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Cytotoxicity of indigenous medicinal plants; *Salacia reticulata, Coriandrum sativum* and *Aerva lanata* on Vero kidney cell line; in-vitro study using MTT assay and SRB assay

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Abstract

Despite pharmaceutical advances, natural treatments are gaining popularity worldwide. Many individuals worldwide who suffer from illnesses use herbal plants to have a therapeutic effect. Use of these plants may harm patients, especially the kidneys. In this study, indigenous medicinal plants *Salacia reticulata, Coriandrum sativum, and Aerva lanata* were tested for nephrotoxicity using Vero kidney cells (Catalogue number CCL-81 TM). Four dilution series in each (5, 10, 15, and 20 grams (g), of *Coriandrum sativum*; 1, 2, 3, and 4 g of *Salacia reticulata*; 3, 6, 9, and 12 g of *Aerva lanata* in 125 milliliters (ml) of Milli-Q water) were exposed to Vero; monkey kidney epithelial cells ($5x10^3$ cells/well). Cell viability was measured using Sulforhodamine B (SRB) and (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assays and the cell viability percentage and the cytotoxic concentration 50% (CC_{50}) values were calculated. In the MTT assay the cell viability percentages in the cells exposed to *Coriandrum sativum, Salacia reticulata* and *Aerva lanata* were ranged from 64.46 to 98.73%, 72.06 to 98.00% and 50.80 to 95.00 % respectively. The cell viability percentages in the cells exposed to *Coriandrum sativum, Salacia reticulata and Aerva lanata* in SRB assay were ranged from 69.66 to 93.33%, 76.64 to 90.98 % and 77.17 to 50.90% respectively. Significantly higher cell viability percentages were recorded in the cells exposed to plant extract than in the cells exposed to the positive control in both MTT and SRB assays ($p< 0.05$). Hence, the results of the present study indicated the water extract of the *Coriandrum sativum, Salacia reticulata and Aerva lanata* plants do not reduce the cell viability and do not cause nephrotoxicity directly. However, further studies are needed to confirm the nephrotoxicity effects of medicinal plants.

Keywords: Cell viability, indigenous medicinal plants, Nephrotoxic effects, plant extracts, Vero kidney cells

1. Introduction

More than five thousand years ago, ancient civilisations predominantly utilised plant products for healing ailments and rejuvenating their bodies.[1] Indigenous medicinal plants have been utilised for the treatment and prevention of various diseases and epidemics.[2] Certain medicinal herbs serve dual purposes as condiments, flavouring agents, colouring agents, and preservatives. Nearly all parts of plants possess therapeutic properties, with their medicinal value attributed to specific chemical active substances that elicit defined physiological effects on the human body.[3] Secondary metabolites present in medicinal plants influence various diseases and are utilised in drug formulation. Throughout history, numerous Sri Lankan plants have served as remedies with antioxidant, anti-inflammatory, anti-insecticidal, anti-parasitic, antibiotic, and anti-hemolytic properties.[4]

Coriandrum sativum, a member of the *Umbelliferae/Apiaceae* family, is widely recognised for its antibacterial, antifungal, and antioxidant properties, attributed to the unique chemical components found in various parts of the plant.[5] The leaves and seeds of plants are

extensively utilised in traditional medicine and as flavouring agents in culinary practices.

The woody climber *Salacia reticulata*, also known as Kothala Himbutu, is frequently used in the Ayurvedic medical system to treat diabetes and obesity.[6] It is also said to have anti-rheumatic effects and is used in traditional medicinal practises for a variety of skin problems.[7] *Salacia reticulata* has been extensively researched in animal models and people for its hypoglycemic and anti-obesity properties.[8]

Furthermore, *Aerva lanata* (Polpala) is frequently mentioned in Ayurveda as a diuretic with moderate analgesic, anthelmintic, antibacterial, and anti-inflammatory properties.[9] It is utilized as an antidote for rat poisoning as well as the treatment of lithiasis, cough, asthma, and headaches.

The safety and toxicity of these medicinal plants remain inadequately investigated. The utilisation of these plants may adversely affect patient health, particularly concerning renal function. Kidneys are particularly susceptible to damage caused by toxins, including harmful substances derived from certain medicinal plants. Healthy kidneys filter approximately 240 millilitres of blood per minute, generating urine from waste products and surplus water.

Nephrotoxicity is a rapid reduction in kidney function induced by medications and chemicals.[10] Nephrotoxins are chemicals that cause nephrotoxicity (10). Some drugs affect renal function in multiple ways. Most medications worsen renal failure in patients and drugs are responsible for around 20% of nephrotoxicity, a figure that is higher in the elderly.[11] Renal tubular toxicity, inflammation, glomerular damage, crystal nephropathy, and thrombotic microangiopathy can cause nephrotoxicity.[11]

In 1962, Chiba University researchers established the Vero cell line from an African green monkey kidney which is one of the most popular continuous cell lines worldwide.[12] A continuous cell lineage can divide repeatedly without senescence. This anchorage-dependent cell line has been utilised in virology research to grow and analyse intracellular bacteria and study the molecular effects of drugs and chemicals on mammalian cells.[13] Vero cells do not release interferon alpha or beta when infected with viruses, unlike normal mammalian cells. Vero kidney cells maintain contact inhibition when they achieve confluency, they stop growing and start dying, thus they must be monitored and subcultured.[13] Since Vero cells retain the attributes of normal cells, they were selected for this study.

Despite advancements in pharmaceuticals, there is a growing global interest in natural treatments. A significant number of individuals globally utilise indigenous medicinal herbs for the treatment of various ailments. The utilisation of these plants may adversely affect patient health, particularly concerning renal function. Given the absence of documented in-vitro nephrotoxicity studies, it is essential to assess the potential adverse health effects of utilising native medicinal herbs. This study investigated the nephrotoxic effects of *Coriandrum sativum, Salacia reticulata, and Aerva lanata* on Vero kidney cells.

2. Methodology

2.1 Plant crude extraction. Seeds of *Coriandrum sativum* were obtained in 5 g, 10 g, 15 g and 20 g. Powdered Stems of *Salacia reticulata* were obtained in 1 g, 2 g, 3 g, and 4 g. Leaves and stems of *Aerva lanata* were obtained in 3 g, 6 g, 9 g and 12 g. All of the plant samples were separately boiled in 250 ml of water until the water is reduced by half (125 ml). Then they were separately put into pre labeled sterile bottles.

A volume of 9 ml of each of the prepared samples were put in to thoroughly cleaned separate evaporation disks and they were placed in the dry oven which was pre heated to 40 °C. Samples were allowed to completely dry for 48 hours. After the samples have been completely dried, 3 ml of Milli-Q water was added to the residue of each dried sample in 1ml strokes. The solutions that were obtained were transferred to clean and sterilized glass bottles. Next all the solutions were filtered into separate clean and sterilized bottles using syringe filters. Milli-Q water was used as the negative control.

2.2 Preparation of exposure solutions. Each plant solution (300 µl) was transferred into 1.5 ml Eppendorf tubes, and they were topped up with 600 µl of complete media under sterile conditions.

2.3 Cell culture and exposure. The Vero cell cryovial was thawed by gently whirling in a 37°C water bath. The cap of the cryovial was kept out of the water during this to avoid any potential contaminations. Then the vial was decontaminated in a laminar flow hood by spraying with 70% ethanol. The Vero cell suspension was transferred from the cryovial into a 15 ml conical tube containing 10 ml of complete media prepared using Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich,UK), 10% Foetal Bovine Serum (FBS) (Sigma-Aldrich,USA) and strep penicillin (Sigma-Aldrich,USA). The cryo-preserving dimethyl sulfoxide (DMSO) (Sigma-Aldrich,USA) in frozen cell supplies can be detrimental to the cells. Therefore, after defrosting the cells, the DMSO was diluted and removed before transferring the cells to tissue culture flasks.[14]

The cells were palletized by centrifuging at room temperature for 5 minutes at 2600 rpm. The supernatant was removed, and the cells were resuspended in 5-10 ml filtered complete DMEM containing 10% FBS and strep penicillin. Then the Vero cell suspension was moved to a T_{25} cell culture flask with a vented top and incubated at 37 °C with 5% CO_2 for 48 hours for cell attachment.

After 48 hours of incubation the T_{25} cell culture flask containing the cells was observed under the microscope and after 75% confluence, cell passaging was done. Then carefully the media was removed from the cell flask and the attached cells were gently washed with 3 ml Dulbecco's Phosphate buffered saline (DPBS) (Sigma-Aldrich,USA) without disturbing the cell monolayer. Next 2 ml of Trypsin EDTA (Sigma-Aldrich,USA) was added into the cell flask to detach the cell monolayer from the bottom of the cell flask, and it was incubated at 37 °C and 5% CO_2 for 2-3 minutes. Afterwards the cell flask was observed under the microscope to confirm that the cells have detached. Then the cell flask was topped up with complete media the cell suspension that was obtained was transferred into a 15 ml centrifuge tube and it was centrifuged at 1600 rpm for 5 minutes. The resulted supernatant was discarded carefully, and the cell pallet was resuspended in complete media. A micro pipette was used to break any clumps of cells.

Then 20 µl of cell suspension was mixed with 20 µl of trypan blue. Next 10 µl of the prepared mixture was loaded in the hemocytometer and a viable and dead cell count was taken by observing under the microscope. The viable cell percentage was calculated. Since the calculated cell viability percentage was above 80%, the cells were seeded in 96 well plates for cell viability assays and the cells were incubated for 48 hours at 37°C and 5% CO_2.

After 48 hours of incubation, the 96 well plates were observed under the microscope. Cells in 96 well plates were exposed to previously prepared plant extracts and cell were incubated for 24 hours at 37 °C and 5% CO_2. Triplicate samples were done for each sample. Negative and positive controls were maintained throughout the experiment and DMSO was used for the positive control. Sterile Milli-Q water was used as the negative control.

2.4 MTT assay. After 24 hours incubation, prepared MTT solution was added to each well and the plate was incubated for 3 hours. After the incubation the solution in each well of the 96 well plate was removed. Lastly the each well were filled with of 10% DMSO and the plate was shaken for 30 minutes. The absorbance was measured at 620nm.[15]

2.5 SRB assay. The cell plate treated and incubated with the exposure solutions were topped up with 50% cold trichloroacetic acid. Then it was incubated at 4 °C for 1 hour. The cell plate was carefully washed with water for 5 times, and it was airdried for few minutes. Then the cells in the wells were stained using prepared SRB solution and it was allowed to incubate for 15 minutes at the room temperature. After 15 minutes the cell plate was washed with 1% acetic acid for 5 times, and it was allowed to air dry for few minutes. Then 10 mM unbuffed tris base solution was added to each well and the plate was shaken for 30 minutes at room temperature. The absorbance was measured at 520 nm.[16]

2.6 Statistical analysis. IBM SPSS version 27 was used to do the statistical analysis. A significant difference was defined as having a *p*-value of less than 0.05.

3. Results

3.1 MTT assay for Coriandrum sativum. As the evidence suggests, the cells that were treated with the highest concentration of *Coriandrum sativum* was recorded the highest cell viability percentage whereas the cells exposed lower concentrations of *Coriandrum sativum* was recorded slightly lower percentages of cell viability. Significantly higher cell viability percentages were recorded in the cells exposed to *Coriandrum sativum* than in the cells exposed to the positive control (DMSO) accordingly ($p<0.05$) (Figure 1).

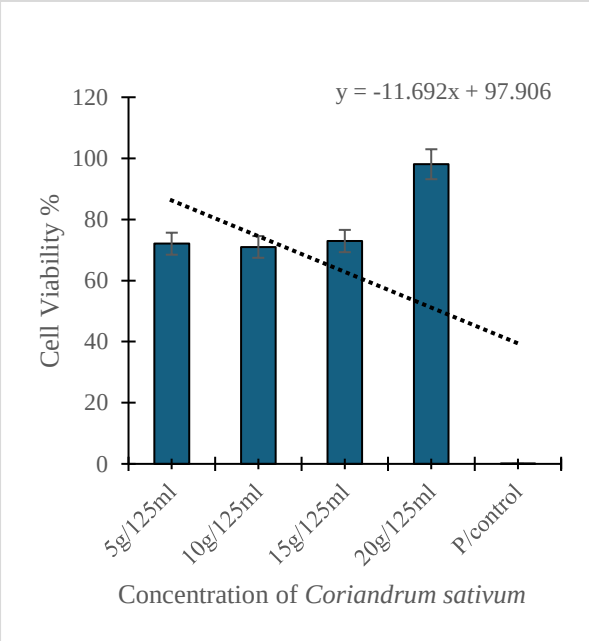

Figure 1. Variation in % viability of Vero cells exposed to different concentrations of *Coriandrum sativum.* Data are presented as mean ± SD.

3.2 MTT assay for Salacia reticulata. In the first three concentrations a decreasing trend for the cell viability percentage was observed. However, the cells that were exposed to the highest concentration of *Salacia reticulata* was recorded the highest percentage of cell viability. Furthermore, significantly higher cell viability percentages were recorded in the cells exposed to *Salacia reticulata* than in the cells exposed to the positive control (DMSO) accordingly ($p<0.05$) (Figure 2).

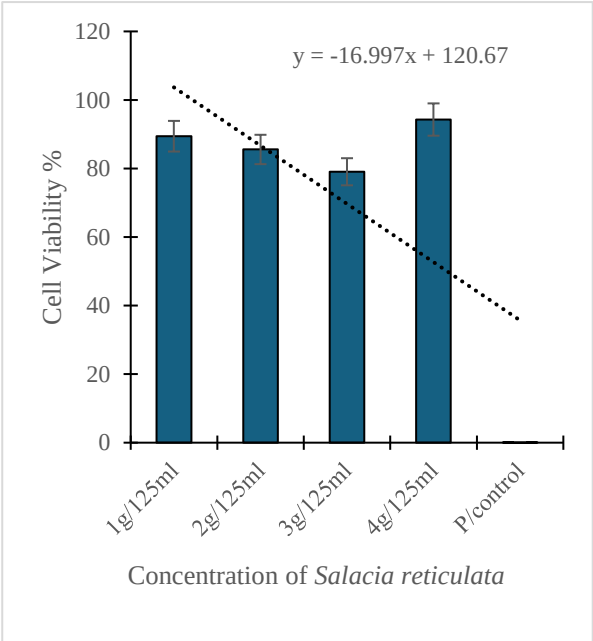

Figure 2. Variation in % viability of Vero cells exposed to different concentrations of *Salacia reticulata*. Data are presented as mean ± SD.

3.3 MTT assay for Aerva lanata. An increasing trend was seen throughout the concentration series where the highest cell viability was observed in the cells that were exposed with the highest concentration of *Aerva lanata*. Accordingly, the cell viability percentages in the cells exposed to *Aerva lanata* were significantly higher than in the cells exposed to the positive control ($p<0.05$) (Figure 3).

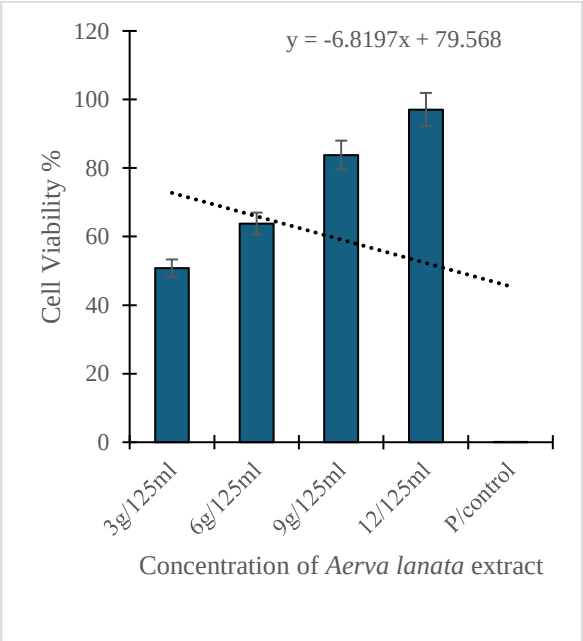

Figure 3. Variation in % viability of Vero cells exposed to different concentrations of *Aerva lanata*. Data are presented as mean ± SD.

3.4 CC_{50} value calculation for results obtained in the MTT assay. CC_{50} is the concentration of test compounds required to reduce cell viability by 50%. According to the results of percentage of viability of cells, there is no significant effect of plant extracts on cell viability. Accordingly, the CC_{50} values obtained from the present study was depicted in Table 1.

Table 1. CC_{50} values calculated according to the MTT assay results.

Plant sample	CC_{50} value
Coriandrum sativum	0.57
Salacia reticulata	-39.19
Aerva lanata	1.198

3.5 SRB assay for Coriandrum sativum. The lower three concentrations resulted in a decreasing trend of cell viability but the cells that were exposed to the highest concentration of *Coriandrum sativum* was recorded the highest cell viability. Significantly higher cell viability percentages were recorded in the cells exposed to *Coriandrum sativum* than in the cells exposed to the positive control (DMSO) accordingly (p<0.05) (Figure 4).

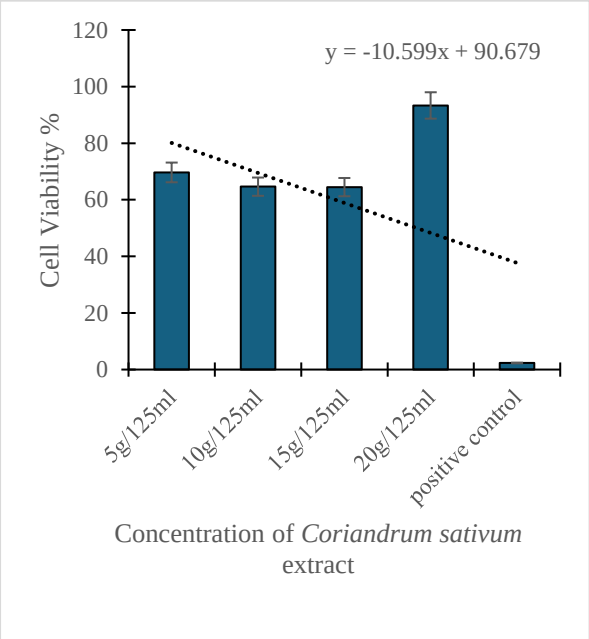

Figure 4. Variation in % viability of Vero cells exposed to different concentrations of *Coriandrum sativum*. Data are presented as mean ± SD.

3.6 *SRB assay for Salacia reticulata.* Except for cells that were exposed to the lowest concentration of *Salacia reticulata* every other cell had a cell viability percentage of more than 80%. Furthermore, significantly higher cell viability percentages were recorded in the cells exposed to *Salacia reticulata* than in the cells exposed to the positive control (DMSO) accordingly (p<0.05) (Figure 5).

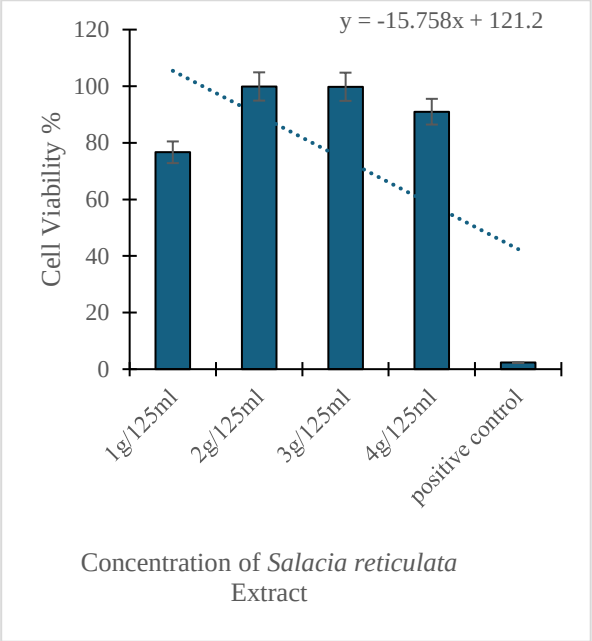

Figure 5. Variation in % viability of Vero cells exposed to different concentrations of *Salacia reticulata*. Data are presented as mean ± SD.

3.7 SRB assay for *Aerva lanata*. A decreasing trend was seen throughout the concentration series where the highest cell viability was observed in the cells that were exposed with the lowest concentration of *Aerva lanata*. However, the cell viability percentages in the cells exposed to *Aerva lanata* were significantly higher than in the cells exposed to the positive control (p<0.05) (Figure 6).

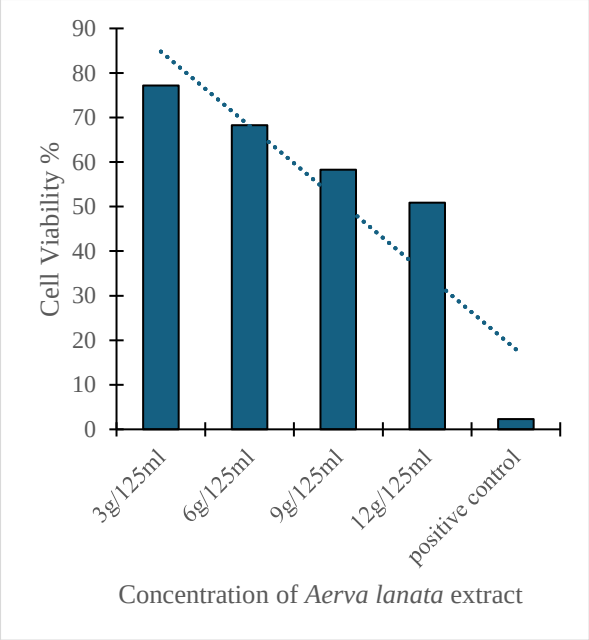

Figure 6. Variation in % viability of Vero cells exposed to different concentrations of *Aerva lanata*. Data are presented as mean ± SD.

3.8 CC_{50} value for the results obtained from the SRB assay. CC_{50} is the concentration of test compounds required to reduce cell viability by 50%. According to the results of percentage of viability of cells, there is no significant effect of plant extracts on cell viability. Accordingly, the CC_{50} values obtained from the present study was depicted in Table 2.

Table 2. CC_{50} value calculated according to the SRB assay results.

Plant sample	CC_{50} value
Coriandrum sativum	-0.752
Salacia reticulata	-7.38
Aerva lanata	4.038

4. Discussion

Some pharmacologically active compounds in medicinal plants have been related to undesirable consequences, increasing reports of adverse responses in human body. Nephrotoxicity is considered as a major side effect of usage of herbal medicines. A recent study done by Xiofen, 2020 found that over 100 herbal medicines harm the kidneys.[17] The nephrotoxicity of popular medicinal plants in Sri Lanka, *Coriandrum sativum*, *Salacia reticulata*, and *Aerva lanata* was examined in this study.

During this investigation series of concentrations were used for each plant extract. They were, the concentration prescribed by a recognized Ayurveda doctor (Dr. Nilumi Buddika, 2022), half of the prescribed concentration and double of the prescribed concentration. This was done to closely monitor the relationship between the concentration of herbal medicine and the cell viability. The plant crude extracts were prepared using only distilled water because in real life these medicines are consumed by people as water extracts.

Based on assay results, cell viability % and CC_{50} were computed. No plant extract concentration reduced cell viability, according to the results. According to the MTT assay results, *Coriandrum sativum* had a cell viability percentage between 64.46 to 98.73% throughout the series. *Salacia reticulata* had a cell viability percentage of above 80% throughout the concentration series whereas *Aerva lanata* had a percentage between 50.80 to 95.00 %. In a study that was done in 2012 by Wen et al., using plant extracts had contradicting findings to this present study where significant toxicity was observed.[18] The SRB assay findings also suggest that none of the concentrations of each plant extract caused distinct nephrotoxicity. Another study which was done by Ming, 2006 confirmed that vegetables like rhubarb caused nephrotoxicity in rats.[19]

A study, which was done by Amita and Juan, 2019 indicated that certain medicinal herbs like milk thistle can increase the concentration of ingested drugs like cyclosporin which can promote kidney inflammation and rejection in kidney transplanted patients by decreasing the immunosuppressive agent concentration.[20] Moreover, in 2021, Kiliś-Pstrusińska and Wiela-Hojeńska stated that many flavonoids present in most of the medicinal plants alter the enzyme activity, which could have potential to alter other drug metabolisms and induce nephrotoxicity.[21] Therefore, the ingestion of herbal medicines and the pharmaceutically developed other drugs *viz* antibiotics and anti-

inflammatory drugs simultaneously may cause adverse effect on kidney function.

The result of the present study is similar to a study conducted by Patel and Patel (2011), which examined the cytotoxic effect of a methanolic extract of Artocarpus heterophyllus by two in vitro cytotoxicity assays MTT and SRB.[22] Both assays SRB and MTT are applicable for assessing the cytotoxicity of herbal plants; however, each assay presents certain limitations.[23] MTT is classified as a carcinogen. Therefore, MTT waste must be properly disposed of following testing by environmental pollution control agencies.

Light contamination should be avoided during SRB staining, as light exposure can lead to the degradation of SRB. The staining process must be executed within a designated timeframe as per the manufacturer's instructions; failure to adhere to this may lead to protein loss and an underestimation of optical density.

Due to the recent significant rise in the accessibility of plant-based raw materials and the resulting decrease in the ability to regulate their usage, the existing system for monitoring the safety of medicinal products is incapable of collecting data on all adverse effects associated with the use of natural products.[24] Furthermore, due to the rising resistance against pharmacologically active compounds, the infections can be difficult to treat. Hence, investigating novel natural compounds provides an avenue for discovering new treatments that may be less prone to resistance development.

5. Conclusion

The present study indicated the water extracts of the *Coriandrum sativum*, *Salacia reticulata* and *Aerva lanata* plants do not reduces the cell viability and do not cause nephrotoxicity directly. However, further studies are needed to confirm the nephrotoxicity effects of medicinal plants.

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