therefore, the interest on green synthesized AgNPS is increasing and also being projected as future generations antimicrobial agent. Nanoparticles synthesis through environment friendly process is known as green synthesis of nanoparticles, where the extract from plant material act as reducing agent for silver ion ($Ag^{+}$) and as encapsulating material to stabilize the newly formed NPs in the synthesis process. This synthesis process is more advantageous than other biological processes as it is less time consuming and inexpensive. The green synthesis of NPs is preferred because of environmental friendliness, single step method process, and safety for human therapeutic use (Khandelwal, N*et al.,* 2014). This synthesis technique could be a potential future method to replace chemical synthesis of NPs in a conventional way. The plant extract during the green synthesis is very important as it provides bio-redactor agents such as enzymes, flavonoids, proteins, phenolic compounds, alkaloids, polysaccharides, amino acids, and terpenoids (Gaikwad, S., 2013).

Among the several nanomaterials, silver nanoparticles (AgNPS) are one of the most important and vastly used materials in recent days. AgNPS have a wide and excellent physicochemical propertyand have potential use in the field of chemistry, material science, environmental science, physics, optoelectronics, and biomedical devices. It is also chemically highly stable, and has high catalytic and antibacterial activities (Luo,*et al*.,2015). AgNPS have presently achieved greater attention due to its antimicrobial activities. Although, several natural material sources have been utilized for the synthesis of AgNPS, *Ipomoea aquatic* (IA) leaf extract mediated green synthesis of AgNPS (IA-AgNPS) is not reported yet. *Ipomoea aquatica* (Water

Spinach) is widely available edible green vegetable in the Indian sub-continent (Nagendra Prasad *et al.,* 2008).

The genus *Ipomoea* contributes about 500–600 species all over the world and honoured as the largest genus of family Convolvulaceae. This family is dominated by twining or climbing woody or herbaceous plants that regularly have heart-shaped leaves and funnel-shaped flowers. Convolvulaceae family described by the extensive presence of Quercetin, Flavanols, Kaempferol, and their *O*-methylated derivatives. Genus *Ipomoea* is broadly dispersed in subtropical and tropical parts of various nations (Zhang et al., 2016). These plants are used in traditional medicine as potent cathartics, ulcers, diuretics, aphrodisiac and, in the management of various skin ailments, inflammation, bronchitis, diabetes, general weakness and, fever. The bioactive compounds found in the plants of this genus are phenolic compounds, ergoline alkaloids, coumarins, diterpenes, triterpenes, nortropane alkaloids, norisoprenoids, benzenoids, isocoumarins, anthocyanins, glycolipids, lignans and indolizidine alkaloids). Many species of *Ipomoea* such as *I. batatas*, *I. aquatica* , *I.cairica* , *I.obscura* , *I. carnea* ,*I.bahiensis, I. Jacq* , *I. sepiaria* , *I. quamoclit* have been reported for their anticancer activity on different cancer cell lines. (Marilena Meira*et al.,* 2012).

Green synthesis is a novel process for the preparation of numerous metallic nanoparticles and provides a safer and eco-friendly process simultaneously achieving functionalization of nanoparticles with bioactive molecules in the plants extract. Green synthesis of silver nanoparticles (AgNPS) is performed using the aqueous extract of *Ipomoea parasiitca* leaves and stems which acts as a bio-reducing agent. The petroleum ether extract from the seeds of *I. parasitica* (HBK) Don. were isolated a unique member of a class of glycoresin (Ivanciuc, *et al.,*2016). From seedsof this species were identified lysergol and elymoclavine besideso there rgolinealkaloids (Amor-Prats & Harborne, 1993a).
However, cytotoxic activities were reported for *Ipomoea parasitica* (Kunth) till date. Hence, in the present investigation methanol extracts of *Ipomoea parasitica*(kunth) were screened for their in vitro cytotoxic activity against two cancerous cell lines using standard procedures.

## 2.0 Materials and Methods

### 2.1 Plantmaterials:

The *Ipomeaparasitica* plant materials collected from Araku valley, Vishakhapatnam, Andhra Pradesh in December of 2021. These were authenticated by Prof. Dr. S.B.Padal, Dept.of. Botany, Andhra University, Visakhapatnam, and M.Santosh

kumari, Senior lecturer in Botany, Govt. College for Women (A), Guntur.

**2.2 Extractionof plant material:**

The*I.parasitica* Plant parts (stem, leaves) were dried at room temperature until they were free from moisture. The plant parts (1000 g) were subjected to size reduction to get coarse powder of desired particle size. The coarse powder was then preserved in a clean dry air tight container and stored at room temperature. The powdered material was subjected to extraction by Soxhlet apparatus with water and methanol for 30 hrs in same way. The obtained extract was finally dried at low temperature under reduced pressure in a rotary evaporator and finally crude powder was obtained.

**2.3 Green synthesis of silver nanoparticles (AgNPS)**

20 ml of aqueous stem extract of *Ipomoea parasitica* was added drop by drop into the 1 mM of AgNO3 solution. The colorless silver nitrate solution turns to brown color that indicates the formation of AgNPS. Then the solution was stirred at 50 °C for 3 h and then centrifuged at 12,000 rpm for 15 min and was repeated 3 times with intermediate washing with Millipore water. The obtained suspension was stored as product for future characterization and anti-cancer evaluation after lyophilization

**2.4 Anti-Cancer Activity By MTT Assay:**(Mosmann, T. 1983)

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

**2.5 Procedure:**

Cell viability was evaluated by the MTT Assay with five concentrations of compound in triplicates. Cells were trypsin zed and preformed the trypan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0 X $10^3$ cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 $^0$ C. After incubation, taken off the old media and added fresh media 100 µl with different concentrations of test compounds (6.25, 12.50, 25, 50, 100 µg/ml) of *H.zeylanicum and I. parasitica*leaves-AgNPS and stem bark-AgNPS represented wells in 96 plates. After 48 hrs., Discarded the solution and added the fresh media with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37 $^0$ C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized

crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100\ (\text{Control} - \text{Treatment})}{\text{Control}}$$

The $IC_{50}$ value was determined by using linear regression equation i.e. y =mx+c. Here,y = 50, m and c values were derived from the viability graph.

## 3.0 Results and Discussion

### 3.1 Anti-cancer activityof *I.parasitica* leaves and Stembarkextractsagainst cancer cell lines:

To evaluate the anticancer activityof *I. parasitica*leaves andstem bark extracts, we treated the cervical (Hela) and lung carcinoma cancer (A-549) cell lines with increasing concentrations of AgNPS extracts at various viz., 6.25, 12.5, 25, 50 and 100 µg/ml for 24 hr. The cell viability was determined using a standard MTT assay (Vithya, *et al*., 2012). The standard drug Cisplatin (HeLa and A-549) were used as control. AgNPS leaves and stembark extracts were higher inpercent of cell viability as compared to the untreated cell lines. It suggests that growth inhibitory principles are present in all organic fractions and we rank them as Cisplatin>AgNPS> untreated cell lines. It is evident from the calculated dose of 50% inhibition of cell viability ($IC_{50}$).

$IC_{50}$ value was determined by using linear regression equation i.e. y =mx+c.

Here, y = 50, m and c values were derived from the viability graph.

The AgNPS extracts stems of *I. parasitica*showed lower $IC_{50}$ value for both Hela (44.90 and 43.45µg/ml) and A-549 (69.42 and70.12µg/ml) cell lines. Leaf-AgNPS of *I. parasitica*showed the $IC_{50}$ valueforboth Hela (63.18 and 64.32µg/ml)andA-549(73.94 and 78.64µg/ml).(fig:1- 4).

The samples established a significant cytotoxicity against cervical and lung carcinoma epithelial cell lines. Based on the literature, we have tested all these extracts for anticancer activity by measuring the growth of actively proliferating cancer cells using Hela and A-549 cell lines. These AgNPS of both plants declined the cell growth in both the cell lines. The magnitude of the inhibition of cell growth is varied for both the extracts and was higher for AgNPS extract as comparison, and is dose dependent. (Tables 1-4).

The cell viability evaluate detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which infers the normal function of mitochondrial and cell viability (Merlin., *et al*., 2010). IPS-AgNPS showed a more suppression of Hela and A-549 growth and also IPL-AgNPS showed low cytotoxic activity to A-549 than Hela cell lines as compared to IPL-AgNPS. To visualize the cancer cell viabilityunder experimental conditions.

**Table 1: Effect of Different doses of IPL-AgNPS on HeLa cell line by MTT assay (% of Cell Viability)**

| **Concentration (µg)** | **Absorbance at 570nm** | **% Inhibition** | **% Viability** | **$IC_{50}$ (µg)** |
|---|---|---|---|---|
| 6.5 | 0.716 | 7.73 | 92.27 | |
| 12.5 | 0.643 | 17.13 | 82.87 | |
| 25 | 0.512 | 34.02 | 65.98 | |
| 50 | 0.392 | 49.48 | 50.52 | |
| 100 | 0.267 | 65.59 | 34.41 | |
| untreated | 0.776 | 0 | 100 | 64.32±1.672 |

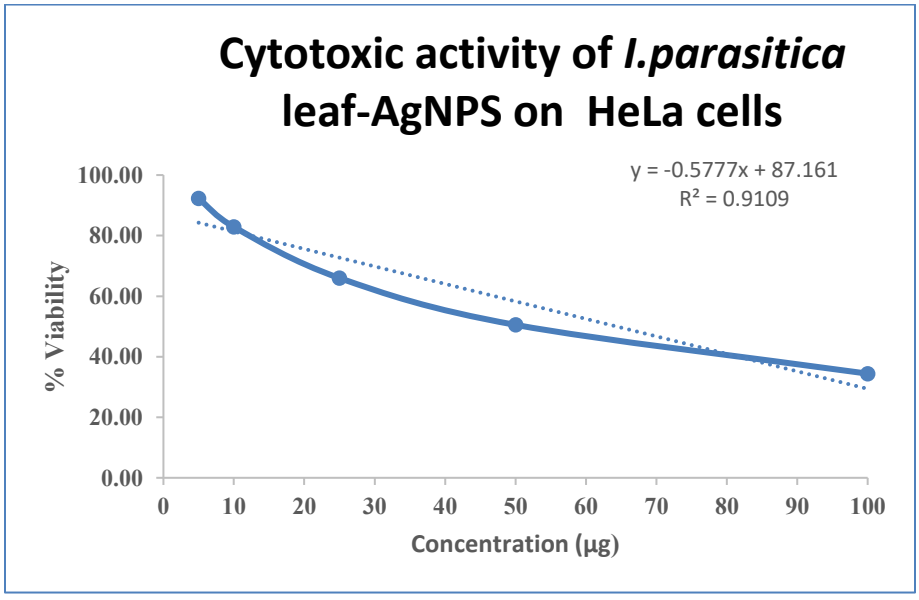



Fig 3:Fig 1:**Cytotoxic activity of *I.parasitica* leaf-AgNPS on HELA cells**

**Table 2:Effect of Different doses of IPS-AgNPS on HeLa cell line by MTT assay (% of Cell Viability)**

| Concentration (µg) | Absorbance at 570nm | % Inhibition | % Viability | $IC_{50}$ (µg) |
|---|---|---|---|---|
| 6.5 | 0.668 | 13.91 | 86.09 | |
| 12.5 | 0.584 | 24.74 | 75.26 | |
| 25 | 0.413 | 46.77 | 53.23 | |
| 50 | 0.259 | 66.62 | 33.38 | |
| 100 | 0.156 | 79.89 | 20.11 | |
| untreated | 0.776 | 0 | 100 | 43.45±1.159 |

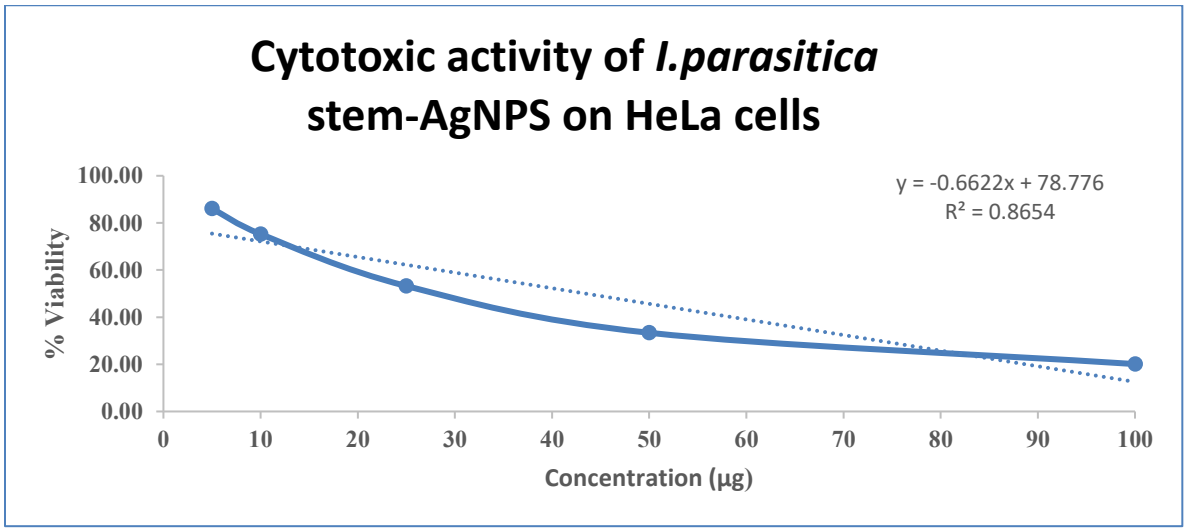
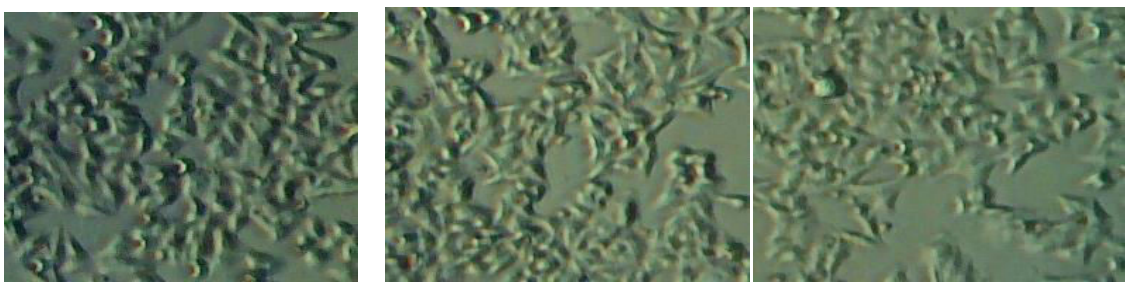


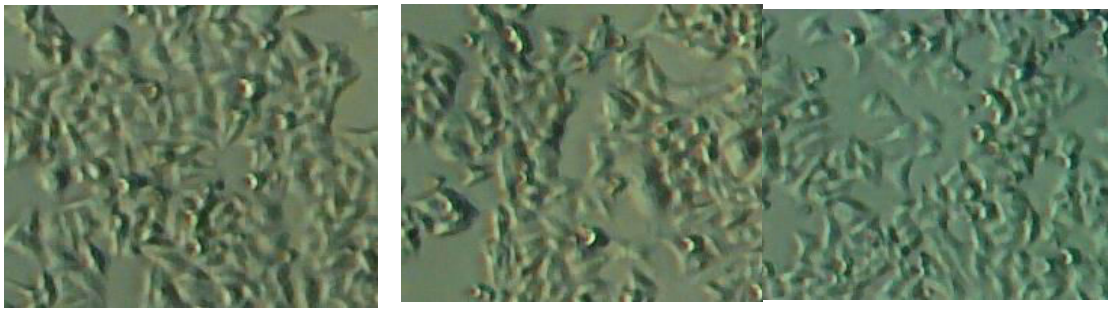

**Fig 2: Cytotoxic activity of*I. parasitica*stem-AgNPS on HeLa cells**

**Image 1:Cytotoxic activity of IPL-NP on HeLa cells**



**6.5µg** **25 µg** **100 µg**

**Image 2:Cytotoxic activity of IPS-NP on HeLa cells**



**6.5µg 25 µg 100 µg**

**Table 3: Effect of Different doses of IPL-AgNPS on A-549 cell line by MTT assay (% of Cell Viability)**

| Concentration (µg) | Absorbance at 570nm | % Inhibition | % Viability | $IC_{50}$ (µg) |
|---|---|---|---|---|
| 6.5 | 0.534 | 4.81 | 95.19 | |
| 12.5 | 0.506 | 9.80 | 90.20 | |
| 25 | 0.448 | 20.14 | 79.86 | |
| 50 | 0.347 | 38.14 | 61.86 | |
| 100 | 0.225 | 59.89 | 40.11 | |
| untreated | 0.561 | 0 | 100 | 78.67±2.146 |

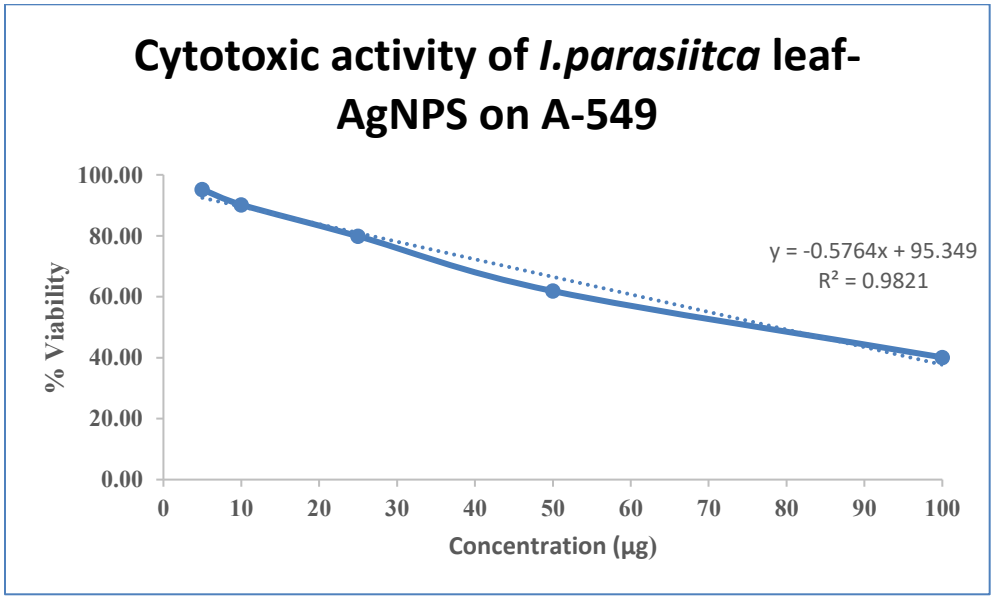



**Fig 3: Cytotoxic activity of *I.parasiitca*leaf-AgNPS on A-549**

**Table 4: Effect of Different doses of IPS-AgNPS on A-549 cell line by MTT assay (% of Cell Viability)**

| Concentration (µg) | Absorbance at 570nm | % Inhibition | % Viability | $IC_{50}$ (µg) |
|---|---|---|---|---|
| 6.5 | 0.519 | 7.48 | 92.52 | |
| 12.5 | 0.492 | 12.29 | 87.71 | |
| 25 | 0.421 | 24.95 | 75.05 | |
| 50 | 0.316 | 43.67 | 56.33 | |
| 100 | 0.197 | 64.88 | 35.12 | |
| untreated | 0.561 | 0 | 100 | 70.12±1.823 |

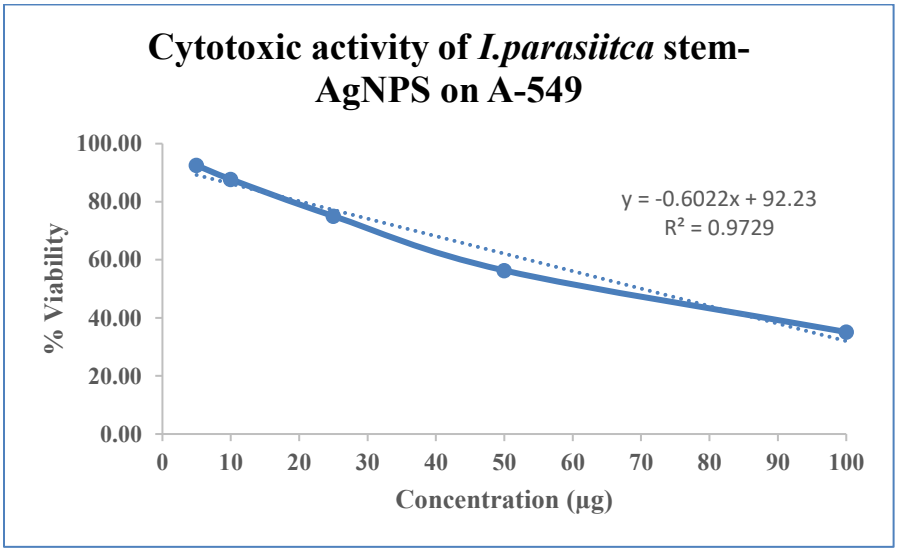
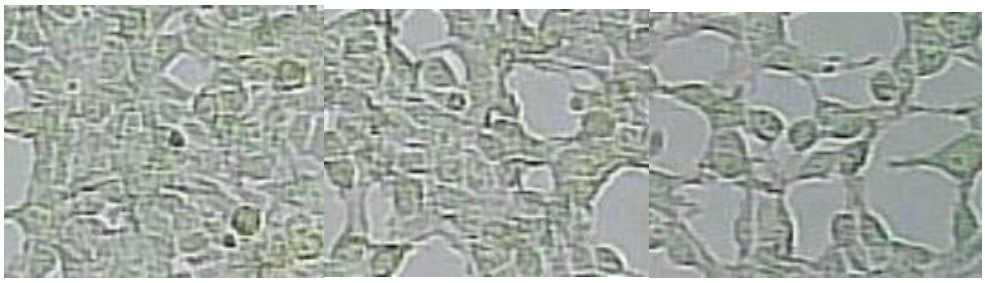


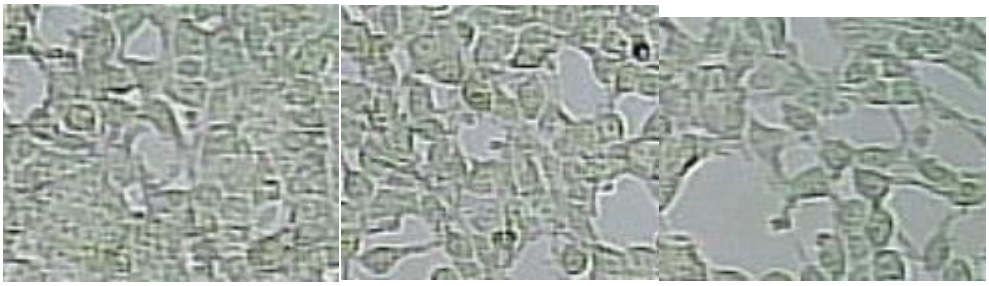

**Fig4: Cytotoxic activity of *I. parasitica* leaf-AgNPS on A-549**

**Image 3: Cytotoxic activity of IPL-NP on A-549**



**6.5µg** **25 µg** **100 µg**

**Image 4: Cytotoxic activity of IPS-NP on A-549**



**6.5µg** **25 µg** **100 µg**

The dissimilar morphological modification was experimental in AgNPS treated Hela and A-549 cells, in opposition no such special effects were seen in untreated cells. It was showing that the morphological abnormalities were detected suchas loss in membrane reliability, inhibition of cell growth, cytoplasmic strengthening and cell clumping. In a separate experiment we captured the images for both Hela and A-549cells using inverted compound microscopy (40X). The cell morphology studies showed a smooth, flattened with normal nuclei in normal culture. The treated cells showed typical morphological changes with membrane blebbing and detached from the surface were noticed in both Hela and A-549cell lines. Based on the $IC_{50}$ values the cell lines were treated with leaf and stem bark extracts and the percent of live and dead cell were counted based on the cell shape and cell size. Untreated cells appeared elongated, attached smoothly on the culture surface. Some cells were grouped together to form colonies. Following treatments with different plant extracts for 24 hrs, the cells became rounded and lost cell contacts (Images 1-4).

Overall, it suggests that, phytoconstituents of AgNPS extract possesses the antioxidant, anti-inflammatory and anti-proliferative activity, which is also evident from formation of apoptotic bodies and membrane distortions in Hela and A-549cells similar observations were observed in phytoconstituentsofaqueous extract ofnaartjie *(Citrus unshiu)*(Mafhala*etal.,*2024).The ethylacetateextract conc. of *H.zeylanicum* 50 µg/ml shows cytotoxicity activity on HaCaT (high sensitivity of human epidermal keratinocytes) cells 28.18 % and cell viability is 71.82%. (Soja, A *et.al.,* 2022). The MTT assay data showed that methanol and chloroform extracts of *I. purpurea* leaves had the antiproliferative effect on lung and breast cancer cells with IC50 of 53.62 ± 0.07 and 124.5 ± 0.01 µg/ml. (Beheshti F. *et al.,* 2021). *trans*-2,3-dibenzylbutyrolactone from *Ipomea caicira* exhibited cytotoxic activity on A-549 cell lines effectively (Subha Veeramani *et al.,* 2018). When compared to the leaves of *I. marginata* (130.4864 IC50 g/ml), the entire plant showed higher cytotoxicity

(284.8381 IC50 g/ml) (Parvathy N *et al.,* 2022). Anticancer activity of leaf extract preparation from *Ipomoea sepiaria* against PC-3 cell line, (Sudhakar et al., 2017). Potential anticancer activity of bioactive compounds from *Ipomoea batatas* (Silva-Correa *et al.,* 2022)

**4.0 Conclusion:**

In this study, a worthy approach for the green synthesis of AgNPS using *Ipomoea parasiitca* (Morning glory) leaf and stem methanol extracts were established for its antiproliferation applications. The bio-active molecules covered on AgNPS shows anti-proliferative action against the cervical (HELA) and lung carcinoma cancer (A-549) cancer cells. AgNPS via green synthesis with this Leaf and stem extracts functionalize by capping/coating of bioactive molecules increasing its potential and further use in developing drug delivery and targeting. Thus, *Ipomoea aquatica* mediated green synthesized IP-AgNPS (IPL-NPS, IPS-NPS) may be an easy and cost-effective potential candidate for several antibacterial applications. This is the first report of high antiproliferative activity of the extract of *I. parasitica* leaf and stem AgNPSon A-549 and HeLa cells. Furthermore, these bioactive compounds could be used in functional food applications for health benefits.

5.0 **References**

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