ANTICANCER ACTIVITY STUDY OF SOME SELECTED INDIAN MEDICINAL PLANTS USED TRADITIONALLY

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Abstract

Due to the availability of several secondary metabolites, plants and their derivatives have historically been relied upon as a primary source of therapeutics. Many different plant families have been found to have compounds with possible anticancer effects. The purpose of this research was to use the SRB assay to assess the cancer-fighting effects of an ethanolic extract of three plants from different families, namely Croton bonplandianum Baill., Heliotropium indicum L., and Quisqualis indica L., on four different human cancer cell lines: melanoma (MDA-MB-231), chronic lung disease (A-549) and prostate cancer (PC-3) and hepatoma (HepG2). These three plants were tested for their cytotoxic activity (SRB assay) against these cancer cell lines. Different test material concentrations (10 gram/milligram, 20 gram/milligram, 40 gram/milligram, and 80 gram/milligram) were used to determine the level of activity. At 80ug/ml, only H. indicum showed a promising impact against melanoma (MDA-MB-231) and lung cancer (A549) cell lines, whereas C. bonplandianum and Q. indica had no effect (A-549).

Keywords: Plant derivatives, Melanoma, Cytotoxic Activity, SRB assay, Secondary Metabolites.

INTRODUCTION

Cancer is a multicellular condition characterised by excessive cell growth [1]. It is the main cause of death globally and the second in developing nations [3, 4]. Population ageing, growth, and cancer-related lifestyle choices including smoking, physical inactivity, and "westernised" diets are increasing the burden in developing nations [5].According to predictions, the overall number of new cancer cases would climb by 25% every decade, reaching 24 million per year in 2050; the total number of fatalities will increase from 6 million in 2000 to 10 million in 2020 to over 16 million in 2050; and there will be 17 million new cancer cases [6–8].

Cancer treatments are based on pathology and symptomatic assessment to cure the illness and prolong life. Patients get chemotherapy, radiation, hormone treatment, surgery, and immunotherapy year-round. Chemotherapy treats metastasized cancer cells because they circulate in the circulation.

Surgery and radiation treat localised malignancies. Systemic cancer treatment uses alkylating agents, antimetabolites, antibiotics, and hormones [9, 10]. Chemotherapeutic medicines are cytotoxic, which means they kill tumour cells and quickly growing normal cells such those in the gastrointestinal system, hair, and bone marrow, causing nausea, vomiting, alopecia, and myelosuppression. Antitumor drugs may cause secondary malignancies. Newer, more effective, and better-tolerated chemotherapeutic therapies are motivated by their present drawbacks.

Plants and their compounds have been medicine since ancient times. Vegetables or their extracts have been utilised to cure and preserve health.

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Thus, humans have long sought therapeutic herbs. Primitive man identified nutritive and medicinal plants for survival. Traditional medicine shows that humans have long sought natural remedies for illness [11].There are at least 250,000 plant species, and more than 1,000 of them contain anticancer characteristics. Plant-based cancer therapies are being researched worldwide. This therapy uses plant chemicals that inhibit or destroy cancerous cells. 460 plant species may be utilised as herbs, including cancer-fighting plants [12]. Various types of anti-cancer plants include the Golden Trumpet Vine. (Allamanda cathartica L.) [13], Elephant foot yam (Amorphophallus paeoniifolius (Dennst.) Nicolson) [14] ,Peacock flower (Caesalpinia pulcherrima (L.) Sw.) [15] , Indian shot(Canna indica L.) [16] , Rubber bush (Calotropis procera (Aiton) Dryand.) [17] , Stinking cassia (Cassia tora L.) [18] , Night bloom jasmine (Cestrum nocturnum L.) , Taro (Colocasia esculenta (L.) Schott) [20] , Dumb cane (Dieffenbachia seguine (Jacq.) Schott) [21], Glory lily (Gloriosa superb L.) [22], Marsh barbel (Hygrophila spinosa (Schumach.) Heine) [23] , Garden balsam (Impatiens balsamina L.) [24] .

There are so many plants that are unexplored and require further research. Out of these plants, some are like Croton bonplandianum Baill. is a plant that contains a variety of phytoconstituents with medicinal properties. The secondary metabolites, including alkaloids and terpenoids [25, 26] , along with irritating cocarcinogenic phorbol esters, are responsible for it [27] . The presence of diterpene resins in several species has been utilised in tumour initiation. Further research could lead to the discovery of powerful phytochemicals for cancer treatment [28].

Heliotropium indicum L. is primarily found on Asian continents, but it is also found in some African countries [29]. H. indicum has a wide range of therapeutic efficacy, including anti-inflammatory [30], wound-healing, anti-cancer, and anti-anticataract properties, according to the scientific manuscript.

Quisqualis indica L. is native to Southeast Asia, and now it is widely found in every part of India as an ornamental plant. Traditionally, this herb was used against various pathogenic infections, like staphylococcal infection and helminth infection, but now it is used for its antidiarrheal, antiinflammatory, antiseptic, and immunomodulatory properties. Many scientific studies indicate that Q. indica also has anticancer properties.

This in vitro SRB test research examined the anticancer activity of Croton bonplandianum Baill., Heliotropium indicum L., and Quisqualis indica L. in several human cancer cell lines.

MATERIALS AND METHODS

Collection and authentication of Plant Materials

Odisha's Khordha district provided fresh plants in November 2019. Dr. Kunja Bihari Satapathy of Centurion University of Technology and Management, Bhubaneswar, Odisha, India, visually validated the plant using taxonomic methods. The voucher specimen was filed at the U.D.P.S. herbarium, Utkal University, Bhubaneswar, Odisha, India.

Table1: Samples of the selected plants

Plant	Family	Sanskrit Name	Part used	Voucher Number
Croton bonplandianum Baill.	Euphorbiaceae	Naga Danti	Whole Plant	UDPS-Dash-1002
Heliotropium indicum L.	Boraginaceae	Hastishundi	Whole Plant	UDPS-Dash-1007
Quisqualis indica L.	Combretaceae	Madhu malati	Leaves and flowers	UDPS-Dash-1010

Preparation of Solvent Extracts

The pure plant parts were gathered in bulk in the morning, washed two to three times under running tap water to remove dust particles, followed by distilled water, and dried under shade at room temperature for 10–15 days to keep them moisture-free. Then the dried plant components were hand-pressed into a coarse powder, sieved, and extracted. Soxhlet apparatus extracted 250 g of dried coarse powdered materials using 700 ml of ethanol for 72 hours. The plant extracts were collected and filtered using Whatman No. 1 filter paper, concentrated with a rotary evaporator (IKA Model RV 10D S96), and frozen in an airtight container at 4° C until use. All extracts underwent phytochemical screening and in-vitro anticancer testing.

The following formula determined the plant material's dry weight % of the dried extracts:

$$Percentage of extract yield = \frac{Weight of dired extract}{Weight of dried plant material} \times 100$$

Analysis of physicochemical parameters

The WHO guidelines (1998) employed multiple analytical methods to assess active physiochemicals in air-dried, coarsely powdered plant material [31].

Preliminary Phytochemical Screening

Using established procedures, phytochemical screening estimates plant extract secondary metabolites such alkaloids, glycosides, flavonoids, saponins, steroids, phenols, and tannins [30] .

Source of Human Cancer Cell Line

National Centre for Cell Science, Department of Biotechnology, Government of India, Pune, Maharashtra - 411007, India provided several human cancer cell lines. -

Selection of Human Cancer Cell Line

Most human cancer cell lines were designated to be maintained in RPMI 1640 media with 10% fetalbovine serum (FBS), 2mM L-glutamine, 100 Units/ml penicillin, and 100µg/ml streptomycin in tissue culture flasks. At all phases, cells were devoid of bacteria, yeast, mould, mycoplasma, and viruses. The research used breast cancer (MDA-MB-231), lung cancer (A-549), prostate cancer (PC-3), and hepatoma cancer (Hep-G2) cell lines cultured at 37°C, 5% CO2, and 100% relative humidity for 24 h before adding experimental medicines.

Chemicals and Reagents

Merck Specialties Pvt. Ltd. and HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India, supplied all analytical-grade chemicals and reagents.

In vitro Cytotoxicity assay of Plants Extract

A semiautomated sulforhodamine-B (SRB) assay was used to test the ethanolic extracts of C. bonplandianu, H. indicum, and Q. indica against four human cancer cell lines. Tissue culture plates were made from cells injected into 96-well microtiter plates in 100 L at various plating densities. Four distinct tissue culture flasks were utilised as control blanks (just growth media without cancer cell line) and control growths to determine maximal (100%) growth (growth medium with cancer cell line). Test growth (growth medium, cancer cell line) and test control indicate growth in test material (growth medium, test material without cancer cell line). After cell inoculation, microtiter plates were incubated at 37 °C in 5% CO2, 95% air, and 100% relative humidity for 24 h before adding experimental medicines. Before usage, experimental medicines were solubilized in 100 mg/mL dimethyl sulfoxide, diluted to 1 mg/mL with water, and frozen. An aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400, and 800 g/ml with complete medium containing the test item upon drug addition. The final drug concentrations of 10 g/ml, 20 g/ml, 40 g/ml, and 80 g/ml were achieved by adding 10 µl of each drug dilution to the corresponding microtiter wells containing 90 µl of medium. Plates were then incubated at 37o C for 48 hours in a CO2 incubator with 5% CO2 and 90% relative humidity. A 48-hour SRB test measured growth.

SRB assay

After 48 hours of incubation under usual circumstances, the plate containing cancer cells and test material was removed and 50 µl of cooled 50% (w/v) Trichloroacetic acid was gently applied to the top surface of the medium-containing tissue culture plates, followed by 10% in all wells. After that, the tissue culture plates were refrigerated for 60 minutes at 4o C to fix the cells on the well bottoms. After one hour, the plates were removed from the refrigerator, all wells were pipetted, and the supernatant was discarded. Trichloroacetic acid growth medium, low molecular metabolites, serum protein, etc. were rinsed off the plates five times with distilled water. Tissue culture plates were filled with distilled water and forcibly flicked over the sink to remove the liquid. Air-dried plates are ready for usage. SRB solution (100 µl) was applied to each plate well and incubated for 30 minutes at room temperature. Washing plate wells five times with 1% acetic vinegar eliminated unattached SRB rapidly. Air-dried plates. The plates then received 100 µl/well Tris buffer. A mechanical shaker gently swirled the plates for 5 minutes, and the absorbance was measured on a plate reader at 540 nm with a reference wavelength of 690 nm. Data were recorded. Growth inhibition was determined as treatment cell survival over control cell survival X 100 (T/C %).

$$\% \text{ Growth inhibition} = 100 - \left[\frac{\text{OD(test sample)} - \text{OD(blank)}}{\text{OD (control)} - \text{OD(blank)}}\right]$$

Statistical Analysis

This research shows mean ± SEM. One-way ANOVA and Dunnett's multiple comparison test analysed data (GraphPad Prism 5, GraphPad Software Inc., La Jolla, CA, USA). at $P<0.05$.

RESULTS & DISCUSSION

The international organisation for research on cancer reports that in 2018, there were 18.1 million new cases of cancer and 96 million deaths worldwide [1]. The results of SRB test of in vitro cytotoxicity research based on the National Cancer Institute's (NCI, USA) proposal that 30 g/mL is the highest IC50 threshold for crude extract purification that is judged promising [40] .Ethanolic extracts of C. bonplandianu and Q. indica had no significant effects on the cell lines (Figure 1&3), however H. indicum exhibited promising action against MDA-MB-231 and A-549 cell lines with IC50 values of 23 and 22 µg/ml, respectively (Figure 2).Doxorubicin, a conventional medication, had IC50 values of 23, 22, 24, and 18 µg/ml on all cell lines in the research (Figure 4).Digital picture of cancer cell lines and Drug treated cells of MDA-MB-231, A-594, PC-3 and Hep –G2 were presented in figure 5-8.

Table 2: The percentage of dry weight that consists of various phytochemicals in plant samples, given in milligrammes per one hundred grammes of dry weight.

S. No.	Name of the plant	Part Used	Total Alkaloids	Total Flavonoids	Total tannins
1	*Croton bonplandianum* Baill.	Whole Plant	3.52	2.26	0.89
2	*Heliotropium indicum* L.	Whole Plant	2.56	1.05	1.17

3	*Quisqualis indica* L.	Leaves and flowers	1.69	2.31	0.78

Table 3: Phytochemical screening of the plant extracts

S. No.	**Phytochemicals**	**Plant extracts**		
		C. bonplandianum Baill.	*H. indicum* L.	*Q. indica* L.
	Alkaloids	++	++	++
	Glycosides	_	+	+
	Tannins	++	++	++
	Saponins	+	_	+
	Terpenoids	+	+	+
	Phenols	++	+	+
	Flavonoids	++	++	+
	Steroids	+	+	_

(++ = strong positive, + = positive,- = negative)

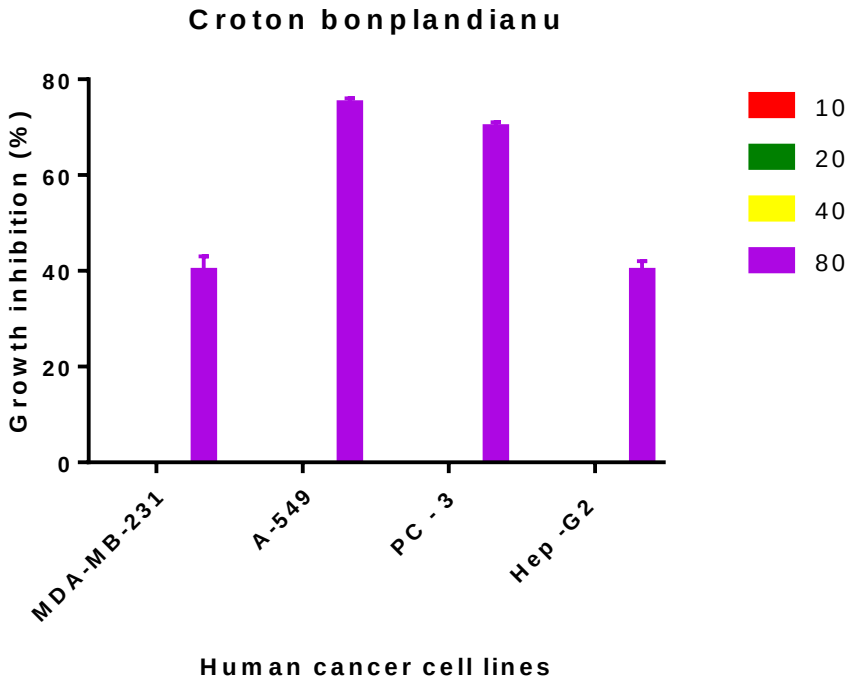

Figure 1:Croton bonplandianu ethanol extract cytotoxicity in the SRB test against many human cancer cell lines in vitro. The IC50 values are estimated to be more than 100 g/mL for MDA-MB-231, A-549, PC-3, and Hep-G2.

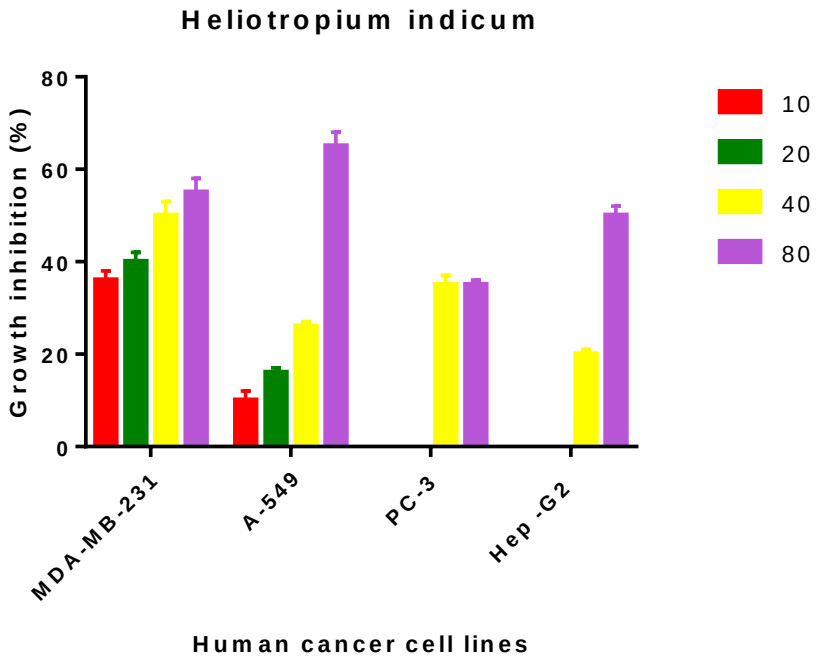

Figure 2: Heliotropium indicum ethanol extract showed cytotoxic activity in the SRB test in vitro against a number of human cancer cell lines. The IC50 values for MDA-MB-231, A-549, PC-3, and Hep-G2 are 23, 18 >100, 75, and 82, respectively.

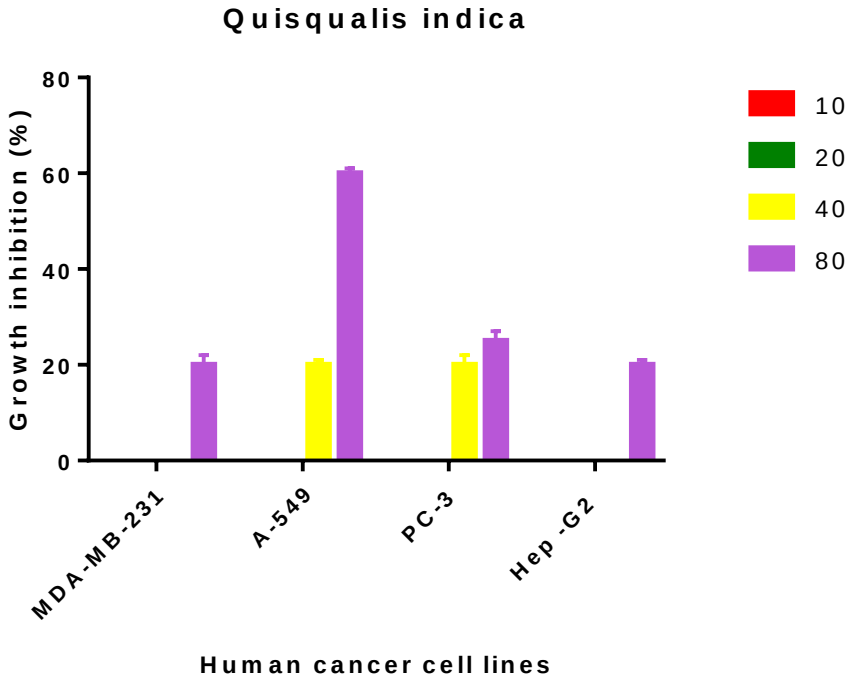

Figure 3: The SRB test demonstrated that an ethanol extract of Quisqualis indica was cytotoxic to many lines of human cancerous cells in culture. The estimated IC50 values for MDA-MB-231, A-549, PC-3, and Hep-G2 are >100, >100, 75, and 82, respectively.

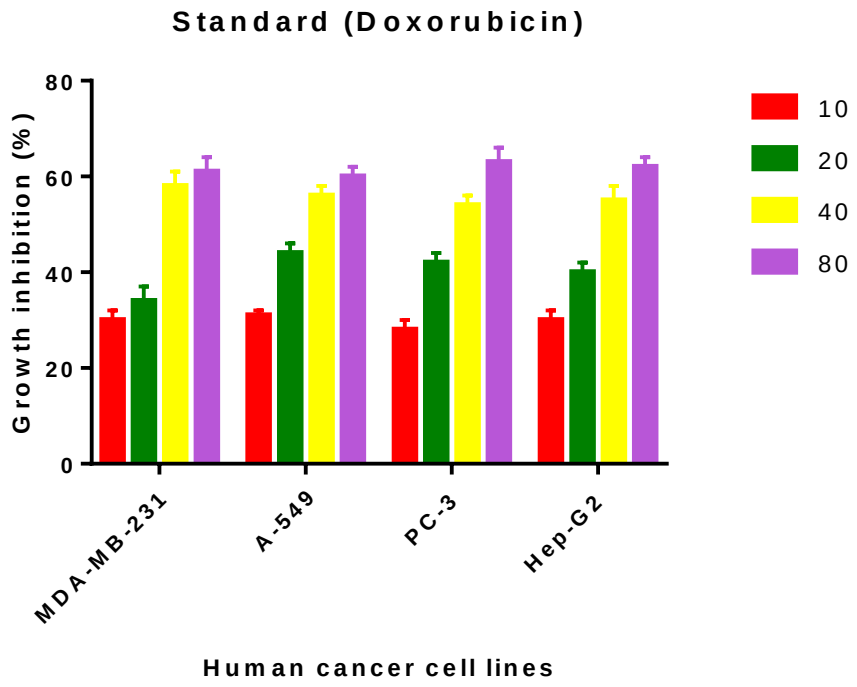

Figure 4: Toxic effects of the reference medication (doxorubicin) in the SRB test on many types of human cancer cells cultured in vitro. The IC50 values for MDA-B-231, A-549, PC-3, and Hep-G2 are 23, 22, 24, and 18, respectively.

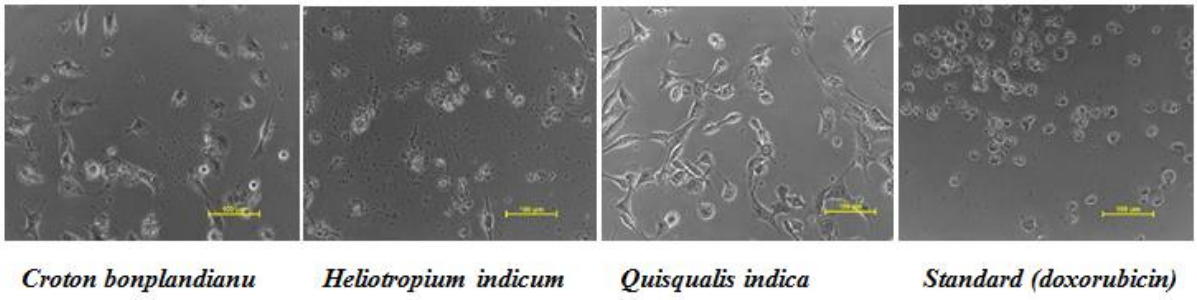

Figure 5. Digital image of Cell Lines and Drug treated cells of MDA-MB-231 Cell Lines

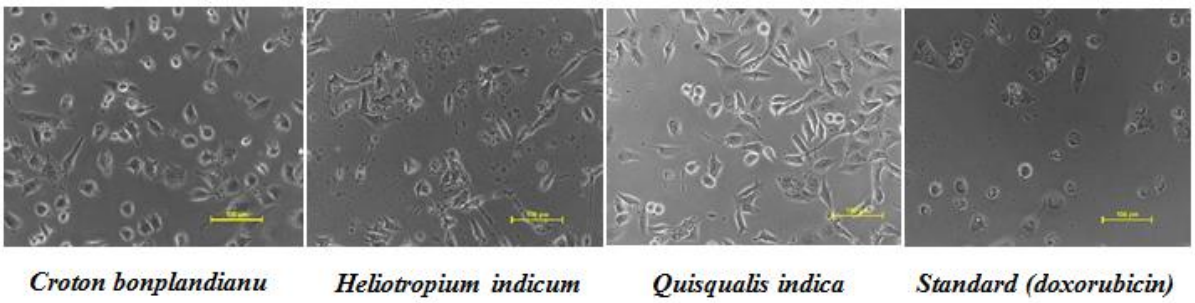

Figure 6. Digital image of Cell Lines and Drug treated cells of A-594Cell Lines

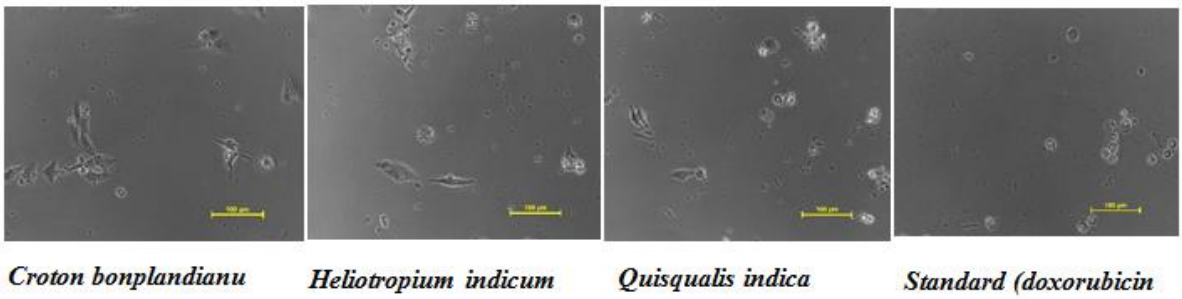

Figure 7. Digital image of Cell Lines and Drug treated cells of PC-3Cell Lines

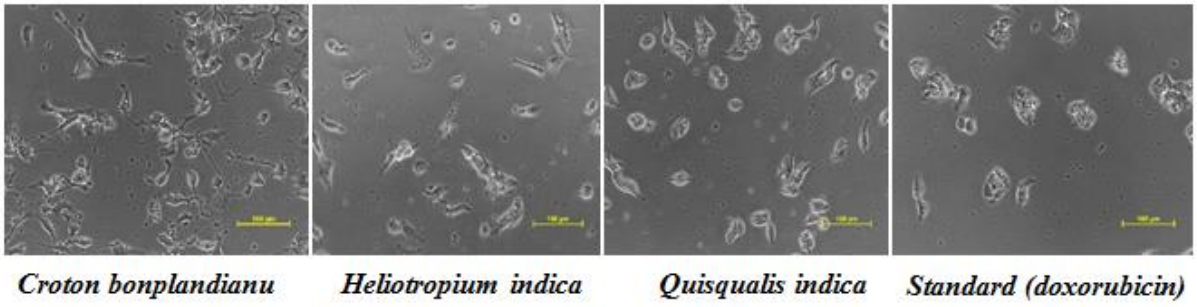

Figure 8. Digital image of Cell Lines and Drug treated cells of Hep –G2Cell Lines

CONCLUSION

The international organisation for research on cancer reports that in 2018, there were 18.1 million new cases of cancer and 96 million deaths worldwide [1]. Herbal remedies, from energising decoctions to the use of herbal extracts in accordance with Western methodology of mainstream medicine, may vary widely in their chemical makeup since herbs are natural products and their chemical composition depends on various aspects. Traditional medicine has been around for a long time, and it encompasses a wide range of techniques for maintaining health, including the prevention, diagnosis, and treatment of diseases, that are grounded on the ideas, beliefs, and experiences of people from many cultures and ages. Cell lines from breast cancer (MDA-MB-231), lung cancer (A-549), prostate cancer (PC-3) and hepatocellular carcinoma (Hep-G2) were used in the SRB in vitro cytotoxicity assay. Heliotropium indicum ethanolic extract exhibited potent anticancer activity against MDA-MB-231 and A-549. Additional research is required to identify, isolate, and characterise the bioactive chemicals responsible for anticancer action and the mechanism(s) by which they work[2,31].

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Dubey, A., Yadav, P., Peeyush,,, Verma, P. and Kumar, R. 2022. Investigation of Proapoptotic Potential of Ipomoea carnea Leaf Extract on Breast Cancer Cell Line. Journal of Drug Delivery and Therapeutics. 12, 1, Pages-51-55. DOI:https://doi.org/10.22270/jddt.v12i1.5172
2. Dubey, Anubhav, Niladry Sekhar Ghosh, Nidhee Agnihotri and Amit Kumar et al.2022. Herbs Derived Bioactive Compounds and their Potential for the Treatment of Neurological Disorders. Clin Schizophr Relat Psychoses 16, Pages-1-11. Doi: 10.3371/CSRP.DANG.081922.
3. S.U. Park, "Anticancer compounds from plants," EXCLI Journal, vol. 11, pp. 386–389, 2012.
4. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. 2011.Ward, and D. Forman, "Global cancer statistics," CA Cancer Journal for Clinicians. 61, 2, Pages- 69–90.
5. G. Schwartsmann, M. J. Ratain, G. M. Cragg et al. 2002.Anticancer drug discovery and development throughout the world," Journal of Clinical Oncology. 20, 18, Pages-47s–59s.
6. G. M. Cragg and D. J. Newman, 2001. Medicinals for the millennia: the historical record," Annals of the New York Academy of Sciences, 953, Pages- 3–25.
7. D. J. Newman, G. M. Cragg, and K. M. Snader, 2000.The influence of natural products upon drug discovery," Natural Product Reports, vol. 17. 3, Pages-219–220.
8. V.P. Shilpa, T. B. Samuel, E.R. Roshni, et.al.2020. Green synthesis Zinc Oxide nanoparticle using Allamanda cathartica leaf extract and their cytotoxic and antibacterial activity. Nanomed Res J. 5(3), Pages- 298-305.
9. M. Majumder, M. Sharma, S. et.al . 2021. Extract Induces Apoptosis and Suppresses Migration of Breast Cancer Cells. Nutrition and cancer. 73(12), Pages- 2477-2490. 2021.
10. N. S. Sakle, D. Lokwani, S. N. Mokale.2019. Caesalpinia pulcherrima Arrests Cell Cycle and Triggers Reactive Oxygen Species-Induced Mitochondrial-Mediated apoptosis and Necroptosis via Modulating Estrogen and Estrogen Receptors. Pharmacognosy Magazine, 15: S2 Pages- 88-97.
11. Ifandari, S. Widyarini, L. H. Nugroho, R. Pratiwi. 2020.Phytochemical analysis and cytotoxic activities of two distinct cultivars of Ganyong rhizomes (Canna indica) against the WiDr colon cancer cell line. Biodiversitas, 21(4), Pages- 1660-1669.
12. T. Juncker, M. Schumacher, M. Dicato, M. Diederich. 2009. UNBS1450 from Calotropis procera as a regulator of signaling pathways involved in proliferation and cell death. Biochemical Pharmacology, 78 (2009), Pages- 1–10.
13. E. E. Abel, P. R. J. Poonga, S. G. Panicker.2016. Characterization and in vitro studies on anticancer, antioxidant activity against colon cancer cell line of gold nanoparticles capped with Cassia tora SM leaf extract. Appl Nanosci, 6(1), Pages- 121-129.
14. K. N. Rashed, A. Ciri, J. Glamoclija, R. C. Calhelha, et.al. 2018. Identification of the Bioactive Constituents and the Antibacterial, Antifungal and Cytotoxic Activities of Different Fractions from Cestrum nocturnum L. Jordan Journal of Biological Sciences, 11(3), Pages- 273-279.
15. P. Chakraborty, P. Deb, S. Chakraborty, B. Chatterjee, J. Abraham.2015. Cytotoxicity and antimicrobial activity of Colocasia esculenta. Journal of Chemical and Pharmaceutical Research, 7(12), Pages- 627-635.
16. S, Muthukrishnan, B. Vellingiri, G. Murugesan.2018. Anticancer effects of silver nanoparticles encapsulated by Gloriosa superba (L.) leaf extracts in DLA tumor cells. Future Journal of Pharmaceutical Sciences, 4(2), Pages-206-214.
17. P. Budchart, A. Khamwut, C. Sinthuvanich, S. et.al.2017. Partially Purified Gloriosa superba PeptidesInhibitColonCancerCell Viability byInducingApoptosis Through p53 Upregulation. Southern Society for Clinical Investigation, 354(4), Pages- 423-429.
18. P. Balachandran R. Govindarajan. 2005.Cancer – an Ayurvedic perspective Pharmacological Res, 51(1), Pages- 19 – 30.

19. X. Yang, D. K. Summerhurst, S. F. Koval, C. et.al. 2001. Isolation of an antimicrobial compound from Impatiens balsamina L. using bioassay-guided fractionation. Phytother Res., 15, Pages- 676-680.
20. T. Chakrabarty, N. P. Balakrishnan.1992. A Pages- revision of Croton L. (Euphorbiaceae) for Indian subcontinent. Bulletin of the Botanical Survey of India 34(1-4) Pages-1-88.
21. A.F.M. Rizk. 1987.The chemical constituents and economic plants of the Euphorbiaceae. Botanical Journal of the Linnean Society 94. Pages-293-326.
22. M. A. Rahman, M. Mia, and I. Shahid,2011. Pharmacological and phytochemical screen activities of roots of Heliotropium indicum Linn. PharmacologyOnLine, vol. 1, (1), Pages- 185–192.
23. A. Togola, D. Diallo, S. Demb´el´e, H. Barsett, et. al. 2005.Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. Journal of Ethnobiology and Ethnomedicine. 1, (7), Pages-7-11.
24. A. A. Adelaja, M. D. Ayoola, J. O. Otulana, O. B. et. al. 2008. Evaluation of the histo -gastroprotective and antimicrobial activities of Heliotropium indicum Linn (boraginaceae). Malaysian Journal of Medical Sciences. 15. (3), Pages- 22–30.
25. K. Srinivas, M. E. B. Rao, and S. Rao, 2000.Anti-inflammatory activity of Heliotropium indicum Linn and Leucas aspera Spreng. in albino rats," Indian Journal of Pharmacology.32. (1), Pages- 37-38.
26. J. S. Reddy, P. R. Rao, and M. S. Reddy, 2002.Wound healing effects of Heliotropium indicum, Plumbago zeylanicum and Acalypha indica in rats," Journal of Ethnopharmacology.79. (2), Pages- 249–251.
27. M. Kugelman, W. C. Liu, M. Axelrod, T. J. McBride, et.al.2015. Indicine-N-oxide: the antitumor principle of Heliotropium indicum," Lloydia, 39. (2-3), Pages-125–128.
28. V. T. Veda, K. S. Sasi, B. R. Asokan, S. et.al. 2016.Anticataract activity of ethanolic extract of Heliotropium indicum leaves on galactose induced cataract in rats.International Journal of Pharmacology & Toxicology, 5, Pages-18–20.
29. World Health Organization, (WHO), 1998.Organization Mondiale De La Sante, Quality control methods for plant materials, 559, Rev. 1, Original English, Pages- 08-67.
30. Anubhav Dubey, Raghuvendra Singh, Ashish Kumar, Gaurav Mishra, Anubha Gupta, Anuj Sonker, & Amit Mishra. (2022). A Critical Review on Changing Epidemiology of Human Monkeypox-A Current Threat with Multi-Country Outbreak. Journal of Pharmaceutical Negative Results, 660–671. Retrieved from https://www.pnrjournal.com/index.php/home/article/view/738.
31. Dubey A, Ghosh NS, Singh R. Zebrafish as An Emerging Model: An Important Testing Platform for Biomedical Science. J Pharm Negative Results 2022;13(3): 1-7.DOI:10.47750/pnr.2022.13.03.001.