IJHSR
ijhighschoolresearch.org
RESEARCH ARTICLE

Phytochemical Analysis and Anticancer Potential of Herbal Formulation Swastharakshak Plus®

Ananya Ravishankar

The International School Bangalore, NAFL Valley, Whitefield – Sarjapur Road, Bangalore, KA, India, 562125, jyotrao@gmail.com
Mentor: Pooja Vijaykumar

ABSTRACT: Integrative oncology comprises a range of therapies that can complement standard cancer treatments, such as chemotherapy, surgery, and radiotherapy. One commonly used approach to complementary medicine involves using naturally occurring antioxidants, such as Ayurvedic herbal extracts, to assist in reducing the adverse effects of cancer treatment on healthy cells. Swastharakshak Plus® (SR plus®) is a polyherbal formulation consisting of *Tinospora cordifolia*, *Curcuma longa*, and *Camellia sinensis*, known for their phytochemical content. This research paper hypothesized that SR plus® has anti-oxidant properties and a cytotoxic effect on HeLa cancer cells. The study found significantly lower IC50 values for SR plus® ethanol extract after both 24 hours (22 µg/ml) and 48 hours (8.65 µg/ml) of exposure, compared to methanol and water extracts of SR plus®. Also, fluorescence staining using Hoechst dye, DAPI, and dual staining (acridine orange and propidium Iodide) revealed the occurrence of apoptosis in HeLa cells treated with the IC50 concentration of SR plus® ethanol extract for 48 hr. HeLa cells showed 43% cell death SR plus® ethanol extract. In conclusion, the SR plus® formulation exhibited cytotoxicity activity against the HeLa cell line, possibly due to apoptosis. By investigating complementary therapies like SR plus®, we may find valuable additions to existing cancer treatments that can enhance their efficacy and reduce side effects.

KEYWORDS: Biomedical and Health Sciences; Nutrition and Natural Products; Swastharakshak Plus®; Complementary and alternative medicine; MTT; IC50; integrative oncology; pro-apoptosis.

Introduction

Typically, when a cell is damaged or reaches senescence, it undergoes apoptosis, and new cells are produced through division to replace it. However, in certain instances, instead of normal cell division, the damaged cell undergoes uncontrolled proliferation, leading to the formation of tumors that can spread to other parts of the body. This condition is known as cancer.[1] Cancer cell lines play an important role in in vitro medical research, providing a valuable tool in areas such as research and drug discovery. Cell lines have a high level of accuracy if created in the right conditions and using the correct protocol.[2] We used Hela cells in this study because HeLa cells are a form of cervical cancer cells. Given that cervical cancer represents a significant cause of mortality in females. HeLa cells are commonly used in anticancer research due to their robust proliferation, genetic stability, and resemblance to cancerous properties.[3] There are many ways to approach cancer treatment. Patients often have to undergo multiple treatments rather than relying on a single one. Some common standard cancer treatments include surgery, chemotherapy, and radiation.[4] Nevertheless, standard cancer treatments often come with some side effects, including appetite loss, drug resistance, hair loss, and relapse.[5] Additionally, patients can also relapse if all the cancer cells were not killed in the first round of treatments or if the cells spread to different parts of the body and create other tumors.[6] Each type of cancer has a different percentage of patients who experience relapse. This variation is influenced by factors such as the cancer stage and genetic factors.[7]

Integrative oncology addresses things such as symptom control. It consists of non-invasive therapies that complement mainstream cancer treatments.[8] There are many complementary therapies with anti-cancerous effects, such as acupuncture, yoga therapies, or herbal and polyherbal drugs.[8] Phytochemicals have many anti-cancerous properties. In particular, they have anti-cancerous effects when talking about phytochemicals, alkaloids, tannins, saponins, flavonoids, and phenolic compounds.[9] Alkaloids are compounds that originate from plants like other phytochemicals. They have many effects on humans. There are various types of Alkaloids; the major ones include true alkaloids, proto-alkaloids, and pseudoalkaloids.[10]

Phytochemicals with high antioxidant properties, such as phenolic compounds, flavonoids, and saponins, are useful in treating cancer, including cellular invasion inhibition and induction of apoptosis.[11, 12] Tannins constitute a large group of polyphenolic compounds found in nature and show significant potential in both preventing and treating cancer.[13]

Ayurvedic formulations have been proven to have various positive effects when used as a complementary treatment to mainstream cancer treatments. For example, various Chinese herbal medicines can be used along with standard cancer treatments such as radiotherapy or chemotherapy, which may reduce the side effects.[14] Integrative oncology blends complementary and integrative medicine (CIM) with traditional therapy to treat cancer patients. Since ancient times, herbal remedies have been utilized to cure various illnesses.[15] India is a hub for medicinal plants that can be used alone or combined to create polyherbal preparations. One such composition is SR plus® (in the form of power), which contains the widely available Indian

DOI: 10.36838/v6i5.2

plants of giloy, turmeric, and green tea. Numerous therapeutic actions of these plants or herbs have been reported.

This study aims to examine the different phytochemicals present in SR plus®, the cytotoxicity of SR plus®, and apoptotic cell death in HeLa cells. It also seeks to compare the effectiveness of SR plus® (a polyherbal formulation) with that of its different solvent extracts.

Methods

Extract formation:

This experiment used one gram of the insoluble SR plus® powder, along with 10 mL of the non-polar solvent acetone, polar solvents like methanol, ethanol, water, and chloroform, a bipolar solvent. After mixing, the solutions were incubated on a rocker for 24 hours and centrifuged for 20 minutes at 3000 rotations per minute (r.p.m.). Approximately 7-8 mL of each resulting extract was collected for further experimentation.[16]

Lambda max:

Lambda max (λmax) refers to the specific wavelength at which a compound exhibits its greatest absorption when measured using a spectrophotometer. Phytocomponents are biologically active substances derived from plants, and this λmax value identifies a peak in the absorption spectra that represents their existence (Figure 1). In this test, undiluted (crude) samples were used to check the λmax. Alkaloids, saponins, flavonoids, polyphenols, and tannins are measured in the sample to determine the total amount of these chemicals during phytochemical screening. This screening process helps identify and quantify the various bioactive substances in the sample, providing valuable insights into their potential medicinal or therapeutic properties.[17]

Qualitative analysis:

Rahman Gul and Kokate carried out qualitative analyses utilizing particular procedures.[18,19]

Table 1: Protocol for qualitative analysis of phytochemicals

Phytochemicals	Procedure Used	Observation
Alkaloid	1 mL Dragendorff's reagent + 0.2 mL of the sample +0.2 mL dilute HCl (1:1)	Orange-brown precipitate in the presence of alkaloids
Saponin	Olive oil test + 0.5 mL of each sample	Formation of a soluble emulsion if saponins are present
Flavonoid	Treatment of 0.5 mL of the sample + 5 drops of 5% NaOH	Alkaline reagent testing for the presence of flavonoids if solution becomes colorless
Polyphenol Test	0.2 mL of the extract, 4 drops of FC reagent, 4 drops of NaCO3	Color change recorded after 20 min incubation in the dark at room temperature indicates the presence of polyphenols
Tannins	0.2 mL of sample + 5 drops of FeCl3 Solution (5% v/v)	Blue color indicates the presence of tannins

Quantitative Analysis

The test for Polyphenols was taken from -. 0.2 ml of each test sample was mixed with 0.6 ml of water and 0.2 ml of Folin Ciocalteu's reagent. They were then incubated for 5 minutes. After incubation, 1 ml of 8% w/v of Na2CO3 was added to the solutions, and the volumes were then made up to 3 ml using water. A 30-minute incubation and centrifuging followed this. The absorbance of the blue color at 765 nm was measured. The phenolic content was calculated as a gallic acid equivalent based on a standard curve.[19]

The pH was maintained at 2-2.5 using 1M HCl, and then 2 mL of Dragendorff's solution was added to measure the alkaloids in the extracts. After centrifugation, 2 mL of 5% disodium sulfide solution was added to the pellet to form a brown-black precipitate. 2 mL of concentrated nitric acid was added, and the volume was up to 10 mL with distilled water. 1 ml of the solution was discarded, and 5 ml of thiourea was added. The absorbance was recorded at 435 nm. Bismuth nitrate was used as a standard.[20]

To quantitatively test for Flavonoids, the procedure was taken from-. 1 ml of the test samples were taken in test tubes, and 4 ml of distilled water was added. Then 0.3 ml of 5% sodium nitrite solution was added to each tub and incubated for 5 minutes. After incubation, 0.3 ml of 10 % $AlCl_3$ and 2 ml of 1 molar sodium hydroxide were added. Then, the volume was made up to 10 ml with distilled water and mixed. The absorbance at 630 nm of the orange color was measured. Quercetin was used as a standard.[21]

To quantitatively test for saponins, the protocol was taken from -. 0.25 ml of the samples were taken, and 0.25 ml of 8% Vanillin in ethanol was added to each sample. Then 2.5 ml of 72% sulphuric acid was added to the solutions and incubated at 60˚C. After the solutions cooled down, the absorbance was measured at 765 nm. Diosgenin was used as a standard for the saponin test.[22,23]

Anti-oxidant assay:

To observe the Anti-oxidant activity of SR plus® 2,2-diphenylpicrylhydrazyl (DPPH) assay was used. Here, 10µL, 50 µL, 150 µL and 200 µL of SR plus® were put in different test tubes. The volumes were made up to 1 ml with methanol, and 3 ml of DPPH assay was added. The test tubes were incubated in the dark and at room temperature for 15 minutes, and the readings were then taken at 517 nm using a spectrophotometer.[24,25]

The percentage inhibition (inhibition of reactive oxygen species, such as the superoxide radical) was calculated according to the formula:

$$\% \text{ inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

A concentration (µg/mL) graph on the x-axis against % inhibition along the y-axis was plotted for the DPPH assay. The IC50 value was found after fitting the data to a linear equation ($y = mx + c$). The point at which 50% inhibition of the reactive oxygen species had occurred was hence determined:

$$(50) = mx + c \rightarrow \therefore x = \frac{50 - c}{m}$$

Cell culture:

HeLa cells were purchased from NCCS (National Centre for Cell Science), Pune. Passage number seven was used for this study. After rapid thawing, cells were cultured in Dulbacco's Modified Eagle Media (DMEM/F-12) with 10% serum (Himedia). After centrifugation (1500rpm, 10 minutes). Cells

were counted using a hemocytometer after centrifugation (1500rpm, 10 minutes). They were cultured and maintained at 37°C with 5% CO2. When reaching 80-85% confluency, the cells were harvested using 0.25% trypsin-EDTA solution (Himedia) and subcultured for further assays.

Cytotoxicity assay:

The MTT (4, 5-dimethyl tetrazolium-2-yl, 2, 5-diphenyl tetrazolium bromide) test parameters were established based on earlier phytochemical analyses, 24 which identified the presence of secondary metabolites (phytochemicals). The essential groups of SR plus® were categorized separately during the phytochemical research.

The MTT assay procedure was adapted from the description by Morgan D.M.L. 26 HeLa cells were counted using a hemocytometer, and 0.3 x 106 cells were seeded in a 96 well plate with Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) (100µL, 3000- 5000 cells per well). The cells were cultured and maintained at 37°C with 5% CO2 for 24 hours to ensure cell adhesion. After 24 hours of incubation, older media was removed to get rid of dead cells. For the treatment of cells, concentrations such as 330, 165, 82.5, 41.25, 20.63, 10.31, and 5.16 µg/mL (two-fold serial dilutions) of SR plus® extracts were used; the incubation periods were 24 hours and 48 hours. After the respective incubation times, 20 µL of MTT solution was added to all the wells, and the plates were further incubated for 4 hours. Subsequently, the medium was removed, and 100 µL of DMSO was added to dissolve the purple formazan crystals. The presence of purple formazan crystals indicated the viability of living cells. Absorbance readings were taken at a wavelength of 545 nm using a spectrophotometer (Thermo Scientific). To minimize errors, three individual sets of experiments were conducted in triplicate. The percentage of cell cytotoxicity was calculated using a specific formula based on the obtained absorbance readings,

$$\frac{Absorbance\ of\ the\ Sample}{Absorbance\ of\ the\ Control} \times 100$$

The formula calculated the percentage cytotoxicity:

$$\frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

The percentage cytotoxicity graphs were plotted against the sample concentration for all groups from the absorbance readings obtained. The IC50.tk software was used to determine the IC50 value obtained from the MTT assay (after both 24 and 48 hours of drug action). Based on earlier study references, cell death exceeded 90% for the highest concentration for SR plus® at 48 hours; the 24-hour and 48-hour time points were selected to determine IC50 values. A 24-hour treatment reveals initial effects while extending to 48 hours assesses cumulative or delayed responses of the polyherbal drug on cell viability. These time points mimic physiological conditions, which are crucial for assessing toxicity within a clinically relevant window due to drug metabolism and excretion. We did not perform the preliminary study for 72 and 96-time points because limiting assays to 24 and 48 hours balances thorough data collection with efficient resource use, capturing significant effects without unnecessary experimental duration.[27]

Staining and microscopy:

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) using a 24-well plate with 10% Fetal Bovine Serum (FBS) (1 mL, 0.1 x 106 cells per well), kept at 37 °C with 5% CO2, and treated with SR plus® at the IC50 dose used in the MTT assay (21.09 µg/mL), along with control cells (untreated). The HeLa control group consists of cells not exposed to SR plus® ethanol extract, while the HeLa treated group comprises cells treated with SR plus® ethanol extract. After 48 hours of drug activity, the medium was removed, and the cells were then washed with 1X PBS (pH-7.4) solution and incubated for 5 minutes at room temperature. The wells were then filled with 4% paraformaldehyde (a fixative chemical) and incubated for another five minutes. The cells were then given one additional PBS washing in ice-cold water. They were employed in the dark and covered to prevent fluorescent stains from exposure to light.

Dual staining: Mascotti *et al*. adopted the dual staining technique.[28] The cells were given 50 µL each of 1 mg/mL propidium iodide and 1 mg/mL acridine orange. The treated and control samples were then examined under a fluorescence microscope in a dark environment at 490 nm (excitation) and 630 nm (emission).

Hoechst staining: The Crowley *et al*. procedure was used to create the Hoechst staining method.[29] Both the treated and control cells received 50 µL (0.5 g/mL) of Hoechst stain, which was then viewed at 350–460 nm under a fluorescence microscope.

DAPI staining was used to see how cells died. DAPI stands for 4′,6-diamidino-2-phenylindole. Both the control and the treated cells received 50 µL (1000 mg/mL) of DAPI dye, which was then applied. The cells were observed at 450 nm under a fluorescence microscope in a dark environment. The pictures were taken with ProgRes® Capture Pro software using an Olympus microscope with a 0.34 numerical aperture (NA) and a 20X magnification. [30,31] Five different areas were chosen to cover the whole 24-well plate and counted the cells based on the morphology. Cellular shrinkage, nuclear and cytoplasmic condensation, bleb formation, and fragmented bodies are considered to be apoptotic cells.

Statistical analysis:

The data was analyzed using Microsoft Office Professional Plus 2016 software. The statistical difference between groups was examined using a paired t-test. n=3, p - value<0.05 considered significant.

■ Results and Discussion

Phytochemical screening of SR plus® was carried out using spectrophotometry. SR plus® was diluted with various solvents to identify the most suitable solvent for further experiments. The screening revealed the presence of phytochemicals at different concentrations in each solution of the SR plus®. Saponins, alkaloids, and phenolic compounds are essential categories of bioactive constituents in herbal products.

DOI: 10.36838/v6i5.2

Lambda max:

The preliminary screening in the Lambda Max run showed that methanol, ethanol, and water absorbed the largest amounts of Phytochemicals (Figure 1).

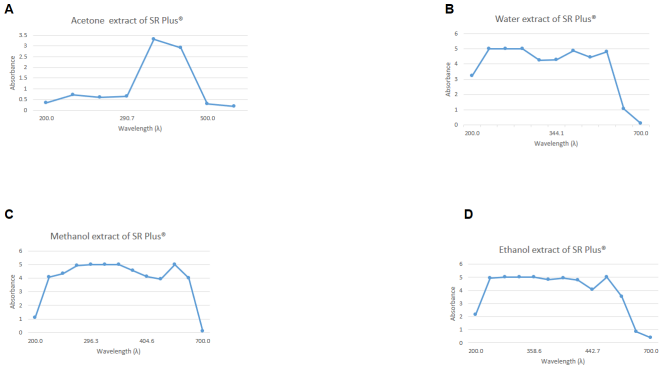

Figure 1: The Lambda max (λmax)

The graphs obtained here through a UV/Vis spectrophotometer show the presence of various phytochemicals in the various solutions through the peaks. A wide range of flavonoids were detected in SR plus® acetone extract (Figure 1A). Saponins, polyphenols, and alkaloids were detected in SR plus® water extract (Figure 1B). Saponins and polyphenols were detected in SR plus® methanol extract (Figure 1C). Flavonoids, polyphenols, and alkaloids were detected in SR plus® ethanol extract (Figure 1D). The range used here was 200 – 800 nm. The results of the phytochemical screening, displayed in Figure 1, with the lambda max values, clearly showed that different solvent extracts of SR plus® had considerable quantities of all the phytochemicals.

Qualitative analysis:

The qualitative analysis showed a high concentration of triterpenoids in the methanol and ethanol solutions. However, Figure 1 shows that saponins were only found in methanol and water (Table 2).

Table 2: The Qualitative analysis of different solvent extracts of SR plus®

	Saponins	Flavonoids	Poly Phenols	Alkaloid	Tannins
Methanol	+	-	+	-	-
Ethanol	-	+	+	+	-
Water	+	-	+	+	-
Acetone	-	+	-	-	-

The Qualitative analysis was measured using "+" and "-" to show the presence or absence of the phytochemicals in the respective solutions. The table shows how to qualitatively find the desired phytochemical for the preliminary screening of phytochemicals in different extracts of SR plus®.

Quantitative analysis:

The concentration of desired phytochemicals present in different extracts of SR plus® are shown below (Figure 2)

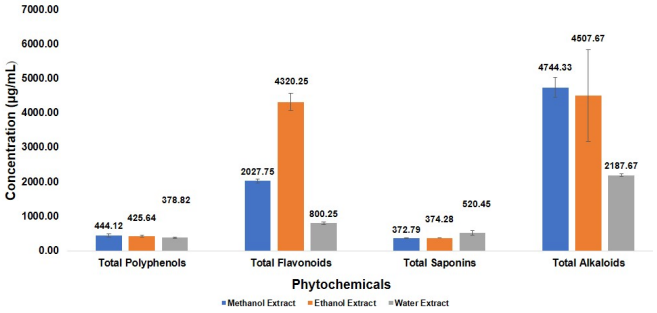

Figure 2: Quantitative analysis of SR plus® extracts. Bar graph showing the quantitative Analyse of different phytochemicals in various plant extracts using other solvents. A. polyphenol, B. flavonoid, C. saponins, and D. alkaloid using other solvents. A. polyphenol, B. flavonoid, C. saponins, and D. alkaloid concentration (µg/ml) in various extracts. Finding the solvent extract with the highest concentrations of a specific desired phytochemical was the first objective of the quantitative phytochemical screening.

Ethanol extract of SR plus® showed higher concentrations of desired phytochemicals than methanol and water extracts. This quantitative analysis data emphasizes the importance of solvent selection on phytochemical extraction efficiency. The outcomes provide valuable insights contributing to the broader understanding of channeling plant-derived compounds for various applications, including pharmaceutical and natural product development.

Anti-oxidant properties:

After the phytochemical analysis, a 2,2-diphenylpicrylhydrazyl (DPPH) assay was carried out to observe the anti-oxidant activity of SR plus®. The IC50 values for the whole plant and methanolic, ethanolic, and water extracts, respectively, were 250.55 µg/mL, 249 µg/mL, and 251.02 µg/mL. SR plus® may be used in conjunction with cancer treatment to protect healthy cells from oxidative damage by free radicals (Figure 3).

This formulation's antioxidant property is significant because it may be used as a supplement to cancer therapy, helping to shield healthy body cells from potential damage brought on by harmful free radicals produced during treatment.

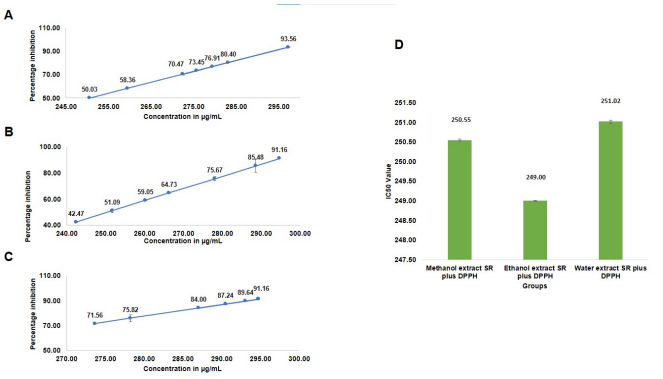

Figure 3: The graph for the DPPH assay. The graph depicts the % inhibition of free radicals with an increase in the concentration of SR plus® in µg/mL. A. methanol extract, B. ethanol extract, C. water extract, and D. IC50 values. This graph represents the formulation's antioxidant properties regarding free radical scavenging capacity. The IC50 value was determined using the graph. As a result, the SR plus® formulation represents an antioxidant activity.

Cytotoxic effect:

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to observe the cytotoxicity of SR plus® on HeLa cells.

To investigate the pro-apoptotic effect of SR plus® ethanol extract on HeLa cells, the extract demonstrated a low IC50 value, indicating its heightened potential for inducing cell death at lower concentrations. Other therapies were not used in this study. The fact that SR plus® ethanol extracts IC50 on HeLa cells was much lower than other extracts of SR plus® indicates its potential for powerful anti-cancer properties (Figure 4).

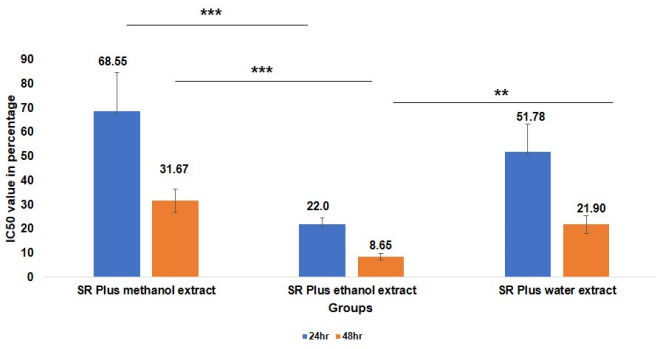

Figure 4: IC50 values for SR plus®. Bar graph showing the IC50 values for the MTT cell viability assay for the five groups tested. The concentration was expressed in µg/ml (N=3, mean±SD). HeLa cell viability decreases with increasing concentrations of - SR plus® derived extracts using MTT assay. The effects of different extracts at 24h and 48h on the HeLa cell line were compared to HeLa cells. Groups such as SR plus methanol extract and water extract were compared to SR plus ethanol extract. These groups were considered statistically significant if $p<0.01$ (**) and $p<0.001$ (***).

The study results have significant ramifications for integrative oncology since they show that the phytochemical extracts from SR plus® have notable cytotoxic effects on HeLa cells. As a result, it is possible that this SR plus® extract could be utilized in addition to or instead of conventional cancer treatments. The objective is to lessen the unfavorable side effects of conventional medicines while improving their anti-cancer capabilities.

Pro-apoptotic properties using fluorescence staining:

Finally, Various stains, including 4′,6-diamidino-2-phenylindole (DAPI), Hoechst, and Dual staining, were used to observe the pro-apoptotic effect of SR plus® ethanol extract on HeLa cells.

Dual staining of the treated and control groups of HeLa cells revealed that the treated group had 50% more cell death than the control group. According to the fluorescence staining study results, the treated HeLa cells had 43% cell death in DAPI compared to the control group (Figure 5).

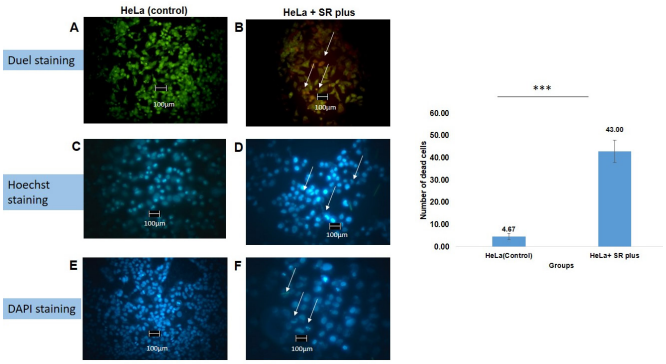

Figure 5: Fluorescence staining to observe the mode of cell death on HeLa cells treated with SR plus®. Apoptosis caused by SR plus® on treated groups: HeLa (control) and HeLa+ SR plus® cells were observed under a fluorescent microscope using acridine orange and propidium iodide (dual) staining (20x) (top) (A, B) and Hoechst (middle) (C, D) and DAPI staining (20x) (bottom) (E, F) with NA of 0.34. The fluorescent nuclear fragmentation and blebs indicate apoptosis marked as a white arrow in acridine orange and propidium iodide (dual) and also in Hoechst and DAPI staining. Scale bar: 100µM. HeLa (control) and HeLa + SR plus® were considered statistically significant, which is shown in the graph if $p<0.001$ (***).

The efficacy of SR plus® in destroying cancer cells is evident, as demonstrated by its impact on HeLa cells treated with IC50 of SR plus®. Furthermore, the Hoechst stain revealed a significantly higher number of apoptotic cells in the group treated with SR Plus®, providing additional evidence of the drug's ability to induce apoptosis in cancer cells.

Conclusion

SR plus® acetone did not show the presence of phytochemicals as compared to methanol, ethanol, and water extracts. In comparison to the methanol and water extracts of SR plus®, the study revealed significantly lower IC50 values for SR plus®'s ethanol extract, particularly at 24 hours (22 µg/mL) and 48 hours (8.65 µg/mL) of exposure to HeLa cells. Additionally, exposure of HeLa cells to the SR plus® ethanol extract at IC50 resulted in apoptosis, as indicated by fluorescent staining with Hoechst dye, DAPI, and dual staining (acridine orange and propidium iodide). In conclusion, SR plus® exhibits significant cytotoxicity against HeLa cancer cells, with ethanol extract proving particularly effective. The observed mechanism of action is apoptosis, although further research is warranted for a comprehensive understanding. Additional experiments are required to confirm the potential synergy of SR plus® with standard treatments, using it as an integrative medicine. In this preliminary study, SR plus® can be considered a prospective option in integrative medicine.

Acknowledgments

I would like to thank Ms. Pooja Kasture for guiding me in my research work, Dr. Jyothsna Rao and Dr. Gururaj Rao for their invaluable guidance throughout my project, and the iCrest team for their support.

References

1. National Cancer Institute (NCI). What Is Cancer? 2007. [Online
2. Mirabelli, P.; Coppola, L.; Salvatore, M. Cancer Cell Lines Are U seful Model Systems for Medical Research. Cancers 2019, 11, 10 98. DOI: 10.3390/cancers11081098.

DOI: 10.36838/v6i5.2

3. Butanis, B. The Importance of HeLa Cells. Johns Hopkins Medi cine. National Cancer Institute (NCI). What Is Cancer? 2007.
4. Mirabelli, P.; Coppola, L.; Salvatore, M. Cancer Cell Lines Are U seful Model Systems for Medical Research. Cancers 2019, 11, 10 98. DOI: 10.3390/cancers11081098.
5. Butanis, B. The Importance of HeLa Cells. Johns Hopkins Medic ine [Online]; Available at: https://www.hopkinsmedicine.org/he nriettalacks/importance-of-hela-cells.html (accessed July 30, 20 23).
6. Cancer Research UK. Why some cancers come back [Online]; 20 14. URL: https://www.cancerresearchuk.org/about-cancer/what- is-cancer/why-some-cancers-come-back (accessed July 30, 2023).
7. Health Media. Cancer Recurrence Statistics. Cancer Therapy Ad visor [Online]; 2018. Available at: https://www.cancertherapyadv isor.com/home/tools/fact-sheets/cancer-recurrence-statistics/ (ac cessed July 30, 2023).
8. Deng, G.; Cassileth, B. Integrative Oncology: An Overview. Am. Soc. Clin. Oncol. Educ. Book 2014, 233–242. DOI: 10.14694/Ed Book_AM.2014.34.233.
9. A Systematic Review of Iran's Medicinal Plants With Anticancer Effects. DOI: 10.1177/2156587215600873.
10. Dey, P.; Kundu, A.; Kumar, A.; Gupta, M.; Lee, B. M.; Bhakta, T. ; Dash, S.; Kim, H. S. Analysis of Alkaloids (Indole Alkaloids, Iso quinoline Alkaloids, Tropane Alkaloids). Recent Adv. Nat. Prod. Anal. 2020, 505–567. DOI: 10.1016/B978-0-12-816455-6.0001 5-9.
11. Huang, M.-T. Phenolic Compounds in Food and Their Effects on Health II. [Online]; Available at: (accessed July 30, 2023).
12. Elekofehinti, O. O.; Iwaloye, O.; Olawale, F.; Ariyo, E. O. Sapon ins in Cancer Treatment: Current Progress and Future Prospects. Pathophysiology 2021, 28, 250–272. DOI: 10.3390/pathophysiol ogy28020017.
13. Kamran, S.; Sinniah, A.; Abdulghani, M. A. M.; Alshawsh, M. A. Therapeutic Potential of Certain Terpenoids as Anticancer A gents: A Scoping Review. Cancers 2022, 14, 1100. DOI: 10.3390 /cancers14051100.
14. Yin, S.-Y.; Wei, W.-C.; Jian, F.-Y.; Yang, N.-S. Therapeutic Appl ications of Herbal Medicines for Cancer Patients. Evid.-Based C omplement. Altern. Med. 2013, 302426. DOI: 10.1155/2013/30 2426.
15. O'Brien K, Ried K, Binjemain T, Sali A. Integrative Approaches to the Treatment of Cancer. Cancers 2022, 14(23), 5933. DOI: 10 .3390/cancers14235933.
16. Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen. Effect of extra ction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, Journal of Food and Drug Analysis, 2014, 22(3), 296–302.
17. Al-Obaidi, Zaid & Ani, Awatef & Hussein, Ali & Al-Juhaishi, Atheer. A comparative study for the quantification of paracetamol in multicomponent oral solution employing standard addition m ethod utilized in UV-Visible spectroscopy and RP-HPLC. Journ al of Pharmaceutical Sciences and Research. 2019, 11, 339-342.
18. Gul, R.; Rahman, M. A.; Ullah, H.; Khan, M. A.; Siddiqui, M. F. Preliminary Phytochemical Screening, Quantitative Analysis o f Alkaloids, and Antioxidant Activity of Crude Plant Extracts fro m Ephedra intermedia Indigenous to Balochistan. The Scientific World Journal 2017, 2017, 5873648. DOI: 10.1155/2017/587364 8.
19. Ellul Micallef, R.; Darmanin Ellul, R. Determination of the Phy tochemical, Antioxidant and Anti-Inflammatory Properties of Se lected Maltese Medicinal Plants. L-Università Ta' Malta, Univer sity of Malta, 2013.
20. Kavitha Chandran, C. I.; Rajathi, K. Quantitative Estimation of Total Phenolic, Flavonoids, Tannin and Chlorophyll Content of Leaves of Strobilanthes Kunthiana (Neelakurinji). Journal of Me dicinal Plants Studies 2016, 4(4), 282-286.
21. Chang, C. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. J. Food Drug Anal. 2002, 10, 178-182.
22. Le, A. V.; Kieu, T. H.; Thi, H. V.; Bach, L. G.; Nguyen, T. T. T.; N guyen, K. H.; Duong, T. T. H.; Nguyen, K. L. T.; Tran, T. D. Impr oving the Vanillin-Sulphuric Acid Method for Quantifying Total Saponins. Technologies 2018, 6(3), 84. DOI: 10.3390/yechnologi es6030084.
23. McDonald, S.; Prenzler, P. D.; Autolovich, M.; Robards, K. Phe nolic Content and Antioxidant Activity of Olive Extracts. Food Chemistry 2001, 73(1), 73–84. DOI: 10.1016/s0308-8146(00)00 288-0.
24. Liyana-Pathirana, C. M.; Shahidi, F.; Alasalvar, C. Antioxidant Activity of Cherry Laurel Fruit (Laurocerasus Officinalis Roem.) and Its Concentrated Juice. Food Chemistry 2006, 99(1), 121–12 8. DOI: 10.1016/j.foodchem.2005.06.046.
25. Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Prio r, R. L. The Chemistry behind Antioxidant Capacity Assays. Jour nal of Agricultural and Food Chemistry 2005, 53(6), 1841–1856. DOI: 10.1021/jf030723c.
26. Morgan, D. M. L. Tetrazolium (MTT) Assay For Cellular Viabi lity And Activity. In Polyamine Protocols; Humana Press, 1998; p p 179-184. DOI: 10.1385/0-89603-448- 8:179.
27. Very Simple IC50 Tool Kit - Calculate, Measure, Determine IC 50 Online. Ic50.Tk, 2020, http://ic50.tk/. Accessed 8 May 2022.
28. Mascotti, K.; McCullough, J.; Burger, S. C. HPC Viability Mea surement: Trypan Blue Versus Acridine Orange And Propidium Iodide. Transfusion 2000, 40(6), 693-696. DOI: 10.1046/j.1537- 2995.2000.40060693.x.
29. Crowley, L. C.; Marfell, B. J.; Scott, A. P.; Waterhouse, N. J. An alyzing Cell Death By Nuclear Staining With Hoechst 33342. C old Spring Harbor Protocols 2016, 2016(9), pdb.prot087205. DO I: 10.1101/pdb.prot087205.
30. Olympus BX63 for Fluorescent Microscopy. Biocompare, Januar y 27, 2016. URL: www.biocompare.com/Product-Reviews/18041 4-Olympus-BX63-for-Fluorescent-Microscopy/.
31. Progres® CapturePro Software Download. Jenoptik, 2020. URL: www.jenoptik.com/products/cameras-and-imaging-modules/mic roscope-cameras/progres-usb-20-fire

Author

Ananya Ravishankar is an 11th grader at The International School Bangalore. She has a strong passion for photochemistry and integrative oncology and finds the research in these domains captivating. She also enjoys participating in various sports activities.