Research Article

Preliminary Phytochemical Analysis of Wheat Grass Leaf Extracts

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Accepted on: 05-07-2016; **Finalized on:** 31-08-2016.

ABSTRACT

Plants have been used for medicinal purposes since time immemorial. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements and cosmetics. Wheat grass is the mature shoot of the plant *Triticum aestivum* Linn. Which belongs to the family Gramineae. Wheat Grass Juice (WGJ) is an extract squeezed from the mature sprouts of wheat seeds. Green Blood Therapy is the use of wheat grass juice (WGJ) to cure multiple diseases. The name "green blood" of wheat grass is attributable to its high chlorophyll content which accounts for about 70% its total chemical constituents and also to its close structural similarity to Haemoglobin. Wheat grass juice contains almost all the nutrients the body requires and is considered to be a complete food. The present study intends to provide an overview of the chemical constituents present in the crude leaf extracts of *T. aestivum* both fresh and dried, with special emphasis on their pharmacological actions. The powdered leaf extracts as well as the fresh juice of wheat grass have been screened for phytochemical constituents in four different solvents as methanol, acetone, ethanol and water. Preliminary phytochemical analysis revealed the presence of ten compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavonoids, cardiac glycosides, sapponins, coumarins, amino acids etc. phytochemical analysis of both the fresh and dried samples were more positive for aqueous extracts. The results suggest that the leaves are a rich source of valuable primary and secondary metabolites.

Keywords: Wheat grass juice, Green blood therapy, *Triticum aestivum*, Nature's finest medicine, Complete food, Pharmacological effects, Therapeutic benefits and phytochemical constituents of WGJ.

INTRODUCTION

Herbal or 'alternative' medicine is gaining popularity and scientific attributes regarding wheatgrass as a "functional food" is becoming more available and popular as a research topic .Wheat Grass Juice (WGJ) is an extract squeezed from the mature sprouts of wheat seeds (T. *aestivum*). Wheat grass can be traced back in history over 5000 years in ancient Egypt and perhaps even early in Mesopotamian civilization. It is reported that ancient Egyptians found sacred the young leafy blades of wheat and freezed them for positive effect on their health and vitality. The consumption of wheat grass in western world began in the 1930's as a result of the experiments conducted by Charles Schnabel an agricultural chemist on his hens using wheatgrass to nurse them back to health 1. Wheat grass is the mature shoot of *T. aestivum* L. It belongs to family Gramineae. *Triticum* is a genus of annual and biennial grasses, yielding various types of wheat, native to south west Asia[2]. Common or bread wheat, is widely cultivated almost all over the world. Generally, 15-20 species are recognized, of which 8 have been reported to occur in India. Wheat grass is cost efficient and a source to provide all kinds of nutrients like vitamins, proteins, minerals, antioxidants and medicinal benefits for a healthy and rejuvenating body. Wheat grass has high concentration of chlorophyll, minerals (calcium, potassium, iron, magnesium, sodium and sulphur), and 17 forms of amino acids. , vitamins (A, B, C, E and K) and active enzymes[3]. It stimulates metabolism and also restores alkalinity to the blood. It's the chlorophyll content in wheat grass that detoxifies the body and strengthens immunity. The three most important effects of wheat grass on the human body are blood purification, liver detoxification and colon cleansing 4. Wheat grass therapy is recommended for patients suffering from chronic diseases like Asthma, Atherosclerosis, Parkinson's disease, Joint pains, Constipation, Hypertension, Diabetes, Insomnia, Bronchitis, Sterility, Haemorrhage, Obesity and Flatulence. It is also useful in the treatment of Cancer.

The use of WGJ for therapeutic purposes was developed and popularized by Dr. Ann Wigmore, as part of her herbal therapeutic nutritional approach. Wigmore, believed that wheat grass, as a part of a raw food diet, would cleanse the body of toxins while providing a proper balance of nutrients as a whole food. The use of wheatgrass, particularly its fresh juice became popular again in the 1970s, through Ann Wigmore's "The Wheatgrass Book" which later on became somewhat of a gospel amongst health supplement fanatics. Ann Wigmore also established the famous Hippocrates Centre treating thousands of clients with herbal grasses and wheatgrass juice 1 Fig.1 & 2. In Asia and Europe wheat grass based products are consumed in the form of juices, powders and extracts for the healthy growth of human body. Wheat grass juice is nature's finest medicine. Two ounces of wheat grass juice has the nutritional

equivalence of five pounds of the best raw organic vegetables. It is over flowing with vitamins, aminoacids, liver enzymes and chlorophyll. It contains 98 of 102 earth elements found in soil, including phosphorous, calcium, iron, magnesium and potassium as well as essential enzymes and 19 aminoacids. It has twice vitamin A as in carrots and is higher in vitamin C than oranges[4]. It contains the full spectrum of complete proteins which is in the form of simple polypeptides, a short chain of aminoacids that the body absorbs more efficiently in the blood stream.

In order to develop a holistic approach for the treatment of chronic diseases, scientists and clinicians world over are now a day's conducting extensive studies to evaluate the efficacy of wheat grass (in the form of powder or juice) and also for the better understanding of therapeutic potential of this medicinal grass [5].

Figure 1

Figure 2

In recent years, phytochemicals previously with unknown pharmacological activities have been extensively investigated, as a source one of the medicinal agents. Green Blood Therapy is the use of Wheat Grass Juice (WGJ) to cure multiple diseases. Wheat grass is called as the green blood. The name "green blood" of wheat grass is attributable to its high chlorophyll content which accounts for about 70% of its total chemical constituents[6&7.]

Wheatgrass juice is a rich source of Vitamins A, C, E and B complex, including B12. It contains a multitude of minerals such as calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron and molybdenum. The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase and super oxide dismutase (SOD). The other notable feature of wheatgrass is its high proportion of amino acids such as aspartic acid, glutamic acid, arginine, alanine and serine. It also has a high content of bioflavonoids like apigenin, quercitin and luteolin. All of these enzymes contribute to its antioxidant activity. Other compounds present, which make this grass therapeutically effective, are the indole compounds, choline and laetrile (amygdalin) [8].

Wheat grass can be cultivated in outdoors, but is commonly grown in indoors on trays filled with potting mix for 15 days. As the leaves grow, they eventually split. At this so called "jointing stage" point the blades can be snipped off, allowing for a second round of leaves to grow. Wheat grass was successfully grown in growth chambers and in field conditions at temperature of 18 to 26 ^{0}C and a relative humidity of 40 to 50% was found to be suitable for the growth of wheat grass [9].

MATERIALS AND METHODS

Collection of plants

The plants for the present study were cultivated in indoors on trays filled with potting mix for about 15 days and we also use commercially available 'chlorophyll instant powder' for comparative phytochemical analysis .

Isolation of pigments

One gram of wheat grass tissue was ground in 50 ml of 80% acetone. The homogenate was filtered, centrifuged at 6000 rpm for 15 minutes and the supernatant was taken as the pigment source.

Absorption spectra

Absorbancy of the pigments were read between wave lengths 400-720 in a spectrophotometer (systronics 104). Absorption spectra were constructed by plotting wavelength on x-axis and absorbance on y-axis.

4. Quantification of pigments by Arnon's formula

From specific absorptions obtained the pigments were quantified based on Arnon's formula.

Mg chlorophyll a/g tissue = $12.7(A_{663})-2.69(A_{645})$ * V/ (1000*W)

Mg chlorophyll b/g tissue = $22.9(A_{645})-4.68(A_{663})$ * V/ (1000*W)

Mg total chlorophyll l/g tissue = $20.2(A_{645}) + 8.02(A_{663})$ * V/ (1000*W)

Total carotenoids = $(D_{490-0.114}) - (D_{663-0.638}) - (D_{645})$

Phytochemical Analysis

Prepared plant extracts were analysed for the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, fixed oils, steroids, terpenoids, tannins, flavanoids, gum mucilages etc. (The presence of phytochemicals extracted in various solvents was confirmed by standard protocols).

Test for carbohydrates

Molishs Test

To 2 ml of Molishs reagent 2 ml of extracts were added and shaken well. To this 2 ml of concentrated sulphuric acid was added through the sides of the test tube. Appearance of reddish violet ring at the junction of the two layers indicates the presence of carbohydrates.

Fehlings Test

To the extract add equal amount Fehling's reagent, mixed well and heated gently. Formation of a brick red precipitate indicates the presence of reducing sugars.

Test for Tannins

To the extracts were added a few drops of 10% ferric chloride solution. Appearance of a green or blue colour indicates the presence of tannins.

Test for steroids

Leaf extracts were mixed with 1 ml of chloroform and later 2-3 drops of conc.H_2SO_4 was added. Appearance of pink or red colour indicates the presence of steroids.

Test for Terpenoids

Salkowiski Test

Five ml of the extracts were mixed with 2 ml of chloroform and 3 ml of conc.H_2SO_4 solution. A reddish-brown colour at the interphase indicates the presence of terpenoids.

Test for Alkaloids

Mayer's Test

To the extracts, 1% hydrochloric acid and six drops of mayer's reagent were added. Apperence of an organic precipitate indicate the presence of alkaloids in the sample.

Detection of Flavanoids

The extracts were treated with conc.H_2SO_4 and observed for a yellowish orange colour for the presence.

Test for Protein

Biuret Test

One ml of 40% NaCl and two drops of 1% $CuSO_4$ were added to the leaf extracts. Appearance of a violet colour confirms the presence of proteins.

Xanthoprotein Test

To the leaf extracts 20% NaOH were added and the formation of an orange colour confirms the presence of proteins which is characteristic for ammonia formation.

Test for Cardiac Glycosides

Keller-Killani Test

Five ml of the extracts were treated with 2 ml of glacial acetic acid containing 2-3 drops of ferric chloride solution and 1 ml of conc.H_2SO_4 acid solution. A green ring initially appears which first turns to violet and then to brown at the interphase indicates the presence of cardiac glycosides.

Test for Fixed oils

Two drops of the extracts were passed between two filter papers. Appearance of an oil strain on the filter paper indicates the presence of fixed oils.

Test for Saponins

Foam Test

Two ml of the extracts were diluted with 20 ml of distilled water, shaken vigoursly and was observed for a stable persistent froth.

Test for Phenolic Compounds

Ferric Chloride Test

Two ml of diluted extracts were treated with dil.$FeCl_3$ solution. Apperence of a violet colour indicate the presence of phenol like compounds.

Detection of Coumarins

To the test solution were added a few drops of alcoholic sodium hydroxide solution. Apperence of an intense yellow colour on addition of conc.HCl indicate the presence of coumarins.

Test for Aminoacids

Ninhydrin Test

Two drops of ninhydrin solution (10mg of ninhydrin in 200 ml of acetone) were added to 2 ml of aqueous filtrates. A characteristic purple colour indicates the presence of aminoacids.

Test for Gum and Mucilage

To 100 ml of each extract added 10 ml of distilled water. To this 25 ml of absolute alcohol was added with constant stirring. Cloudy precipitate indicate the presence of gum and mucilage

RESULTS AND DISCUSSION

Physical Characteristics of wheatgrass (*T.aestivum* L.) juice is given in table 1.Chlorophyll is the first product of light and therefore it contains more healing properties than any other elements. It is the life blood of plants. In the present study the pigment levels of wheatgrass at each day is recorded by using a UV – VIS spectrophotometer. From the findings it is very evident that the eighth day has maximum absorbancy value and is the apt day for wheat grass consumption.

The second experiment is the analysis of the phytochemical constituents of the crude extracts of wheatgrass (*T. aestivum.L).* Both the dried powdered as well as the fresh wheat grass extracts have been screened for phytochemical constituents in four different solvents as methanol, acetone, ethanol and water. Preliminary

phytochemical analysis of both extracts revealed the presence of compounds such as carbohydrates, tannins, steroids, terpenoids, alkaloids, flavanoids, cardiac glycosides, saponins, coumarins etc (table 2 &3).

Among the four different solvents used, chloroform & **water were more positive of fresh leaf extracts, whereas** the dried extracts were more positive for methanol and water extract of leaf samples. From the above results obtained it can be concluded that water is one of the best solvents used. The various leaf extracts of wheatgrass have clearly indicated the presence of all the major phytochemicals and this may be one of the reasons why the plant have been preferred to use as medicine as well as a food supplement in our daily routine. The role of tannin is to protect from predation, pesticides and in plant growth regulation. Previous studies by various other workers prove that flavanoids provide health benefits through cell signaling pathways and antioxidant effects.

Table 1: Physical Characteristics of wheatgrass (*T. aestivum* L.) juice[4].

Physical Constants	T. aestivum L.
Macroscopic Characteristics	
Nature	Grass
Colour	Bright green/Dark green
Odour	Characteristic
Taste	Acrid
Loss on drying (%w/w)	21.1%

Table 2: Showing absorbancy of pigments at different wavelengths.

Day	Absorbancy at Different Wavelength(nm)				
	490	645	663	665	680
4th	0.555	0.251	0.406	0.438	0.485
5th	0.566	0.254	0.408	0.440	0.310
6th	0.572	0.281	0.415	0.448	0.338
7th	0.693	0.295	0.425	0.458	0.399
8th	0.538	0.301	0.572	0.393	0.446
9th	0.472	0.134	0.210	0.243	0.368
10th	0.452	0.129	0.207	0.237	0.361

Table 3: Phytochemical analysis of fresh wheatgrass extract.

Test	Solvents				
	Methanol	Acetone	Ethanol	Water	Chloroform
Detection of Carbohydrates					
Molisches test	-	-	-	-	-
Fehlings test	+	+	+	+	+
Test for tannins	-	-	-	+	+
Test for steroids	+	-	+	-	+
Test for terpenoids					
Solkowiski test	-	+	-	-	+
Detection of alkaloids					
Mayers test	+	+	+	+	+
Detection of flavanoids	-	-	+	+	+
Test for protein					

Biuret test	-	-	-	-	-
Xanthoprotein test	-	-	-	-	-
Test for cardiac glycosides					
Keller killani test	-	+	-	-	+
Test for fixed oils	-	-	-	-	-
Test for sapponins					
Foam test	-	-	-	-	-
Test for phenolic compounds					
FeCl3 test	-	-	-	-	-
Detection of coumarins	+	+	+	+	+
Test for aminoacids					
Ninhydrin test	-	-	-	-	-
Gum and mucilage	-	-	-	-	-

Table 4: Phytochemical analysis of chlorophyll instant powder.

Test	Solvents				
	Methanol	**Acetone**	**Ethanol**	**Water**	**Chloroform**
Detection of carbohydrates					
Molisches test	+	+	+	+	+
Fehlings test	-	-	-	-	-
Test for tannins	+	+	+	+	-
Test for steroids	-	+	+	+	-
Test for terpenoids					
Solkowiski test	+	+	+	+	+
Detection of alkaloids					
Mayers test	+	+	+	+	+
Detection of flavanoids	+	+	-	-	+
Test for protein					
Biuret test	-	-	-	-	-
Xanthoprotein test	-	-	-	-	-
Test for cardiac glycosides					
Keller killani test	-	+	+	+	+
Test for fixed oils	-	-	-	-	-
Test for saponins					
Foam test	+	+	+	+	+
Test for phenolic compounds					
$FeCl_3$ test	-	-	-	-	-
Detection of coumarins	+	-	+	-	-
Test for aminoacids					
Ninhydrin test	-	-	+	-	-
Gum and mucilage	-	-	-	-	-

Plant are enriched with various phytochemical molecules such as vitamins, terpenoids, phenolics, lignins, tannins, flavonoids, quinines, alkaloids, and other metabolites, which are rich in antioxidant activity [10]. Studies have shown that many of the phytocompounds possess anti-inflammatory, anti-diabetic and antimicrobial activities [11]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents[12]. Phytochemical qualitative analysis of *Triticum aestivum* presented in the Table 3 & 4 signifies *T. aestivum* L .as a valuable therapeutic natural source which will serve as a natural food supplement [13]. The screening analyses were performed in order to identify the various secondary metabolites present in the leaf samples of *T. aestivum* L.by using a wide range of solvents namely aqueous, methanol, ethyl acetate and chloroform. The screening analysis of *T. aestivum* L. using various solvents revealed the presence of carbohydrate, proteins, alkaloids,tannins, phenols, in both methanolic and aqueous extracts. While the presence of saponins was noted only in chloroform extract. The result of our present study is further supported with similar studies reported by Gaurav Kumar *et al* in 2010[14]. Thus this qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which could be exploited effectively as herbal option against various dreadful infectious diseases.

CONCLUSION

Medicinal plants were the potent source of human health due to the active phytochemical compounds that is responsible for its various pharmacological activities. On the basis of the results obtained, the present study concludes that plants were rich in phytochemical constituents exhibiting antimicrobial properties. The results of the phytochemical screening of the leaf extracts of the sample varied, with the presence and absence of certain compounds. It was observed that most of the components were present in water extracts. Phytochemicals such as glycosides, carbohydrates, coumarins, terpenoids, alkaloids, phenolic compounds.etc were present in almost all solvents. Again from the results obtained it is very evident that the eighth day wheat grass harvest had high chlorophyll content and hence is the apt day for wheat grass consumption due to its close structural similarity to Hemoglobin of blood. Thus the present study highlights the far reaching application of this miracle plant in the treatment for minor ailments and serious life threatening issues as well as a preventative dietary supplement. The study had shown that the results of extraction yield, total alkaloid and flavanoid compounds as well as the bioactivity tests varied depending upon the type of the solvent been used. Hence it can be concluded that the leaves of *T. aestivum L.* would direct to the establishment of some compounds that could be used to invent new and more potent useful products like medicines.

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Source of Support: Nil, **Conflict of Interest:** None.