International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://theaspd.com/index.php

IN VITRO ANTICANCER POTENTIAL OF WHEATGRASS (TRITICUM AESTIVUM) EXTRACTS

Satya Prakash Maurya[1], Shaily Choudhary[1], Naveen Gupta[1], Dharmendra Singh Rajput[1]
[1]Department of Pharmacy, Madhyanchal Professional University, Bhopal, M. P.
Address for correspondence
Satya Prakash Maurya
Research Scholar
Department of Pharmacy,
Madhyanchal Professional University Bhopal M.P.
satyaz6050@gmail.com

Abstract
Wheatgrass have multitude effects in thalassemia, hemolytic anemia, asthma, allergy, inflammatory bowel disease and detoxification. Wheatgrass seems to be very promising herbal drug and extensive research work is needed in order to explore its therapeutic application in various diseases. An attempt was made to evaluate the anti-proliferative potentials of the Wheatgrass extracts on human breast cancer (MCF-7) cell lines, human colorectal cancer (HT-29) cell lines and human cervical cancer (HeLa) cell lines by MTT assay. Ethanol extract of wheatgrass were found more effective in in vitro anticancer activity in MTTT assay.
Keywords: *MTT assay, anticancer activity, wheatgrass, triticum aestivum*

INTRODUCTION

Cancer is one of the most important causes of death worldwide and is continuously stimulating the search for new bioactive molecules from natural sources. There is an urgent need of new anticancer drugs because tumor cells are developing resistance against currently available drugs, like vinca alkaloids and taxanes and some anticancer drugs have side effects that can threaten life because they do not discriminate normal and tumoral cells [1-3].

The term cancer can be described as a set of complex processes involving impaired cells death, unlimited cell proliferation and temporal–spatial changes in cell physiology that often leads to malignant tumor formation resulting in invasion of distant tissues to form metastasis. Multistage carcinogenesis is a widely accepted hypothesis in the development of cancers and is operationally divided into three stages, namely, initiation, promotion and progression [4].

Cancer rising incidence worldwide combined with improved survival rates especially in high-income countries as a result of advances in treatment and early detection in the past decades, the number of people living with or after cancer is growing. Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in the 21st century. The situation is so alarming that every fourth person is having a lifetime risk of cancer. India registers more than 11 lakh new cases of cancer every year, whereas, this figure is above 14 million worldwide. All cancers are curable if they are caught early enough. The fundamental abnormality resulting in the development of cancer is the continual unregulated proliferation of cancer cells. Rather than responding appropriately to the signals that control normal cell behavior, cancer cells grow and divide in an uncontrolled manner, invading normal tissues and organs and eventually spreading throughout the body. The generalized loss of growth control exhibited by cancer cells is the net result of accumulated abnormalities in multiple cell regulatory systems and is reflected in several aspects of cell behavior that distinguish cancer cells from their normal counterparts [5-6].

Carcinogenesis may result from extensive DNA damage, often caused by exposure to a variety of exogenous and endogenous agents including ultraviolet radiation, ionizing radiations, mutagenic chemicals, environmental agents, therapeutic agents or diagnostic imaging. DNA damageas a term encapsulates both frank single and double-stranded DNA breaks, as well as stable modifications to nitrogen bases in DNA or its sugar-phosphate backbone, caused by external (e.g., IR) or internal sources [e.g., reactive oxygen species generated during oxidative metabolism], which impact the cell by disrupting gene function and/or impairing transcription, DNA replication and cell proliferation. Maintaining genomic integrity is therefore crucial for the organism since it is a key feature in the maintenance of cell

function and inappropriate DNA repair is associated with both the initiation and progression of cancer [7,-8].

Recent studies have investigated associations between dietary factors and survival or prognosis of various cancers, and findings indicated beneficial outcomes for higher intake of plant-based diets and components in cancer survivors. In this context, it is important to consider several aspects, including the timing of dietary assessment (e.g. assessment after cancer diagnosis or postdiagnostic diet), different cancer sites (e.g. breast, colorectal, prostate cancer) and specific prognostic outcomes, such as cancer-specific mortality, recurrence or overall survival [9-10].

Wheatgrass refers to the young grass of the common wheat plant, Triticum aestivum Linn., family Poeaceae (Graminae), which is freshly juiced or dried into powder for animal and human onsumption – both the forms provide chlorophyll, amino acids, minerals, vitamins, and enzymes. It is also known as "living food" and is a superior source of chlorophyll – appropriately referred to as the "green blood" .Various chlorophyll-rich greens are being used from variable sources, since prehistoric times, as blood builders. Thus, wheatgrass, containing about 70% chlorophyll, has been proclaimed to improve blood flow, aid in digestion and in general detoxification of the body [11-14].

In the present study attempts have been made to evaluate the anticancer activity of Wheatgrass (Triticum aestivum) extract using MTT assay in vitro model . An attempt was made to evaluate the anti proliferative potentials of the Wheatgrass extracts on human breast cancer (MCF-7) cell lines, human colorectal cancer (HT-29) cell lines and human cervical cancer (HeLa) cell lines by MTT assay.

MATERIALS AND METHODS

Human estrogen-receptor positive breast cancer human breast cancer (MCF-7) cell lines are collected from the American Type Culture Collection (ATCC), USA., Human colorectal cancer (HT-29) cell lines, Human cervical cancer (HeLa) cell lines were collected from National Centre for Cell Science (NCCS), Pune, India. MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and Dulbecco's Modified Eagle's Medium (DMEM), were obtained from Sigma Chemical Co., St.Louis, MO. Fetal bovine serum (FBS) from Arrow labs, penicillin-G, sodium streptomycin sulphate, phosphate buffered saline (PBS), trypsin-EDTA from Highveld Biological, L- glutamine from Cambrex Bioproducts. Trypan blue from Sigma-Aldrich Co., St.Louis, Mo.96 well flat bottom tissue culture plates- Tarson, Centrifuge Sigma 2-16KC, Refrigerated -Sartorius AG, Germany. UV-visible spectrophotometer, (Shimadz).

Preparation of wheatgrass extracts: Wheat grasses were collected from fully grown plants from the near places of Bhopal, India. It was washed thoroughly to remove any soil and dirt. It was authenticated by Botanist. Fresh grass was used for Pharmacognostical studies. Wheat grasses were dried under shade separately. Wheat grasses were powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies. The Leaves were initially separated from the main plants body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles. The macroscopic description of wheat grasses includes size, shape, nature of outer and inner surfaces, and organoleptic characters like color, odour, taste etc. were studied.

The powdered materials were successively extracted with three different solvents viz., hexane, ethyl acetate and methanol by soxhlet extraction method. The powdered plant material is placed in a Soxhlet apparatus, which is fitted on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to flask and the setup is heated under reflux below the boiling point of the solvent. The crude extracts were further evaporated in a rotary evaporator. The powdered material was then subjected to successive soxhlet extraction with hexane, ethyl acetate and ethanol. The solvents thus obtained were separately concentrated under vacuum at temperature of 55^{o}c by using rotavapor. The concentrated extracts were collected and stored in desiccators for further use.

Preparation of plant samples

Stock solutions of plant extracts (HEWG- Hexane extract of wheat grass; EAWG: Ethyl acetate extracts wheat grass; EOWG: Ethanol extract of wheat grass) in methanol at a concentration of 50 mg/ml were prepared. The following final well concentrations were prepared using experimental medium. 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml. The following controls were prepared. (i) Methanol in experimental media as a negative control, (ii) plant extract with experimental media and no cells.

HEWG: Hexane extract of wheat grass

EAWG: Ethyl acetate extracts wheat grass

EOWG: Ethanol extract of wheat grass

5.3.1. MTT assay

This colorimetric assay indirectly estimates the viable cell number by staining total cellular proteins. The ability of extracts of selected plants to inhibit the in vitro growth of three human cancer cell lines was evaluated. By colorimetric assay using MTT reagent. MTT (3-[4,5- dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a water soluble yellow colored tetrazolium salt, which is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by succinate dehydrogenase present within the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in healthy cells. Since reduction of MTT can only occur in metabolically active cells, the intensity of purple color is a measure of viability of the cells. The intensity can be measured by spectrophotometer after dissolving formazan crystals in DMSO.

Cell lines and cell culture

The MCF-7, HT-29 and HeLa cell lines were obtained from the National Cancer Institute (USA). All cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% FBS, 1 ml per 500 ml media of 10 mg/ml penicillin-G, 10 mg/ml sodium streptomycin sulphate and 5 ml per 500 ml media of 2 mM L-glutamine. The cells were incubated at 37°C in a humidified atmosphere of 5% CO2.

Once the cells were at 75% confluency, they were subcultured by aspirating the media and replacing it with 0.1 mM (500 µl) phosphate buffered saline (PBS) (pH 7.4) and 1 ml 0.05% (2 mg/ml) trypsin-EDTA. The flasks were incubated at 37°C for 10 - 15 min, until the majority of cells had lifted. The trypsin was then inactivated by the addition of experimental media (antibiotic- free, serum supplemented DMEM medium).

A single cell suspension was formed by the gentle pipetting action. The cell suspension was centrifuged at 1000 rpm for 3 min and the supernatant discarded. The pellet was resuspended in its respective medium, an aliquot of which was stained with 0.2% (w/v) trypan blue and the cells were counted using a haemocyto meter. This single cell suspension, with a cell viability of greater than 95%, was then diluted in culture medium to obtain a standard cell suspension of 150 000 cells/ml.

Procedure for MTT Assay

Aliquots of 100µL of 150000 cells were seeded into a 96-well microtiter plate. The plates were incubated at 37°C for 24 hours to facilitate the attachment of the cells to the bottom of the wells. Plant extract or compound to be tested was added at different concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml with serial two-fold dilutions of 122 cell culture medium (100µL) to the wells already containing 100µL of cell suspension, in triplicate.

The plates were incubated for a further 48 hours at 37°C. On completion of the 48 hour incubation period, 10µL of MTT reagent was added to each well. The plates were then incubated at 4°C for 1 hour, after which the supernatant was washed from the wells (washed out 5 times) with PBS to remove any excess amounts of MTT reagent, experimental media or any other low molecular weight metabolites. The plates were inverted and left overnight to air dry. Once dried, 100µL of DMSO was added to all wells to stain the fixed cells and aid in assessing the cell growth and the plate was shaken at 960 rpm for 3 min on a micro titer plate reader, dissolving bound dye present in the wells. The absorbance was then read at 570nm. The colour intensity of each well corresponds to the number of viable cells, an indication of the inhibitory effect of the extracts or test compounds added. The percentage growth inhibitions (absorbance of control cells- absorbance of treated cells)/ absorbance of control cells] x100} were calculated and plotted against the concentrations.

RESULT AND DISCUSSION

An attempt was made to evaluate the ant- proliferative potentials of the Wheatgrass extracts on human breast cancer (MCF-7) cell lines, human colorectal cancer (HT-29) cell lines and human cervical cancer (HeLa) cell lines by MTT assay. The results are expressed as percentage cell growth inhibition at 100 µg/ml, while those indicating a percentage inhibition of greater than 80% at 100 µg/ml were expressed as IC50 values, later defined as the concentration causing 50% cell growth inhibition. After a 48 hour continuous exposure of the cells to the extracts, in accordance with the "NCI three-cell line screen", the proliferative activity observed was most certainly reflective of a cancer type-specific sensitivity.

IC50 values for those plant extracts that showed more than 80% inhibition of cell viability at 100µg/ml concentration was calculated for all cell lines tested. The hexane extracts at 100 µg/ml showed percentage growth inhibitions of less than 80%, failing the prescreen-select criterion and were thus not subjected to

further testing. Hence the IC50 values for hexane extracts were not determined. The ethanolic and ethyl acetate extracts of selected species induced an inhibitory effect greater than 80% at concentration of 100 µg/ml on the cancer cells. The inhibitory activity of the extracts showed dose-dependant activity, which increased with an increase in concentration. The IC50 values of those tested extracts which showed inhibitory effect greater than 80% at 100 µg/ml concentration were calculated and presented.

According to the American National Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extracts is an IC50 < 30 µg/ml. The present study indicate that ethyl acetate extract and ethanolic extracts of Wheatgrass evinced strong antiproliferative activity on MCF-7 cell line.

Ethyl acetate extract exerted maximum activity against HT-29 cell line while ethanolic extracts were found to be more potent against HT-29 cell line. Hexane extracts have not shown any potent activity on HT-29 cell line. Ethyl acetate extracts of wheat grass, evinced strong anti proliferative activity on HeLa cell line. Hexane extracts evinced any anti proliferative activity on HeLa cell line.

Table 1: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer MCF-7 cell line with Wheatgrass extracts

Treatment	% CGI	IC50(µg)
Hexane extract	65.8 ± 1.27	**
Ethyl acetate extract	80.8 ± 1.27	25.01 ± 0.43
Ethanol extract	94.8 ± 1.27	20.01 ± 0.43

MCF-7 human breast cancer cell lines ** no mortility

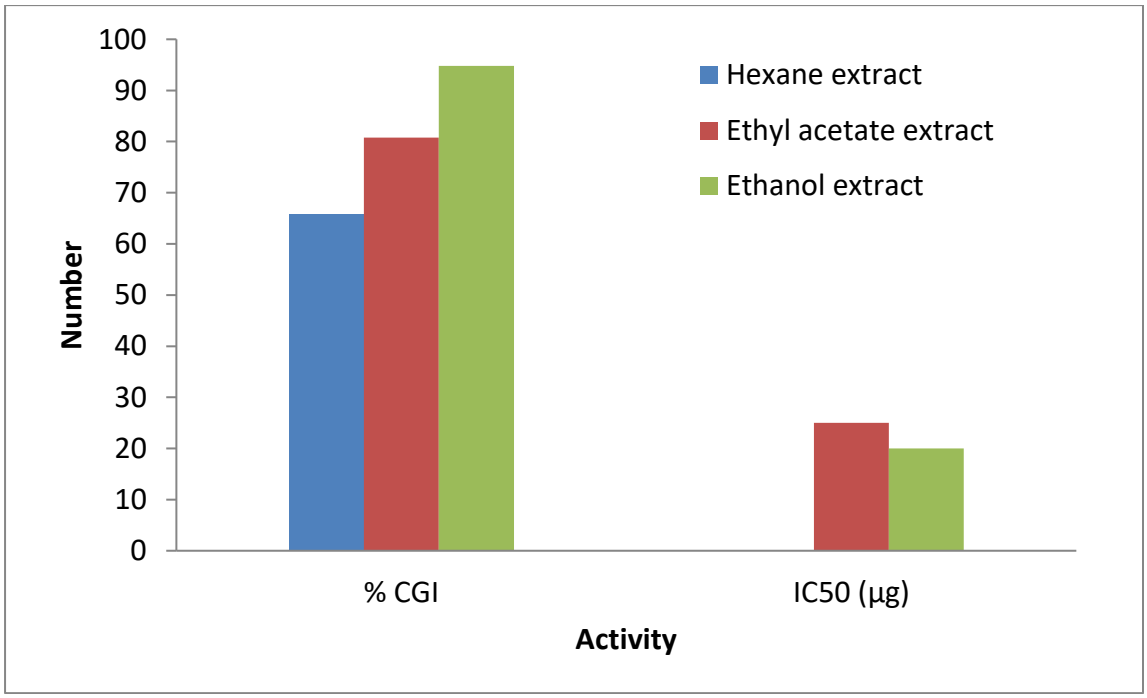

Figure 1: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer MCF-7 cell line with Wheatgrass extracts

Table 2: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer HT-29 cell line with Wheatgrass extracts

Treatment	% CGI	IC50(µg)
Hexane extract	11.8 ± 1.27	**
Ethyl acetate extract	78.8 ± 1.27	41.01 ± 0.43
Ethanol extract	91.8 ± 1.27	29.01 ± 0.43

HT-29 human colorectal cancer cell lines ** no mortility

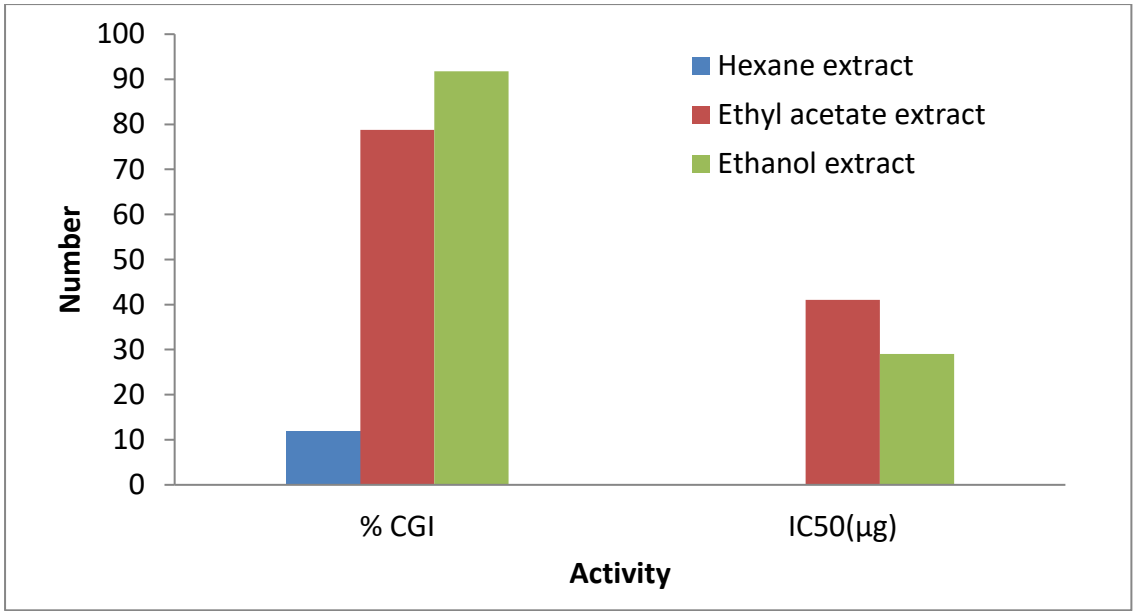

Figure 2: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer HT-29 cell line with Wheatgrass extracts

Table 3: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer HeLa cell line with Wheatgrass extracts

Treatment	% CGI	IC50(µg)
Hexane extract	17.9 ± 1.27	**
Ethyl acetate extract	81.8 ± 1.27	31.01 ± 0.43
Ethanol extract	87.8 ± 1.27	22.01 ± 0.43

HT-29 human cervical cancer cell lines ** no mortility

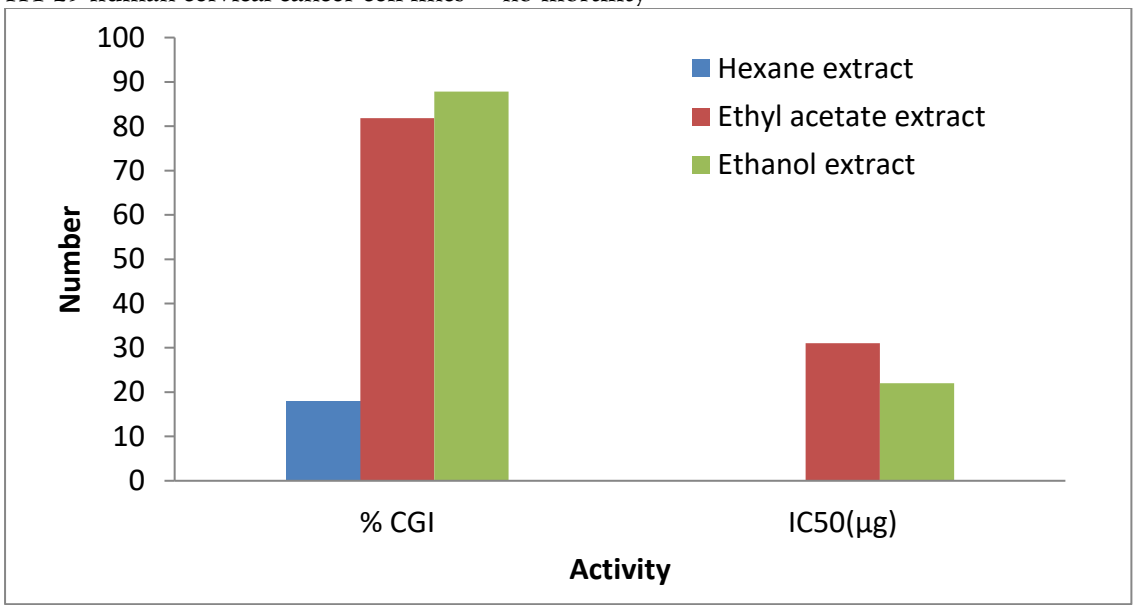

Figure 3: Percentage cell growth inhibition (CGI) and the IC50 values of human cancer HeLa cell line with Wheatgrass extracts

A number of natural products are used as chemo protective agents against commonly occurring cancers. The phytochemicals, which act against cancer are curcumin, genistein, resveratrol, diallyl sulfide, (S)-allyl cystein, allicin, lycopene, ellagic acid, ursolic acid, catechins, eugenol, isoeugenol, isoflavones, protease inhibitors, saponins, phytosterols, vitamin C, lutein, folic acid, beta carotene, vitamin E and flavonoids, to name but a few. Flavonoids are a group of polyphenolic secondary metabolites present in a wide variety of plants and display a large number of biochemical and pharmacological properties, including cancer preventative effects. A number of mechanisms by which flavonoids are able to prevent carcinogenesis have been reported. These mechanisms include their free radical scavenging ability, the modification of

enzymes to activate or detoxify carcinogens and the inhibition of the induction of the transcription factor activator protein activity by tumour promoters.

Anti proliferative potentials of the wheatgrass extract in the present study revealed some promising results. As evidenced from the data ethanol extract was shown to induce strong anticancer activity against all the three tested cell lines. Recent studies indicate that, apart from the reported phytoconstituents, Phenolic compounds, found in plants, play a significant role in cancer prevention and treatment by influencing various cellular processes. They can modulate signaling pathways involved in cell survival, proliferation, and apoptosis, as well as exhibit antioxidant, anti-inflammatory, and anti-angiogenic properties. Phenolic compounds can induce cell cycle arrest, preventing cancer cells from dividing and multiplying. They can trigger programmed cell death (apoptosis) in cancer cells, eliminating them from the body. Phenolic compounds can inhibit the growth and spread of cancer cells by targeting various pathways involved in these processes, such as angiogenesis (formation of new blood vessels to feed tumors). They can neutralize harmful free radicals and reduce inflammation, both of which can contribute to cancer development and progression. The presence of these phytoconstituents might have contributed for the ant proliferative activity of the extracts on the three cell lines tested in the present study.

CONCLUSION

The benefits of natural compounds as reduced adverse effects and the capacity to impact on multiple signaling pathways involved in the carcinogenesis process could be considered an explanation for the fact that from 240 antitumor drugs approved in the last 40 years, only 29 are strictly synthetic drugs. Moreover, in the past 10 years, synthetic compounds with pharmacophore of natural origin that mimic the natural product effect were approved as antitumor drugs. Phytochemicals have been in the spotlight of cancer research since its early beginnings, as they were among the first antineoplastic drugs discovered. The current study represents showing the anticancer activity of wheatgrass extract. An attempt was made to evaluate the ant- proliferative potentials of the Wheatgrass extracts on human breast cancer (MCF-7) cell lines, human colorectal cancer (HT-29) cell lines and human cervical cancer (HeLa) cell lines by MTT assay. Anti proliferative potentials of the wheatgrass extract in the present study revealed some promising results. As evidenced from the data ethanol extract was shown to induce strong anticancer activity against all the three tested cell lines. This study demonstrated that ethanol extract of wheatgrass has significant anticancer potential.

REFERENCES

1. Shen GP, Xu FH, He F, Ruan HL, Cui C, Chen LZ, et al. Pretreatment lifestyle behaviors as survival predictors for patients with nasopharyngeal carcinoma. PLoS ONE. 2012;7(5):e36515
2. Demark-Wahnefried W, Aziz NM, Rowland JH, Pinto BM. Riding the crest of the teachable moment: promoting long-term health after the diagnosis of cancer. J Clin Oncol. 2005;23(24):5814–5830.
3. Fassier P, Zelek L, Lécuyer L, Bachmann P, Touillaud M, Druesne-Pecollo N, et al. Modifications in dietary and alcohol intakes between before and after cancer diagnosis: results from the prospective population-based NutriNet-Santé cohort. Int J Cancer. 2017;141(3):457–470.
4. Tollosa DN, Holliday E, Hure A, Tavener M, James EL. A 15-year follow-up study on long-term adherence to health behaviour recommendations in women diagnosed with breast cancer. Breast Cancer Res Treat. 2020;182(3):727–738. doi: 10.1007/s10549-020-05704-4.
5. Tollosa DN, Tavener M, Hure A, James EL. Adherence to multiple health behaviours in cancer survivors: a systematic review and meta-analysis. Journal of cancer survivorship: research and practice. 2019;13(3):327–343.
6. Schwedhelm C, Boeing H, Hoffmann G, Aleksandrova K, Schwingshackl L. Effect of diet on mortality and cancer recurrence among cancer survivors: a systematic review and meta-analysis of cohort studies. Nutr Rev. 2016;74(12):737–748.
7. Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G. Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis. Nutrients. 2017;9(10).
8. Fadelu T, Zhang S, Niedzwiecki D, Ye X, Saltz LB, Mayer RJ, et al. Nut consumption and survival in patients with stage III colon cancer: results from CALGB 89803 (Alliance) J Clin Oncol. 2018;36(11):1112–1120.
9. Jiang R, Poschet G, Owen R, Celik M, Jansen L, Hell R, et al. Serum concentration of genistein, luteolin and colorectal cancer prognosis. Nutrients. 2019;11(3).
10. Lochhead P, Nishihara R, Qian ZR, Mima K, Cao Y, Sukawa Y, et al. Postdiagnostic intake of one-carbon nutrients and alcohol in relation to colorectal cancer survival. Am J Clin Nutr. 2015;102(5):1134–1141.
11. Reynolds C, A DNA technology-based cellular assay used to measure specific biological activity in wheatgrass extract, Journal of Australasian Integrative Medicine Association., 2005:1-3.
12. Fernandes CJ, O'Donovan DJ, Natural antioxidant therapy for patients with haemolytic anemia, Indian Pediatrics, 2005; 42: 618-619.
13. Marwaha RK, Bansal D, Kaur S, Trehan A. Wheat grass juice reduces transfusion requirement in patients with thalassemia major: A pilot study. Indian Pediatrics, 2004; 41: 716-720.

14. Braca A, Fico G, Morelli I, De Simone F, Tomè F, De Tommasi N. Antioxidant and free radical scavenging activity of flavonol glycosides from different Aconitum species. J Ethnopharmacol. 2003 May;86(1):63-7. doi: 10.1016/s0378-8741(03)00043-6. PMID: 12686443.