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International Journal of Current Research in Life Sciences
Vol. 09, No. 10, pp.3338-3341, October, 2020

ISSN: 2319-9490

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL CHARACTERIZATION AND ANTI CANCER ACTIVITY OF *TRITICUM AESTIVUM* L. (WHEAT GRASS-12TH DAY PLANT)

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Received 07th August, 2020; Accepted 20th September, 2020; Published 30th October, 2020

ABSTRACT

Many drugs commonly used today are of herbal origin. Some are made from plant extracts and most of them are synthesized artificially to mimic the natural compound.Wheatgrass (*Triticum aestivum* L.) is one of the most widely used health foods, but its functional groups and mechanisms remain unidentified. Wheat germinated over a period of 8-12 days is generally called wheatgrass. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts. Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides in their ethanolic leaves extract. The present investigation, we examined the phytochemical and cytotoxic effect of ethanolic extract from *Trticum aestivum* on A549 cell line *in vitro*. In order to determine the cytotoxic effects we used an MTT viability assay. The results showed that cell growth is significantly lower in extract treated cells compared to untreated control. Based on the results it is determined that Wheat grass is a significant source of biologically active substances that have cytotoxic activity *in vitro*. The present study was designed to evaluate relative contribution of different phytochemicals and anticancer effect in ethanol extract of wheat grass.

Key words: Wheatgrass, phytochemical, ethanol extract, Anticancer activity.

Citation: Vijayalakshmi, R., Aishwarya, P, Dr. Vetriselvi, A. and Dr. Sridharan, R.. 2020. "Preliminary Phytochemical Characterization and Anti cancer activity of Triticum aestivum L. (Wheat Grass-12th day plant)" *International Journal of Current Research in Life Sciences*, 09, (10), 3338-3341

INTRODUCTION

Plant Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic qualities. Herb plants produce and contain a variety of active substances that act upon the body. Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug discovery. Wheat, (Triticum species) a cereal grass of the Gramineae (Poaceae) family, is the world's most edible grain cereal-grass crop. Nowadays, researchers have known Wheatgrass is a nutrient-rich type of young grass in the wheat family, is many times richer in levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant (1). At present, wheatgrass is quickly becoming one of the most widely used supplemental health foods and is available in many health food stores as fresh produce, tablets, frozen juice, and powder. Wheatgrass provides a concentrated amount of nutrients, including iron; calcium; magnesium; amino acids; and vitamins A, C and E and large amounts (70%) of chlorophyll (2). Some therapist used wheatgrass as a treatment for cancer (3), ulcerative colitis (5) and joint pain, and as antioxidant agent (4). It has been suggested that wheatgrass has a greater nutritional value than several everyday foods, and ingesting wheatgrass is comparable to eating a large amount of vegetables (9). In their study, Marwaha et al had thalassemia patients drink wheatgrass juice daily, and as a result, half required over 25% less packed red blood cells. The effectiveness of the plant extracts is mainly due to the presence of bioactive constituents like phenolics, flavonoids and others (6). During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts, and wheat sprouts reach the maximum antioxidant potential (8). Wheatgrass is used to treat many conditions, but so far there isn't enough scientific evidence to support effectiveness for any of these uses. By this study, we have embodied the most effective solvent extract of wheat grass, to determine the total phenolic and flavonoid and other phytochemical contents. Details on the qualitative compositions of various solvent extracts of wheatgrass would provide useful information on therapeutic use.

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MATERIALS AND METHODS

Cultivation of *Triticum aestivum* Wheat, (Triticum species) a cereal grass of the Gramineae (Poaceae) family, is the world's largest edible grain cereal grass crop. The wheat plant is an annual grass.

In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves.

Extraction of plant material: The 12th day grass of *Triticum aestivum* was cultivated, collected and chopped with the help of knife. It was dried in shade and then powdered with a mechanical grinder. The powder was passed through sieve and stored in a labeled air tight container for further studies. Wheatgrass powder was subjected to soxhlet extraction by using ethanol, for about 24h. The solvent extract was evaporated to dryness and used for further studies.

Qualitative Screening Carbohydrates: In a test tube containing 2.0 ml of plant sample, 2 drops of freshly prepared 20% alcoholic solution of a naphthol was added and mixed. To this solution 2.0 ml of concentrated sulphuric acid was added so as to form a layer below the mixture, formation of the red violet ring at the junction of the solution and its disappearance on the addition of an excess of alkali solution indicate the presence of carbohydrates.

Proteins: 1 part of extract was digested with 2 parts of HNO3 and the resulting solution was diluted with 2 volumes of water. To a small quantity of decoction, 5-6 drops of Million's reagent was added. A precipitate which turned red on heating was formed and it indicates the presence of proteins.

Alkaloids: 1.36gm of mercuric chloride was dissolved in 60ml distilled water and 5gm of potassium iodide and diluted to 100ml with distilled water. To 1.0ml of acidic aqueous solution of samples, few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids (Harborne, 1973).

Flavonoids: In a test tube containing 0.5ml of various extracts of the samples, 5-10 drops of dilute HCl and a small piece of Zn or Mg were added and then solution was boiled for few minutes. In the presence of flavonoids, the reddish pink or dirty brown colour was produced (Harborne, 1973).

Tannins: In a test tube containing about 5.0 ml of an extract, a few drops of 1% solution of lead acetate was added. A yellow or red colour precipitate was formed, indicating the presence of tannins (Harborne, 1973).

Phenols: To 1.0ml of alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added and the formation of blue or green colour indicates the presence of phenols (Harborne, 1973).

Saponins: In a test tube containing 5ml of various extract of sample, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously for 3mins. A honey comb like froth was formed and it showed the presence of saponins (Harborne, 1973).

Glycosides: A small amount of various extract of sample was dissolved in 1ml of water and aqueous solution of sodium hydroxide was added. Formation of a yellow colour indicates the presence of glycosides (Harborne, 1973).

Steroids: To 2.0ml of various extracts of samples, 1.0 ml of concentrated H_2SO_4 was added carefully along the sides of the test tube. Formation of red colour chloroform layer indicates the presence of steroids (Harborne, 1973).

Terpenoids: 0.5 ml of extract was mixed with 2 ml of chloroform in a test tube. 3 ml of concentrated sulfuric acid was carefully added to the mixture to form a layer. A reddish brown coloration was formed for the presence of terpenoids.

Anticancer activity

Cell line and culture: A549 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100µ/ml), and streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO2 at 37 °C. In Vitro assay for anticancer activity: (MTT assay) (Mosmann, 1983) Cells (1 × 105 /well) were plated in 24-well plates and incubated in 370C with 5% CO2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically.

RESULTS AND DISCUSSION

Plant are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinines, alkaloids, and other metabolites, which are rich in antioxidant activity (11). Studies have shown that many of the phytocompounds possess anti-inflammatory, anti-diabetic and antimicrobial activities (12).

Anticancer effect of Sample on A549 cell line

S.No	Concentration (µg/ml)	Dilutions Neat	Absorbance (O.D)	Cell viability (%)
1	1000	1:1	0.134	21.57
2	500	1:2	0.175	28.18
3	250	1:4	0.218	35.10
4	125	1:8	0.264	42.51
5	62.5	1:16	0.308	49.59
6	31.2	1:32	0.352	56.68
7	15.6	1:64	0.395	63.60
8	7.8	1:1	0.440	70.85
9	Cell control	-	0.621	100

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (13). Plant derived substances have recently become a great interest owing to the versatile applications. Medicinal plants and herbs are the richest bio-resource of drugs of traditional systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs. Phytochemical screening of *Triticum aestivum* using ethanol extract positive to many metabolites. The screening analysis of *Triticum aestivum* using ethanol extract revealed the presence of carbohydrate, protein, phenol terpenoids cardiac glycosides etc.

The result of our present study is further supported with similar studies reported by Gaurav Kumar et al. The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies the *Triticum aestivum* as a valuable therapeutic natural source which will serve as an effective herbal option to compact dreadful infectious diseases. Cancer-related research is conducted worldwide every day, since cancer is a leading cause of death. These studies often involve the investigation of the effects of biologically active substances on cancer cells, and they frequently originate from plants (22). There is a great need to examine reliable and inexhaustible sources of natural substances. In addition, it is important to understand the mechanisms of anticancer agents for future application in cancer therapy (23). This experiment investigated the cytotoxic activity of the ethanolic extract of *Triticum vulgare* (in a concentration of 1–1000 µg/mL) in cell line (A549), using MTT assay.

The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in Xaxis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

Conclusion

Scientific research is increasingly confirming what was known to our ancestors form experience. While plants continued to provide us pleasure with their beauty (colour and fragrance) and enhance the taste of our food by their flavour, we seemed to have become moreish. Young cereal plants were valued in ancient times. It had been said that people in the ancient Middle East ate the green leaf tips of the wheat plant as a delicacy. It helps to prevent tooth disorders, constipation, skin diseases etc. With this wide potential, medicinal application and therapeutic value, the present work has been undertaken and the inferences are summarized as follows. Phytochemical qualitative screening exhibited a good range of primary metabolites and a wide range of secondary metabolites (alkaloids, tannins, phenols, saponins and glycosides) present in *Triticum aestivum.* Herbs are staging comeback and 'herbal renaissance' is happening all over the globe, the herbal products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to human and environment. Although, herbs had been raised for the medicinal, flavoring and aromatic qualities for centuries. The synthetic products of the modern age surpassed their importance for a while. However, the blind dependence on synthetic drug is over and people are retaining to the natural with hope of safety and security. With this rational evidence and on scientific basis, hence study justify and supports the use of *Triticum aestivum* in traditional folk medicine.

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