



Antioxidant Profiling of *Triticum aestivum* (wheatgrass) and its Antiproliferative Activity In MCF-7 Breast Cancer Cell Line

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ABSTRACT

The present study was undertaken to put forward the scientific evidences of wheatgrass as an alternative therapy to treat cancer. The aim was to investigate the antiproliferative effects of wheatgrass extract on the selected model of breast cancer cells i.e. MCF-7 cell line and effect of extraction medium and sample preparation on total antioxidant capacity (TAC) of wheatgrass plant. Antioxidant properties of different wheatgrass extracts were studied by estimating Total Phenol Content (TPC), DPPH ((1, 1-diphenyl-2-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) Assay. Significant antiproliferative effect and cell death was observed in a dose dependent manner of wheatgrass extract on MCF-7 cells. The results indicates that TAC of the samples processed with one step procedure is more substantial as compared to two step procedure and three step procedure. The best results were obtained when the one -step procedure for sample preparation and 70 % ethanol (ethanolic) as an extracting agent were applied. Thus, this study provides preliminary data about the anticancer activity of wheatgrass extract and its potential to be considered as an anticancer agent.

Key words: *Triticum aestivum*, TPC, FRAP, DPPH, MTT, MCF-7.

INTRODUCTION

Variables like solvent, extraction method and extraction time, all influence the amount of antioxidants which can be extracted. Recently, a new three step method was proposed for sample preparation based on discriminating water-soluble, water-insoluble and protein antioxidants within same plant; the same protocol is followed in our work for extraction of different extracts from wheatgrass¹.

Antioxidants are chemical compounds that can bind to free oxygen radicals thus preventing these radicals from damaging healthy cells which could lead to cancer. The young grass of the common wheat plant, *Triticum aestivum* is known as wheatgrass, family Poaceae. Wheatgrass is known to be a rich source of vitamins, antioxidants and minerals. It also contains Vitamin A, B1, C and E, many minerals and trace elements including calcium, iodine, selenium and zinc. Wheatgrass is known to contain antioxidant enzymes superoxide dismutase (SOD) and cytochrome oxidase that have the potential to convert reactive oxygen species (ROS) to a hydrogen peroxide and an oxygen molecule². Chlorophyll, one of the primary components in the wheatgrass extract, was found to augment blood formation and strengthen the immune system through inhibition of metabolic activation of carcinogens^{3,4}. It also possesses the ability to inhibit oxidative DNA damage⁵. Few clinical trials have been accomplished that have shown on consumption of wheatgrass juice, the number of transfusions in patients with thalassemia major is decreased⁶. Reduction in the overall disease activity index and the severity of rectal bleeding in cases of distal ulcerative colitis on consumption of wheatgrass juice has also been observed⁷. Some preliminary studies have suggested that the observed beneficial results could be because of the antioxidant activity, but a detailed study has not been done⁸.

In the present study, we investigated the free radical scavenging activity and antiproliferative activity of various extracts of *Triticum aestivum* by estimating Total Phenolic Content, DPPH and FRAP, followed by cell proliferation assay using MTT assay on breast cancer cell line MCF-7.

MATERIALS AND METHODS

Materials

Plant material

Seeds of *Triticum aestivum* (strain PBW343) were obtained from HAU (Chaudhary Charan Singh University, Hisar) for the following study. The seeds were soaked

overnight. Next day, the seeds were germinated in trays containing soil. The trays were monitored daily and watered as per the need. Wheatgrass plant of 6-7 inches was used as an earlier study revealed that maximum antioxidant activity was observed in a wheatgrass plant of the aforesaid length⁹.

Cell Culture

Human Breast cancer cell line- MCF-7 was used as an *in vitro* model for our study and obtained from NCCS, Pune, India. The cells were cultured in DMEM medium supplemented with 1% (v/v) antibiotic combination of Penicillin-Streptomycin and 10% (v/v) FBS, maintained at 37°C in a 5% CO₂ incubator. Cells at exponential stage were used for experimentation.

Preparation of different extracts

Fresh wheatgrass was dried, cut into small pieces and 10% (w/v) wheatgrass extracts were prepared by maceration using pestle and mortar, with different solvents such as water, 70% ethanol, 0.1% TCA, sodium acetate buffer pH 5 and potassium phosphate buffer pH 7.4. The homogenates were centrifuged at 13500 g for 30 minutes at 4°C and the resulting supernatants (one- step, crude fraction) were analyzed for future experiment. In the two-step procedure residues left behind, except that from the ethanol slurry, were re-extracted with acetone under agitation for 30 min at room temperature, centrifuged at 13500 g for 15 min at 4°C and the supernatants were collected for further analysis (acetone fraction). For the three-step procedure, initial supernatants from phosphate and acetate buffers were further processed by adding 60% TCA to a final concentration of 1%. After centrifugation at 8500 g for 15 min at 4°C, obtained supernatants (low-molecular fraction) were evaluated further. The protein residues were resuspended in a detergent solution (6 M Urea, 0.1 M acetate buffer, pH 5) and used in the analyses (protein fraction)¹.

Methods

Phytochemical Screening

The aqueous extract of wheatgrass was subjected to various chemical tests in order to determine the presence of secondary plant constituents.

Test for Reducing Sugars:

To 2 ml of the extract, 5 ml of a mixture (1:1) of Fehling's solution A and Fehling's solution B was added and boiled in a water bath for five minutes. A brick-red precipitate indicated the presence of free reducing sugars¹⁰.

Test for the presence of Anthraquinones:

0.5 g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken; the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones¹¹.

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Test for Saponins:

Aqueous extract was obtained by boiling on the water bath. The extract was transferred into a test tube and shaken vigorously then was left to stand for 10 minutes and the result noted. A thick persistent froth indicates saponins¹⁰.

Test for Flavonoids:

Extract of the sample was reduced to dryness on the boiling water bath. The residue was treated with dil. NaOH, followed by addition of dilute HCl, solubility and colour was noted. A yellow solution with NaOH, which turns Colourless with dil. HCl, confirms flavonoids¹⁰.

Test for Tannins:

0.5 g of the extract was dissolved in 5 ml of water followed by a few drops of 10 % ferric chloride. A blue-black, green, or blue-green precipitate would indicate the presence of tannins¹².

Test for Alkaloids:

0.5 g of extract was stirred with 5ml of 1 % aqueous hydrochloric acid on a water bath, filtrate was second was treated with Dragendorff's reagent. Turbidity or precipitation with either of these reagents would indicate the presence of alkaloids in the extracts¹³.

Test for terpenoids (Salkowski test):

5 ml of extract was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids¹⁴.

Determination of Total Phenolic Content

The phenolic compounds in the various extracts were determined using the Folin-Ciocalteu reagent in a manner proposed by Singleton and Rossi¹⁵, based on the reduction of mixture of phosphate tungsten and molybdenum oxides complex by phenolics to give blue coloured product. To 50 µl of a sample/standard, 3.5 ml water, followed by 250 µl FC (2 N Folin-Ciocalteu) reagent was added. The mixture was mixed and incubated it for 8 minutes at room temperature. To the above mixture 750 µl of 20% sodium carbonate solution was added and incubated for 2 hrs at room temperature. After incubation, the absorbance was taken at 765 nm. The total phenolic content (TPC) was expressed as mg Gallic Acid Equivalents/gram of dry extract.

Evaluation of *in vitro* Antioxidant activity

DPPH radical-scavenging activity

The free radical scavenging activity of wheatgrass extracts based on principle, when DPPH ((1, 1-diphenyl-2-picrylhydrazyl) reacts with phenolic compounds, it is reduced to 1, 1-diphenyl-2-picrylhydrazine due its ability to donate H atom. The change in colour (DPPH) + (H-A) purple → DPPH-H + (A) yellow was measured. The DPPH method is described as a simple, rapid and the most convenient method. It is independent of sample polarity for screening of many samples for free radical scavenging activity¹⁷. A solution of 0.002 % (w/v) DPPH in methanol was prepared and 1.5 ml of this solution was mixed with of different concentrations of the extracts in 1:1 dilution in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm¹⁶. The IC₅₀ value was defined as the concentration (µg/ml) of extracts that scavenges the DPPH radicals by 50%. % scavenging activity was calculated by the

$$\text{following formula} = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100$$

Where A_{cont} = Absorbance of DPPH radical + methanol;
A_{test} = Absorbance of DPPH radical + standard/ sample extract.

Ferric Reducing Antioxidant Power Assay

FRAP assay was followed according to modified method of Benzie and Strain. Fresh FRAP reagent was prepared by mixing the following reagents: 25 ml Sodium acetate.trihydrate buffer (300 mM) pH 3.6, 2.5 ml TPTZ (10mM, in 40 mM HCl), 2.5 ml of FeCl₃.6H₂O (20 mM) and 3 ml H₂O. The temperature of the solution was raised to 37°C before using for further work. Wheatgrass extracts (150 µl) were allowed to react with 2850 µl of FRAP solution for 30 minutes in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) was taken at 593 nm¹⁶.

Evaluation of *in vitro* anti-proliferative activity

MTT Assay:

The 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) assay that differentiates dead from living cells was adapted from the literature¹⁸⁻²¹. Cells were seeded into a 96-well microplate (10⁵cells/well) and incubated for

48 hrs at 37°C to achieve 70-80 % confluency; they were treated with varying concentrations (40-400 µg/ml) of wheatgrass extracts for a further period of 48 hours at 37°C. Following this, 30 µl of MTT reagent was added to each well and was incubated for 4 hrs at 37°C. Isopropanol-HCl (50 µL) was added to each well after the incubation was over to solubilize the formazan product and kept at room temperature for 15-20 min. The absorbance (A) was then read at a wavelength of 570 nm²². The decrease in absorbance in the assay measures the extent of decrease in the number of viable cells following exposure to the test substances calculated by using the following formula:

$$\% \text{ Inhibition of cells} = \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \times 100.$$

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using GraphPad Instat. P values < 0.05 were regarded as significant and P values < 0.01 as very significant.

RESULTS AND DISCUSSION

The following work was carried out to explore the antioxidant and antiproliferative activities of wheatgrass plants by deducing the effect of different extraction methods and sample preparations. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radicals are molecules with incomplete electron shells which make them more chemically stable than those with complete electron shells. On exposure to various exogenous factors like tobacco, smoke, alcohol and UV radiation, leads to free radical formation²³. Significant work has been done showing positive linear correlation between the total content of Phenolic compounds and the antioxidant activities. The methanolic and aqueous extracts of Chinese medicinal plants show a positive correlation between the Total Phenolic Content and antioxidants²⁴. The extraction of polyphenols entirely depends upon the extraction method and solvent system used and in support of this it was reported that use of methanol as an extraction solvent resulted in higher antioxidant activities²⁵. Aqueous ethanol and methanol extracts of red and black currant contained more polyphenols as compared to its crude aqueous extract²⁶. Also it was found methanol was a better solvent in extraction of low molecular weight antioxidants, while aqueous acetone extracts yielded more of high molecular weight antioxidants²⁷. Most commonly used solvent for polyphenols extraction was reported to be ethanol²⁸. Till date, one step procedure and two step procedure were followed by researchers, but a new approach was developed, which used three step procedure based on extraction of water soluble, water insoluble and protein antioxidants from the same sample was done¹. In our study we used three step procedures to check the total antioxidant capacity and antiproliferative activity based on different extraction medium and solvent system. The results of phytochemical screening of the crude aqueous extract revealed that wheatgrass is a rich in secondary metabolites as evidenced by the presence of reducing sugars, saponins, flavonoids, alkaloids, anthraquinones, tannins and terpenoids (Table 1). Work done by other workers also indicated the presence of saponins, flavonoids, tannins, anthraquinones, reducing sugar and triperenoids²⁹.

Table 1: Qualitative phytochemical screening of aqueous wheatgrass extract

| Compound | Observation | Results |
|----------------|---|---------|
| Reducing sugar | Blue colour of Fehling turns to brick red | +ve |
| Saponins | Appearance of frothing | +ve |
| Flavonoids | Appearance of yellow colour | +ve |
| Alkaloids | Appearance of turbidity | +ve |
| Terpenoids | Reddish green colour | +ve |
| Anthraquinones | Pink colour | +ve |
| Tannins | Bluish green | +ve |

Total Phenolic Content

Previous studies revealed that most commonly used synthetic antioxidant Propylgallate (PG), butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) were toxic to liver and also carcinogenic³⁰⁻³¹, so research has been directed towards more of naturals than that of synthetic antioxidants. Plants are rich in natural antioxidants³²⁻³³. Among these, the Phenolic compounds have ability to scavenge free radicals, super oxide and hydroxyl radicals through oxidation reactions. The estimation of TPC of wheatgrass extracts was carried using straight line equation (y = 0.0132x - 0.0093, r² = 0.999). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of dry weight of extract. The results obtained in Table.2 indicated that ethanolic extract has highest TPC content in comparison to other wheatgrass extracts. Similar work was done by which showed ethanolic extract showed highest TPC content as compared to aqueous extract⁹.

Table 2: Total Phenolic Content of different extracts of wheat grass

| Sample (crude extracts) | TPC (mg GAE/gram of dry extract) |
|-------------------------|----------------------------------|
| Ethanolic | 06.48 ± 0.23 |
| Potassium phosphate | 02.44 ± 0.06*** |
| Sodium acetate | 02.75 ± 0.19*** |
| 0.1% TCA | 02.56 ± 0.10*** |
| Aqueous | 03.08 ± 0.30*** |

Values are Mean ± SEM; n=3), Significant at ***p < 0.001 One way ANOVA was used to compare different extracts of wheatgrass with extract showing highest activity (70 % Ethanolic extract).

FRAP

According to Benzie and Strain³⁴, at low pH- the reduction of ferric tripyridyl triazine (Fe⁺³) TPTZ complex to ferrous form (Fe⁺²) gives intense blue color product, which can be monitored by measuring the change in absorption at 593 nm. The reaction is non-specific, any half reaction that has lower redox potential, under reaction conditions than that of ferric ferrous half reaction will drive the ferrous Fe III to Fe II ion formation. Therefore change in absorbance is directly related to reducing power of the electron donating antioxidants present in the reaction mixture. Table.3 represents the FRAP value of different extracts of wheatgrass. The FRAP value were calculated using the standard curve equation $y = 0.6178x - 0.0053$ and $r^2 = 0.997$. The FRAP value of known antioxidant Ascorbic acid obtained was 12.13 ± 0.98 mM Fe II/gram.

Table3: FRAP value of different extracts of wheatgrass

| Sample (crude extracts) | FRAP Value(mMFe (II)/gram of dry extract) |
|-------------------------|---|
| Ethanolic | 0.61 ± 0.010 |
| Potassium phosphate | 0.10 ± 0.000 *** |
| Sodium acetate | 0.16 ± 0.005 *** |
| 0.1% TCA | 0.14 ± 0.000 *** |
| Aqueous | 0.10 ± 0.002 *** |

Values are Mean ± SEM; n=3), Significant at ***p < 0.001 One way ANOVA was used to compare different extracts of wheatgrass with extract showing highest activity (70 % Ethanolic extract).

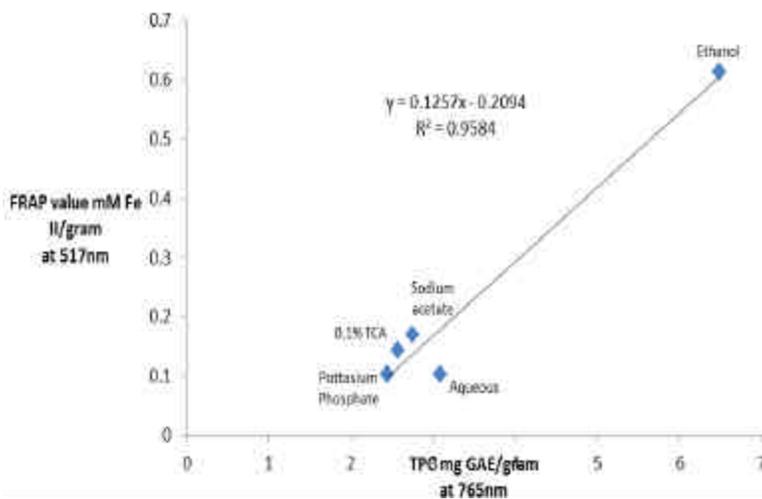


Figure1: Correlation between the TPC and FRAP value of different wheatgrass extracts.

The figure indicates the positive correlation between the total phenolic content and the FRAP value of different extracts of wheatgrass, signifying the relation between the polyphenols and antioxidant activity.

DPPH

The DPPH method is considered as a simple, rapid and the most convenient method. It is independent of sample polarity for screening of many samples for radical scavenging activity¹⁷. The use of methanol as extract solvent restricts the cellular compounds responsible for scavenging the DPPH radical, only the substances soluble in methanol (e.g. carotenoids, fatty acids) are involved in this scavenging process. High reduction of DPPH is related to the high scavenging activity performed by particular sample³⁵. IC₅₀ was calculated as amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The lower the IC₅₀ value, the higher is the free radical scavenging activity of the sample. Table.4 shows that ethanolic

extract has highest IC₅₀ of 140.32 µg/ml as compared to other extracts of wheatgrass, while IC₅₀ of known antioxidant Ascorbic acid was 6.33 µg/ml; similar work was done using two extracts of wheatgrass (Aqueous and ethanol) according to which ethanolic extract showed significantly higher IC₅₀ value as compared to aqueous extract⁹.

Table 4: IC₅₀ value of free radical scavenging activity of different extracts of wheat grass.

| Sample(crude extracts) | IC ₅₀ (µg/ml) |
|------------------------|--------------------------|
| Ethanolic | 177.701 |
| Potassium phosphate | 1076.68*** |
| Sodium acetate liquid | 764.316* |
| 0.1% TCA liquid | 993.411** |
| Aqueous | 646.056 * |

Values are Mean ± SEM; n=3), Significant at *p < 0.05 One way ANOVA was used to compare different extracts of wheatgrass with extract showing highest activity (70 % Ethanolic extract). [*p – least significant, **p – significant, ***p – extremely significant]

Antiradical activity assay is based on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolorization is stoichiometric with respect to the number of electrons captured. The anti-radical activity of known antioxidant Ascorbic acid was found to be 0.168±0.003 Table.5, shows that ethanolic extract has highest anti-radical activity as compared to other extracts.

Table 5: Anti- Radical activity of different wheatgrass extracts.

| Sample(crude extracts) | Anti-radical activity (1/IC ₅₀) |
|------------------------|---|
| Ethanolic extract | 0.0055 ± 0.00 |
| Aqueous extract | 0.0013 ± 8.81*** |
| 0.1%TCA | 0.00098 ± 1.15*** |
| Potassium phosphate | 0.0009 ± 0.00*** |
| Sodium acetate | 0.0012 ± 3.33*** |

Sodium acetate 0.0012 ± 3.33***, Values are Mean ± SEM; n=3), Significant at ***p < 0.001 One way ANOVA was used to compare different extracts of wheatgrass with extract showing highest activity (70 % Ethanolic extract). [*p – least significant, **p – significant, ***p – extremely significant].

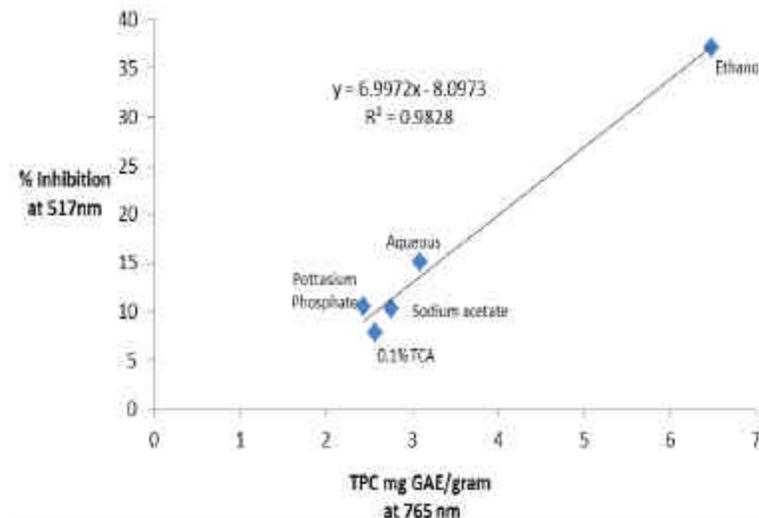


Figure 2: Correlation between TPC and % (percent) inhibition of DPPH by different extracts of wheatgrass.

The figure indicates the significant relation between the free radical scavenging activity and total phenolic content between the different extracts of wheatgrass, indicating the positive correlation between the polyphenols and antioxidants.

MTT assay

The MTT assay was performed to evaluate the antiproliferative effect of the wheatgrass extraction on breast cancer cell line i.e. MCF-7. From the above experiment IC₅₀ of the ethanolic extract was found to be 140.32 µg/ml i.e. 50% of cell death was observed at this particular concentration of ethanolic wheatgrass extract which proved to be highest as compared to other extracts, whereas

the IC₅₀ of Cisplatin which was used as a positive control was found to be 12 µg/ml. Table.6 represents the IC₅₀ of different extracts of wheatgrass.

Table 6: IC₅₀ of different extracts of wheatgrass.

| Sample(crude extracts) | IC ₅₀ (µg/ml) |
|------------------------|--------------------------|
| Ethanollic | 140.32 |
| 0.1 % TCA | 196.05*** |
| Aqueous | 168.46*** |
| Potassium phosphate | 456.87*** |
| Sodium acetate | 408.27*** |

Values are Mean ± SEM; n=3), Significant at ***p < 0.001 One way ANOVA was used to compare different extracts of wheatgrass with extract showing highest activity (70 % Ethanollic extract). [*p – least significant, **p – significant, ***p – extremely significant].

The results obtained from the various assays for total antioxidant activities and cell cytotoxicity studies have indicated that wheatgrass is rich in water soluble antioxidants as compared to water-insoluble and protein soluble antioxidants ,which showed no detectable activities.

CONCLUSION

The work done so far indicates the importance of wheatgrass in terms of antioxidant and antiproliferative properties. From the results obtained in this study, crude ethanollic extract proved to show highest free radical scavenging activity as well as the highest cell killing, which correlates well with the role of antioxidants in reduction of viability of cancer cells. However, further investigations are required to identify the mechanism behind the killing of cancer cells.

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REFERENCES

- Kerchev P and Ivanov S, Influence of extraction techniques and solvents on the antioxidant capacity of plant material, biotechnol. & biotechnol, 2008.
- Padalia S et al., Multitude potential of wheatgrass juice (Green Blood) An Overview, Chronicles of young scientists, 1, 2011, 23-24.
- Lai CN, Chlorophyll: the active factor in wheat sprout extracts inhibiting the metabolic activation of carcinogens *in vitro*, Nutr Cancer, 1, 1979, 19–21.
- Lai CN, Dabney BJ, Shaw CR, Inhibition of *in vitro* metabolic activation of carcinogens by wheat sprout extracts, Nutr Cancer, 1, 1978, 27–30.
- Falconi G, Fedeli D and Tiano I, Antioxidant activity of wheat sprout extracts *in vitro*: Inhibition of DNA oxidative damage, J Food Sci, 67, 2002, 2918-2922.
- Marwaha R et al., Wheat Grass Juice Reduces Transfusion Requirement in Patients with Thalassemia Major: A Pilot Study, Indian Pediatrics, 2004, 41.
- Ben-Arye E et al., Wheatgrass juice in the treatment of active distal ulcerative colitis: a random double-blind placebo-controlled trial, Scand J Gastroenterol, 37, 2002, 444–449.
- Peryt B., Szymczy KT Lesca P, Mechanism of antimutagenicity of wheat sprout extracts, Mutat Res, 269, 1992, 201–205.
- Kulkarni S, Tilak J, Acharya R, Rajurkar N, Devasagayam P.A and Reddy A, Evaluation of the Antioxidant Activity of Wheatgrass (*Triticum aestivum* L.) as a Function of Growth under Different Conditions, Phototherapy Res, 20, 2006, 218–227.
- Onwukaeme D, Ikuegbvweha T and Asonye C, Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of *Pycnanthus Angolensis* Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits Tropical, Journal of Pharmaceutical Research, 6, 2007, 725-730.
- Ngbede J et al., Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North Plateau, Nigeria, Research Journal of Biological Sciences, 3, 2008, 1076-1078.
- Das P and Talukdar A.D, Phytochemical Screening and Bioactivity Evaluation of *Homalonema aromatica* (Roxb.) Schott, Biological and Environmental Sciences, 6, 2010, 71-74.
- Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA, Mboti CI, Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotics resistant bacteria, International Journal of Applied Research in Natural Products, 3, 2010, 6-12.
- Edeoga H.O, Okawa D.O and Mbaebie B.O, Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology, 4, 2005, 685-688.
- Singleton V.L. and Rossi J.A, Colorimetry of total phenolics with phosphomolybdic -phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 1965, 144-158.
- Adedapo et al., Antibacterial and Antioxidant properties of methanol extracts of the leaves and stems of *Calpurnia aurea*, BioMed Central Complementary and Alternative Medicine, 8, 2008, 53.
- Koleva I.I, van Beek T.A, Linssen J.P.H, de Groot A, Evstatieva L.N, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, *Phytochem. Analysis*, 13, 2001, 8–17
- Lee K.R, Kozukue N, Han J.S, Park J.H, Chang E.Y, Baek E.J, Chang J.S, Friedman, Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells, J. Agric. Food Chem, 52, 2004, 2832–2839.
- Friedman M, Lee K.R, Kim H.J, Lee I.S, Kozukue N, Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells, J. Agric. Food Chem, 53, 2005, 6162–6169.
- Friedman M, Mackey B.E, Kim H.J, Lee I.S, Lee K.R, Lee S.U, Kozukue E, Kozukue, N, Structure-activity relationships of tea compounds against human cancer cells, J. Agric. Food Chem, 55, 2007, 243–253.
- Alley M.C, Scudiero D.A, Monks A, Hursey M.L, Czerwinski M.J, Fine D.L, Abbott B.J, Mayo J.G, Shoemaker R.H, Boyd M.R, Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay, Cancer Res, 48, 1988, 4589–601.
- Slambrouck V.S et al., Effects of crude aqueous medicinal plant extracts on growth and invasion of breast cancer cells, Oncology Reports, 17, 2007, 1487-1492.
- K. J. A. Davies, Oxidative stress: the paradox of aerobic life. *Bioch. Soc. Sym.* 61, 1994, 1-34.
- Cai Y, Luo Q, Sun M, Corke H, Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer, *Life Sci*, 74, 2004, 2157- 2184.
- Negi P.S, Chauhan A.S, Sadia G.A, Rohinishree Y.S, Ramteke R.S, Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts, *Food Chem*, 92, 2005, 119-124.
- Lapornik B, Prosek M, Wondra AG, Comparison of extracts prepared from plant by-products using different solvents and extraction time, *J. Food Eng.* 71, 2005, 214-222.
- Metivier R.P, Francis F.J, Clydesdale, F.M, Solvent extraction of anthocyanins from wine Pomace, *J. Food Sci.* 45, 1980, 1099-1100.
- Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y, Extraction of polyphenolics from plant material for functional foods-engineering and technology, *Food Rev. Int*, 21, 2005, 139- 166.
- Kothari S, Jain A.K, Mehta S.A and Tonpay S, Hypolipidemic effect of fresh *Triticum Aestivum* (wheat grass) juice in hypercholesterolemic rats, *Acta Poloniae Pharmaceutica N Drug Research*, 68, 2011, 291-294.
- Grice, H. C. 1986, Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract, *Food Chem. Toxicol*, 24, 1986, 1127-1130.
- Wichi H.P, Enhanced tumor development by butylatedhydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium, *Food Chem. Toxicol*, 26, 1988, 717-723
- Kamatha VG, Chandrashekarb A, Rajini P.S, Antiradical properties of sorghum (*Sorghum bicolor*L. Moench) flour extracts, *J Cereal Sci*, 40, 2004, 283-288.
- Hollman P.C.H, Katan M.B, Dietary flavonoids: intake, health effects and bioavailability, *Food Chem Toxicol*, 37, 1999, 937-942.
- Benzie, I. F. F., & Strain, J. J, Ferric reducing / antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, *Methods Enzymol Journal*, 299, 1999, 15-27.
- Blois MS, Antioxidants determination by the use of a stable free radical, *Nature*, 181, 1958, 1199-1200.

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