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In Vitro Anticancer and Cytotoxic Activities of Siddha Formulation Parangi Rasayanam Against Hela Cell Line

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ABSTRACT:

Background: Cervical cancer is a significant global health concern since it is the fourth most frequent disease in women globally, accounting for about a quarter of a million fatalities each year. Drug resistance, recurrence, and metastasis are the systematic risks associated with conventional therapies. As a result, there is a dire need to develop new drugs with high efficacy and low side effects. Traditional forms of medicine used in India, such as siddha, offer an excellent resource for discovering novel candidates against cervical cancer. Objective: The main aim of the present clinical investigation is to evaluate the anticancer potential of the siddha formulation Parangi rasayanam (PR) against the human cervix adenocarcinoma cell line (HeLa). Methods: Viable HeLa cells were incubated with test drug PR at the concentration of 10, 25, 50, 75, and 100 µg/ml along with standard doxorubicin over a period of time, and the percentage cell viability was ascertained using SRB and MTT assay model. Results: Results of the SRB assay signifies that PR reveals significant percentage inhibition (33.17) at 100µg/mL. A similar type of inhibition was observed at a lower dose range of PR at 75µg/mL (50), 50µg/mL (75.57), and 25 µg/mL (94.03), respectively. The test drug's activity was compared to conventional doxorubicin (3.5uM/ml), revealing a viability of 49.73%. Results of the MTT assay show that the percentage of cell viability of the HeLa cell line decreases as the concentration of the test substance PR increases. The lowest cell viability was observed at 100µg/ml (31.23%), and the corresponding IC50 value was 75.98 µg/ml. Conclusion: It was concluded from the results of the present investigation that the siddha formulation PR reveals promising anticancer properties against the HeLa cells; further studies need to be elaborated before clinical recommendation of the drug in the future.

1.INTRODUCTION

Cervix cancer is the most prevalent form of malignancy in women worldwide [1]. In India, cervical cancer accounts for roughly 6-29% of all malignancies in women [2]. Asia had the highest percentage of cervical cancer cases recorded (58.2%); according to recent estimates for the year 2018, there were 569847 new cervical cancer cases diagnosed yearly. About 2785 million women are at risk of developing cervical cancer, of which Four-fifths are from less developed nations [3]. Age-standardized incidence rates are 14.7 per 100,000 women and 9.2 per 100,000 women, respectively, in India [4]. As a result, there is a compelling need for collective action on a worldwide scale to enhance the treatment of cervical cancer.

Surgery, radiation, and chemotherapy are the three primary types of treatment for cervical cancer [5]. Some surgical procedures used to treat cervical cancer include radical hysterectomy and pelvic lymphadenectomy. In addition, the medications often recommended for treating cervical cancer have been shown to cause several adverse side effects and drug

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resistance [6]. Cisplatin, one of the most powerful anticancer medications, can be resistant to the effects of the drug due to a self-defence mechanism [7]. Regarding people with cervical cancer, 5-fluorouracil (5-FU) has been documented to have resistance and a number of adverse effects [8].

In recent years, natural compounds originating from plants have been regarded to be among the most promising options for use as cancer treatments. They can selectively destroy tumor cells while having a minimal harmful effect on healthy cells. This is because of their wide variety of chemicals, the complexity of their structures, their intrinsic biological activity, and the few negative impacts they have [9,10]. Flavonoids, terpenoids, alkaloids, and phenols are some plant-derived natural products that have been found to have antitumor activities. These antitumor activities can inhibit tumor-cell proliferation, induce apoptosis, reduce telomerase activity, suppress angiogenesis, improve immune function, reverse multidrug resistance, and other similar activities [11,12].

It is believed that more than sixty percent of the anticancer drugs now on the market are derived from plants. The unprocessed plant material utilized in traditional medicine has the potential to be a significant source for the discovery of antitumor medications intended to treat gynecological malignancies. As a result of their prevalence across all age ranges, these tumors have a detrimental effect on women's overall health and result in significant medical expenses [13]. The siddha system of medicine is a traditional method that has helped people with their healthcare needs for many years. A key component of siddha therapy is determining the underlying causes of a disease's genesis based on the balance of vata, pitha, and kaba. The philosophy of traditional medicine emphasizes that altering or changing the tridosha opens the door to disease and disorders [14]. Herbal ingredients play a vital role in formulating siddha drugs. Bioactive components present in the herbs with novel therapeutic capabilities, making siddha medicine a pioneer in treating inflammation and other degenerative disorders. The excellent safety index of herbal supplements is well-known because phytocomponents improve the immune system by suitably enhancing the cellular biochemical pathway [15].

The siddha formulation *Parangi Rasayanam* comprises the combination of potential medicinal herbs with a previous track history of imposing several pharmacological activities. The main aim of the present clinical investigation is to evaluate the anticancer potential of the siddha formulation *Parangi Rasayanam* against the human cervix adenocarcinoma cell line (HeLa).

2.MATERIALS AND METHODS

2.1. Source and Authentication of raw drugs

All the raw materials required for the formulation were procured from the authorized traditional medicine shop in Chennai District, Tamil Nadu, India. A professional expert in the relevant field authenticated the raw drugs for genuinity before subjecting the material for processing and preparation.

2.2. Formulation of *Parangi Rasayanam*

The herbs such as *Cassia Auriculate* (Parangi chakkai), *Curculigo orchi*oides (Nilapanai kizhangu), *Withania somnifera* (Amukkura kizhangu), *Asparagus racemosus* (Thanneervittan kizhangu), *Hemidesmus indicus* (Nannari verpattai), *Bauhinia triandra R*oxb (Mutchankan verpattai), *Terminalia chebula*. (Kadukkai), *Phyllanthus emblica* (Nellikai),*Belleric Myrobalan* (Thanrikkai), *Cassia fistula* (Lavangapathiri), *Mesua ferrea* (Sirunagapoo), *Embelia ribes* (Vaividangam), *Coriandrum sativum* (Kothamalli), *Cuminum cyminum* (Seeragam), *Nigella sativa* (Karuncheeragam), *Trachyspermum ammi* (Omam), *Trachyspermum Roxburghianum* (Kurosani omam), *Alpinia Officinarum* (Chitrarathai), *Santalum album* (Santhanam), *Solanum surrattense* (Siruthekku / kandankathiri), *Plumbago zeylanic* (Chithira moola ver pattai), *Coleus Vet*tiveroides Root (Vilamichai ver), *Illicium verum* (Thakkolam), *Piper cubeba* (Valmilagu), *Vitis vinifer*a (Dhiratchai), *Phoenix dactylifera* (Perichai), *Wrightia tinctoria* (Vetpalai arisi), *Nelumbo nucifera* (Thamarai kizhangu), *Myristica fragrans* (Jathikkai / Jathipathiri), *Syzygium aromaticum* (Lavangam), *Murraya koenigii* (Karuveppillai),*Zingiber officinale* (Chukku), *Piper nigrum* (Milagu), *Piper longum* (Thippili) and *Cinnamomum verum* (Lavangapattai) being partially heated and the subjected to pulverisation and sieving procedure before being used. The powdered ingredients were processed by the addition of a measured proportion of milk, sugar, ghee and honey. To this, other components like *Crocus sativus* (also known as Kunkumapoo), *Cow Bezoars* (also known as Korosanai), and *Cinnamomum C*amphora (also known

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as Pachai karpooram) were added in order to complete the *Parangi Rasayanam* preparation [16].

2.3. Cell line and culture

HeLa, a cell line derived from a human cervical adenocarcinoma, was obtained from India's National Institute for Cell Research in Pune. Cultured cells were maintained in Dulbecco's modified eagle medium containing 10% foetal bovine serum and 1% penicillin-streptomycin. In a humidified incubator with 5% CO2, growth was maintained at 37°C.

2.4. *In vitro* cell growth by Sulforhodamine B assay (SRB)

Cells were seeded at a density of $1.5X10^4$ cells per well in 200 µl of the appropriate culture media into 96-well plates. Following a 24-hour pre-incubation period, cells were treated with increasing concentrations of PR (10, 25, 50, 75, and 100 µg/ml) before being incubated with the test drug PR. After 60 minutes' incubation at 4°C, cells were fixed by the careful addition of 50 µl of ice-cold 30% (w/v) trichloroacetic acid (final concentration, 10% TCA). The dishes were then cleaned under cold running water four times, and the surplus water was blotted dry using paper towels. A blow dryer was used to dry the plates thoroughly. After the plates were completely dry, 100 µl of a 0.057% sulforhodamine B (SRB) solution was poured into each well. After letting the stain sit for 30 minutes, the plate was rinsed quickly with 1% acetic vinegar four times to get rid of any unbound colour. Next, 200 µl of 10 mM Tris base solution (pH 10.5) was added to each well. Following this, the plates were shaken for 5 minutes to disperse the protein-bound dye. Microplate readers were used to measure the optical density (OD) at 510 nm. Using the following formula, we were able to determine the cell viability in a given sample [17].

Percentage viability % = Absorbance of test / Absorbance of sample×100

2.5. Assessment of cytotoxicity using MTT assay

Pre-confluent HeLa cells were seeded in 96-well plates at a density of 20,000 cells/200 µl/well. 24 hours after plating, cells were exposed to various doses of the test drug PR (10, 25, 50, 75, and 100 µg/ml) before being incubated at 37°C for a day. The cells were exposed to the medication for 20 hours before being incubated for 4 hours at 37°C with 5 mg/ml MTT. The media was withdrawn from the experiment after which the insoluble formazan product was dissolved in 200 µL of dimethyl sulfoxide and left in the dark for at least 15 minutes. MTT reduction was measured using a 96-well plate reader (ELX-800, BioTek, CA, USA) to measure absorbance at 570 nm and 630 nm [18,19].

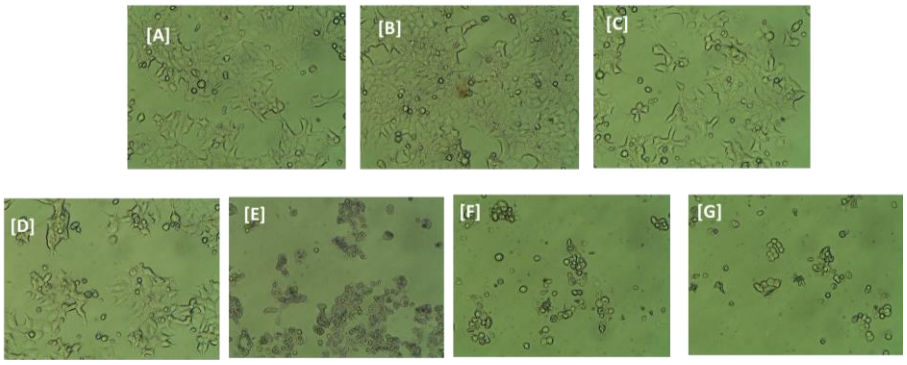
Cell viability (%) = (Abs of treated cells/Abs of control cells) ×100

3.RESULTS

3.1. Result Analysis on anti-cancer potential of PR by Sulforhodamine B assay (SRB)

The anticancer potential of the test drug PR was evaluated using the Sulforhodamine B assay. The study's outcome signifies that PR reveals significant percentage inhibition (33.17) at 100µg/mL. A similar type of inhibition was observed at a lower dose range of PR at 75µg/mL (50), 50µg/mL (75.57), and 25 µg/mL (94.03), respectively. The test drug's activity was compared to conventional doxorubicin (3.5uM/ml), revealing a viability of 49.73%. As shown in Table 1 and Figure 1.

Figure 1: Morphology of control and drug-treated HeLa cells in SRB assay



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Morphology of control and drug-treated wells subjected to SRB assay visualized under inverted microscope: (A) Untreated control HeLa cells, (B) PR 10 µg/ml, (B) PR 25 µg/ml, (C) PR 50 µg/ml, (D) PR 75 µg/ml, (E) PR 100 µg/ml and (F)Standard Doxorubicin 3.5uM/ml.

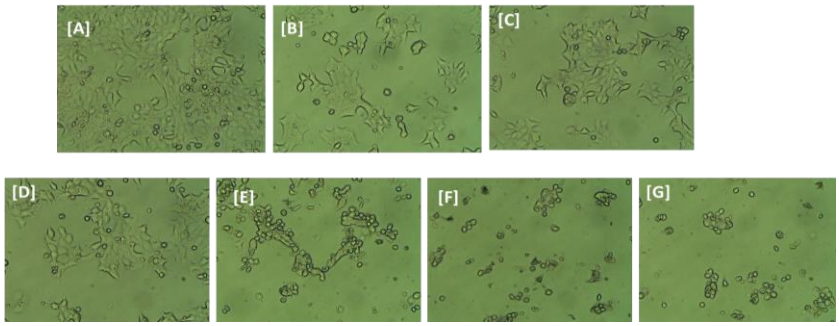
Table 1: Effect of PR on viability of HeLa (cervical adenocarcinoma) cell line

S. NO	Drug Concentration (ug/ml)	Test Parameter-SRB
		% Cell Viability
1	Control –Un treated	100.00
2	STD (Dox-3.5uM)	49.73
3	PR -10 µg/ml	98.26
4	PR -25 µg/ml	94.03
5	PR -50 µg/ml	75.57
6	PR -75 µg/ml	50.00
7	PR -100 µg/ml	33.17

3.2. Result Analysis on Cytotoxic potential of PR by MTT assay

The study's findings show that the percentage of cell viability of the HeLa cell line decreases as the concentration of the test substance PR increases. The lowest cell viability was observed at 100µg/ml (31.23%), followed by 75µg/ml and 50µg/ml, with percentage viability of 52.64 and 69.99% found in MTT assay. The test substance PR's corresponding IC50 value was determined to be 75.98 g/ml. The corresponding IC_{50} value of the test drug PR was 75.98 µg/ml. The test drug's activity was compared to conventional doxorubicin (3.5uM/ml), revealing a viability of 53.10%. As shown in Table 2 and Figure 2.

Figure 2: Morphology of control and drug-treated HeLa cells in MTT assay



Morphology of control and drug-treated wells subjected to MTT assay visualized under inverted microscope: (A) Untreated control HeLa cells, (B) PR 10 µg/ml, (B) PR 25 µg/ml, (C) PR 50 µg/ml, (D) PR 75 µg/ml, (E) PR 100 µg/ml and (F)Standard Doxorubicin 3.5uM/ml.

Table 2: Percentage cell viability values and observed IC_{50} value of PR against HeLa cells.

Culture condition	% cell viability	IC_{50} conc (µg/ml)
Control –Un treated	100.00	
STD (Dox-3.5uM)	53.10	

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PR -10 µg/ml	92.42	75.98
PR -25 µg/ml	84.59	
PR -50 µg/ml	69.99	
PR -75 µg/ml	52.64	
PR -100 µg/ml	31.23	

4.DISCUSSION

Cancer is the most severe disease that impacts people all over the globe, and its prevalence is predicted to rise because of lifestyle factors known to promote cancer [20]. The most significant barriers presently exist to applying chemotherapeutic drugs rely on toxicity and resistance. Thus, it is regarded as a substantial problem to locate novel therapy choices that are also secure [21].Chemoradiotherapy is currently the most effective treatment for locally advanced cervical cancer patients. This treatment has dramatically enhanced patients' overall survival (OS) and their disease-free survival rates (DFS) [22,23]. On the other hand, more than fifty percent of patients with stage III to IV cervical cancer are resistant to treatment and will, in the end, either develop recurrent disease or distant metastases [24].In addition, significant toxicity, such as suppression of bone marrow and impairment of liver and kidney function, decreases a patient's likelihood of complying with their treatment plan. As a result, it is of the highest significance in treating cervical cancer to increase the therapeutic benefits of chemotherapeutic medications and limit the harmful side effects.

Natural substances produced from plants have recently been investigated as promising anticancer treatments that preferentially destroy tumor cells while posing little risks [25]. An increasing body of research has demonstrated that natural products can effectively combat cervical cancer through various methods, including apoptotic induction, angiogenesis and telomerase activity reduction, immune system improvement, and reversal of multidrug resistance [26].

The value of herbal medicine as a supplemental and alternative therapy for cancer patients is growing. Research has shown that herbal remedies work well in conjunction with conventional treatments, especially in terms of cancer patients' quality of life, immune systems, and survival rates [27]. Plants have always been a paradigmatic source of medications, and a large number of the pharmaceuticals that were previously accessible were produced either directly or indirectly from plants. Activity commensurate with their potential application in the treatment of different illnesses has been established by a wide variety of plant-derived active principles representing multiple chemical compounds [28]. These active principles come from plants. In recent years, there has been a substantial increase in the amount of attention paid, among some subsets of the scientific community [29], to ethnobotanical knowledge in medicinal plant research. From the beginning of recorded history, people have used different components of plants and isolated phytochemicals to prevent and treat a wide range of medical conditions [30]. The formulation ***Parangi Rasayanam*** selected for the present investigation comprises more than thirty herbs as a core ingredient to exert significant biological action as listed in siddha literature.

The MTT technique uses mitochondrial dehydrogenases to gauge the activity of live cells. The MTT technique offers precise, accurate, and reproducible findings. When incubated, (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide), often known as MTT, transforms into a tetrazolium salt that is water soluble. The mitochondrial dehydrogenase enzymes of live cells cleave the tetrazolium ring of MTT to produce an insoluble purple formazan [31]. A spectrophotometric analysis of the resultant colored solution is performed. The quantity of formazan produced changes in direct proportion to the number of cells present, providing a direct indication of the cytotoxicity of the test substance [32]. The study's findings show that the percentage of cell viability of the HeLa cell line decreases as the concentration of the test substance PR increases. The lowest cell viability was observed at 100µg/ml (31.23%), followed by 75µg/ml and 50µg/ml, with percentage viability of 52.64 and 69.99% found in MTT assay. The test substance PR's corresponding IC50 value was determined to be 75.98 g/ml. The corresponding IC_{50} value of the test drug PR was 75.98 µg/ml. The test drug's activity was compared

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to conventional doxorubicin (3.5uM/ml), revealing a viability of 53.10%.

Sulforhodamine B (SRB) cell cytotoxicity test is one of the most extensively used techniques for detecting cell viability or drug cytotoxicity. This test is based on SRB's capacity to bind cellular protein components and calculate total biomass [33]. SRB is a pink amino xanthene dye that forms an electrostatic compound with essential amino acid residues of proteins in slightly acidic conditions [34]. The anticancer potential of the test drug PR was evaluated using the Sulforhodamine B assay. The study's outcome signifies that PR reveals significant percentage inhibition (33.17) at 100µg/mL. A similar type of inhibition was observed at a lower dose range of PR at 75µg/mL (50), 50µg/mL (75.57), and 25 µg/mL (94.03), respectively. The test drug's activity was compared to conventional doxorubicin (3.5uM/ml), revealing a viability of 49.73%.

5.CONCLUSION

Cervical cancer is a primary cause of cancer mortality in women. The number of young women afflicted by cervical cancer has grown dramatically during the last three decades. Cancer therapy typically involves the administration of medicines that suppress proliferation, invasion, and metastasis. Traditional remedies have a long history of treating severe illnesses like cancer. In our present investigation, the SRB and MTT assay results signify that the drug PR reveals significant percentage inhibition of growing HeLa cells with the lowest IC_{50} value of 75.98 µg/ml. Hence it was concluded that the siddha formulation *Parangi Rasayanam* reveals promising anticancer properties and shall be recommended for the management of cervical cancer with the prior clinical investigation in the near future.

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Conflict of Interests

Declared none

REFERENCES

1. Freddie B, Jacques F, Isabelle S, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. doi: 10.3322/caac.21492
2. Bobdey, S., Sathwara, J., Jain, A., & Balasubramaniam, G. (2016). Burden of cervical cancer and role of screening in India. Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology, 37(4), 278–285. https://doi.org/10.4103/0971-5851.195751
3. Singh, M., Jha, R. P., Shri, N., Bhattacharyya, K., Patel, P., & Dhamnetiya, D. (2022). Secular trends in incidence and mortality of cervical cancer in India and its states, 1990-2019: data from the Global Burden of Disease 2019 Study. BMC cancer, 22(1), 149. https://doi.org/10.1186/s12885-022-09232-w
4. ICO Information Centre on HPV and cancer . Human papillomavirus and related diseases in India (summary report 2019-06-17) 2019.
5. Kim J.Y., Byun S.J., Kim Y.S., Nam J.-H. Disease courses in patients with residual tumor following concurrent chemoradiotherapy for locally advanced cervical cancer. Gynecol. Oncol. 2017;144:34–39. doi: 10.1016/j.ygyno.2016.10.032.
6. Federico C., Sun J., Muz B., Alhallak K., Cosper P.F., Muhammad N., Jeske A., Hinger A., Markovina S., Grigsby P., et al. Localized Delivery of Cisplatin to Cervical Cancer Improves Its Therapeutic Efficacy and Minimizes Its Side-Effect Profile. Int. J. Radiat. Oncol. 2020 doi: 10.1016/j.ijrobp.2020.11.052.
7. Shen D.-W., Pouliot L.M., Hall M.D., Gottesman M.M. Cisplatin Resistance: A Cellular Self-Defense Mechanism Resulting from Multiple Epigenetic and Genetic Changes. Pharmacol. Rev. 2012;64:706–721. doi: 10.1124/pr.111.005637.
8. Sun C., Brown A.J., Jhingran A., Frumovitz M., Ramondetta L., Bodurka D.C. Patient Preferences for Side Effects Associated With Cervical Cancer Treatment. Int. J. Gynecol. Cancer. 2014;24:1077–1084. doi: 10.1097/IGC.0000000000000149.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 826-832 | ISSN:2251-6727

9. Mann J. Natural products in cancer chemotherapy: Past, present and future. Nat. Rev. Cancer. 2002;2:143–148. doi: 10.1038/nrc723. [PubMed] [CrossRef] [Google Scholar]
10. Aung T.N., Qu Z., Kortschak R.D., Adelson D.L. Understanding the Effectiveness of Natural Compound Mixtures in Cancer through Their Molecular Mode of Action. Int. J. Mol. Sci. 2017;18:656. doi: 10.3390/ijms18030656.
11. Ouyang L., Luo Y., Tian M., Zhang S.-Y., Lu R., Wang J.-H., Kasimu R., Li X. Plant natural products: From traditional compounds to new emerging drugs in cancer therapy. Cell Prolif. 2014;47:506–515. doi: 10.1111/cpr.12143.
12. Kikuchi H., Yuan B., Hu X., Okazaki M. Chemopreventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. Am. J. Cancer Res. 2019;9:1517–1535.
13. Bayala, B., Zouré, A. A., Zohoncon, T. M., Tinguerie, B. L., Baron, S., Bakri, Y., Simpore, J., & Lobaccaro, J. A. (2020). Effects of extracts and molecules derived from medicinal plants of West Africa in the prevention and treatment of gynecological cancers. A Review. American journal of cancer research, 10(9), 2730–2741.
14. D.Sivaraman. Evaluation of Anti- Urolithiasis Potential of Indian Medicinal Herbs Ficus hispida, Morinda tinctoria and Sapindus emarginatus by Struvite Crystal Growth Inhibition Assay. International Journal of ChemTech Research.2017,10(10):436-447.
15. D.Sivaraman, N.Anbu, N.Kabilan, M.Pitchiah Kumar, P.Shanmugapriya , G.J.Christian. Exploration of Anti-Urolithiasis Potential of Traditional Siddha formulations Amukkara Chooranam and Karisalai Karpam Chooranam by Struvite Crystal Growth Inhibition Assay. Pharmacognosy Journal.2019;11(4) :683-688.
16. Agasthiyar vaithiya rathina surukkam-300
17. Orellana, E. A., & Kasinski, A. L. (2016). Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio-protocol, 6(21), e1984. https://doi.org/10.21769/BioProtoc.1984
18. Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. (2021). The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. International journal of molecular sciences, 22(23), 12827. https://doi.org/10.3390/ijms222312827
19. Tavares-Carreón, F., De la Torre-Zavala, S., Arocha-Garza, H. F., Souza, V., Galán-Wong, L. J., & Avilés-Arnaut, H. (2020). In vitro anticancer activity of methanolic extract of Granulocystopsis sp., a microalgae from an oligotrophic oasis in the Chihuahuan desert. PeerJ, 8, e8686. https://doi.org/10.7717/peerj.8686
20. Torre L.A., Bray F., Siegel R.L., Ferlay J., Lortet-Tieulent J., Jemal A. Global cancer statistics, 2012. CA Cancer J. Clin. 2015;65:87–108. doi: 10.3322/caac.21262.
21. Abdulaev F.I. Plant-derived agents against cancer. In: Gupta S.K., editor. Pharmacology and Therapeutics in the New Millennium. Narosa Publishing House; New Delhi, India: 2001. p. 345.
22. Mélanie D, Antonin L, Michele M, et al. The combination of the antiviral agent cidofovir and anti-EGFR antibody cetuximab exerts an antiproliferative effect on HPV-positive cervical cancer cell lines' in-vitro and in-vivo xenografts. Anticancer Drugs. 2013;24(6):599–608. doi: 10.1097/CAD.0b013e3283612a71
23. Yee Grace PC, de Souza P, Khachigian LM. Current and potential treatments for cervical cancer. Curr Cancer Drug Targets. 2013;13(2):205–220. doi: 10.2174/1568009611313020009
24. Alina S, Richard P, Ulrik FL, et al. Image guided brachytherapy in locally advanced cervical cancer: improved pelvic control and survival in RetroEMBRACE, a multicenter cohort study. Radiother Oncol. 2016;120(3):428–433. doi: 10.1016/j.radonc.2016.03.011
25. He, M., Xia, L., & Li, J. (2021). Potential Mechanisms of Plant-Derived Natural Products in the Treatment of Cervical Cancer. Biomolecules, 11(10), 1539. https://doi.org/10.3390/biom11101539
26. Li X., Chu S., Lin M., Gao Y., Liu Y., Yang S., Zhou X., Zhang Y., Hu Y., Wang H., et al. Anticancer property of ginsenoside Rh2 from

ginseng. Eur. J. Med. Chem. 2020;203:112627. doi: 10.1016/j.ejmech.2020.112627.

27. Yin SY, Wei WC, Jian FY, Yang NS. Therapeutic applications of herbal medicines for cancer patients. Evid Based Complement Alternat Med. 2013;2013:302426
28. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. J Ethnopharmacol. 2002;81:81–100. doi: 10.1016/S0378-8741(02)00059-4.
29. Ayyanar M, Ignacimuthu S. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. J Ethnopharmacol. 2011;134:851–864. doi: 10.1016/j.jep.2011.01.029.
30. Pedersen ME, Vestergaard HT, Hansen SL, Bah S, Diallo D, Jäger AK. Pharmacological screening of Malian medicinal plants used against epilepsy and convulsions. J Ethnopharmacol. 2009;121:472–475. doi: 10.1016/j.jep.2008.11.006.
31. Gonzalez, R.J. and Tarloff, J.B. Evaluation of hepatic sub cellular Fractions for alamar blue and MTT reductase activity. Toxicology in vitro.2001;15, 259-9.
32. R. Kiruthiga and D. Sivaraman. Evaluation of Anti-Cancer Potential of Indian Medicinal Herbs Morinda Tinctoria and Ficus Hispida Using Hela Cell Line by MTT Assay Method. World Journal of Pharmacy and Pharmaceutical Sciences.2016; 5(9): 1658-1670.
33. Duong HH, Yung LY. 2013. Synergistic co-delivery of doxorubicin and paclitaxel using multi-functional micelles for cancer treatment. International journal of pharmaceutics, 454 (1): 486-95.
34. Mauludin R, Müller RH, Keck C. 2009. Development of an oral rutin nanocrystals formulation. International journal of pharmaceutics, 370: 202-209.