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PHYTOCHEMICAL SCREENING OF WHEATGRASS EXTRACT

Nikita Mahalle* and Monica Borikar

P.G Department of Cosmetic Technology, Nikalas Mahila Mahavidhyalaya, Khamla, Nagpur, Maharashtra, India.

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***Corresponding Author**
Nikita Mahalle
P.G Department of Cosmetic Technology, Nikalas Mahila Mahavidhyalaya, Khamla, Nagpur, Maharashtra, India.
nikitamahalle5422@gmail.com

ABSTRACT

Wheatgrass (*Triticum Aestivum Linn*) is one of the most widely used health cosmetics and pharmaceutical industries. Wheat germinated over a period of 6-10 days is generally called wheatgrass, which have been used as traditional herbal medicine and is highly valued for its therapeutic and nutritional properties. The chemical compositions of the leaves are proteins, flavonoids, alkaloids, glycosides, terpenoids, saponins, fibers, tannins, and phenolic compounds The present study was designed to evaluate relative contribution of different phytochemicals in methanolic extracts of wheat grass. Wheatgrass leaves were collected over a specific period of nine days, and the leaves of the selected medicinal plant were washed, air dried, and then powdered. Extraction of wheatgrass was done by maceration process. Methanol is used as a solvent to obtain methanolic extract. The extract of the leaves sample was used for the phytochemical analysis to find out the phytochemical constituents in the plant. Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, steroids, and glycosides in their methanolic leaves extract. Spectrophotometric analysis of wheatgrass extract is done with the UV spectrophotometer to determine the absorbance of quercetin in extract, it was observed that on wavelength 320 nm gives absorbance of 0.627 shows the highest peak (λ max) on the graph.

KEYWORDS: Wheatgrass, *Triticum Aestivum Linn*, Phytochemical screening, Methanolic extract.

I) INTRODUCTION

Scientific research is increasingly confirming what was known to our ancestors from experience. While plants continued to provide us pleasure with their beauty (color and fragrance) and enhance the taste of our food by their flavor, we seemed to have become moreish. 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, or savoury 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.[1][2][3][4]

Triticum Aestivum Linn is commonly known as common wheatgrass belonging to the family *Poaceae (Gramineae)* and have high chlorophyll content about 70% of its chemical constituents. Wheatgrass is highly valued because of its therapeutic and nutritional properties which has been used as traditional herbal medicine. The therapeutic qualities of *Triticum Aestivum* have been imposed to its rich nutritional content, including chlorophyll, vitamins, (A, C, and E), minerals (calcium and magnesium), bioflavonoids, iron, and 17 amino acids. The goal of the present study was to assess the phytochemical screening of crude methanolic extracts of ten days mature *T. aestivum Linn*.[5][6][7]

II) DETAILS OF ACTIVE

Triticum Aestivum Linn. is commonly known as wheatgrass belonging to the family *Poaceae (Gramineae).*[8]

i) Geographical source

The wheat plant was originated from the levant region of the Near East. *Triticum Aestivum* plant is mainly cultivated in temperate, irrigated to dry, high rainfall areas, warm, humid to dry, and cold regions. It is native to southwest Asia and the Mediterranean region and is grown almost all over the world.

A total of 15-20 species of *Triticum Aestivum* are documented worldwide widely out of which 8 species of wheatgrass are reportedly found in India. The other varieties of wheatgrass are *Agropyron trachycaulam* (slender wheatgrass), *Elytrigia, Eremopyrum, Pascopyrum,* and *Pseudoroegneria* commonly found in temperate regions of the United States and Europe.

It is cultivated on a large scale all over India and also occasionally cultivated in a garden. For the present study samples of the Wheatgrass, leaves were collected over a specific period of nine days. It is a perennial, tufted grass plant that belongs to the family *Poaceae*. The shoot or young grass of *Triticum Aestivum* is known as wheatgrass which reaches up to the height of 30–100 cm (12 to 40 inches).[9]

ii) Chemical Compositions

The leaves contain proteins, flavonoids, alkaloids, glycosides, terpenoids, saponins, fibers, tannins, and phenolic compounds. The phytochemical analysis of the crude extract executed the presence of alkaloids, carbohydrates, glycosides, saponins, glycosides, steroids, tannins, flavonoids, terpenoids, and phenols.

Wheatgrass is also a rich source of amino acids such as glutamic acid, arginine, alanine, aspartic acid, and serine. Wheatgrass is a rich source of Vitamin A, C, E, and B complex and minerals present are phosphorus, calcium, magnesium, selenium, alkaline earth metals, boron, zinc, potassium, and molybdenum. Wheatgrass extract contains chemical constituents like chlorogenic acid, rutin, tocopherol, and gallic acid.[8][9]

1)Bioflavonoid: apigenin, quercetin, luteolin, amely choline, indole compounds, choline, and laetrile (amygdalin).
2)Amino acid: aspartic acid, asparagine, valine, arginine, threonine, glutamine, proline, glycine, alanine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan and serine, P4D1 (gluco-protein), mucopolysaccharides, and chlorophyll.
3)Enzymes: Protease, transhydrogenase, lypase, amylase, cytochrome oxidase, superoxide dismutase (SOD).
4)Phytoconstituents: Rutin, chlorogenic acid, tocopherol, gallic Acid.
5) Vitamins and minerals: Vitamins A, B1, B2, B3, B5, B6, B8, and B12; C, E, and K, ascorbic acid, dehydrated ascorbic acid, sodium, aluminium, carotene, sulphur, copper, calcium, iodine, phosphorus, magnesium, alkaline earth metal, potassium, selenium, Iron, Zinc, boron, and molybdenum.[8]

iii) Benefits of wheatgrass

Wheatgrass is used due to its antioxidant property because of the presence greater number of bioflavonoids such as luteolin, quercetin apigenin, and indole components such as laetrile (amygdalin) and choline. wheatgrass includes properties such as sun screening, anti-bacterial,

anti-microbial properties (inhibiting the growth of microorganisms), antioxidant, anti-allergic, anti-inflammatory, and antiaging.

1) Antioxidant property- The antioxidant activity of wheatgrass extract was observed at various levels of protection such as primary and secondary radical scavenging and inhibition of free radical induced membrane damage. This can be explained based on its chemical content. It has been shown that these extracts contain significant amounts of phenolic compounds including flavonoids.
2) The B-complex vitamins, especially thiamine, riboflavin, and niacin offered by natural brown Wheat promote youthful energy and nourishment to the skin and blood vessels.
3) Wheatgrass juice acts as an excellent mouth wash for sore throats and pyorrhoea. It also prevents tooth decay and toothaches.[10][11]

III) MATERIALS AND METHODS

i) Collection of Herbs and Chemicals

Collection of wheatgrass

Wheat grains *(Triticum Aestivum Linn)* of the best quality were taken from the local market for experimentation. After proper washing of wheat grains with distilled water, cultivation was carried out in plastic trays at laboratory scale under controlled temperature and light conditions. On the ninth day, the grass of *Triticum Aestivum* was cultivated, collected, and chopped with a knife and grind with mechanical grinder. The powder was passed through a sieve and stored at 4^0 c in a labeled airtight container for further studies.[12][13]

ii) Preparation of herbal extracts

The maceration technique was used for the extraction. 10g powder of *Triticum Aestivum* (wheatgrass) was suspended in 100ml of methanol using a 250ml conical flask and kept on an orbital shaker for 48 h at 37°C. After 48h, the supernatant was filtered through Whatman filter paper no.1 and evaporated to dryness at room temperature with a yield of 6.3 gm (w/w). The viscous material was stored in a sterile, air-tight container, and kept at 4^0 C for further studies. The residue was dried and further used for successive extraction.[12]

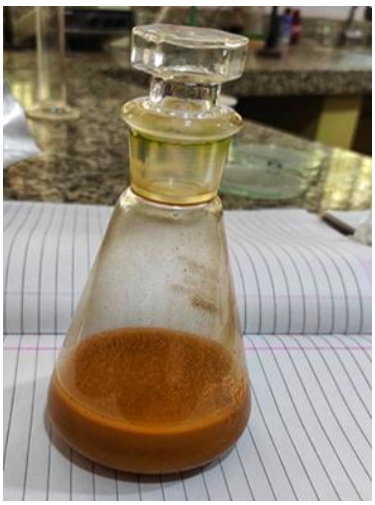


Image 1: Maceration of wheatgrass.

Image 2: After 48 Hrs.

Image 3: Crude Extract

iii) Phytochemical testing of extract

The residue from the Extraction is now used in the phytochemical evaluation. The extract was suspended in a proper solvent and to identify the phytochemical constituents present in *Triticum Aestivum* grass extracts, a preliminary screening was carried out by the application of various testing methods of Draggendorff's and Mayer's test, Liebermann-Bur chard test, Foam formation test, Lead acetate test, Molisch's and Fehling's test and Ferric Chloride test for determining the presence of alkaloids, terpenes, steroids, saponins, flavonoids, polysaccharides, and tannins, respectively.[12]

- **Alkaloids**

1) Mayer's test

   Alkaloids gave cream colour precipitate with mayer's reagent (potassium mercuric iodide solution).

2) Dragandroff's test

   Alkaloids gave reddish brown precipitate with dragandroff's reagent (potassium bismuth iodide solution).[13]

- **Tannins**

1) Gelatin test

   Extract with 1% gelatin solution containing 10% sodium chloride gave white precipitate.

2) Ferric chloride test

   Test solution gave blue green color with ferric chloride.[13]

- **Flavonoids**

1) Shinoda test (Magnesium hydrochloride reduction test).

To the test solution, a few fragments of magnesium ribbon were added, and concentrated hydrochloric acid was added dropwise, pink scarlet, crimson red, or occasionally green to blue colour appeared after a few minutes.

2) Alkaline reagent test

To the test solution, a few drops of sodium hydroxide solution were added. An intense yellow colour was formed, which turned colorless on the addition of a few drops of dilute hydrochloric acid, indicating the presence of flavonoids.[13]

- **Proteins and amino acids**

1) Millons test.

The test solution was mixed with 2 ml of Millon's reagent (mercuric nitrate in nitric acid containing traces of nitrous acid), and white precipitate appeared, which turned red upon gentle heating.

2) Ninhydrin test.

Amino acids and proteins when boiled with 0.2% solution of ninhydrin (indane 1, 2, 3 trione hydrate), a violet colour appeared.[13]

- **Carbohydrates**

In a test tube containing 2.0 ml of plant sample, 2 drops of freshly prepared 20% alcoholic solution of a naphthol were added and mixed. To this solution 2.0 ml of concentrated sulphuric acid was added 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 indicates the presence of carbohydrates.[13]

- **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.[14]

- **Saponins**

A few drops of sodium bicarbonate were added to a test tube containing 5ml of various extracts of the sample. The mixture was shaken vigorously for 3mins. A honeycomb like froth was formed and it showed the presence of saponins.[14]

- **Glycosides**

A small amount of various extracts of the sample was dissolved in 1ml of water and an aqueous solution of sodium hydroxide was added. The formation of a yellow colour indicates the presence of glycosides.[14]

- **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. The formation of red colour chloroform layer indicates the presence of steroids.[14]

- **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.[15]

iv) Spectrophotometric analysis of methanolic wheatgrass extract with UV spectrophotometer

Spectrophotometric analysis is one of the quantitative tests this is used to find out of absorbance of actives at their specific wavelength range. It is also helpful to calculate total amount of actives present in the sample solution i.e., the concentration of apigenin present in wheatgrass extract. To study how the chemical compounds interact with different wavelength in a given region of electromagnetic radiation is called spectroscopy of spectrochemical analysis.

The word spectroscopy implies that the electromagnetic spectrum is produced when matter emits electromagnetic radiation. Thus, modified ultraviolet means the information will come from a specific region of the electromagnetic spectrum called the ultraviolet region (190 to 400nm visible region).

Ultraviolet spectroscopy involves the absorption behavior of substance, which can be either qualitative or quantitative. The quantitative application is for determining an unknown concentration of specific species. The data was derived from UV spectrum coefficient (E) and k-value. UV absorbance gives a fair idea of the sample's absorbance in the UV range. This was evaluated with help of UV spectrophotometer.[16]

Image 4. UV Spectrophotometer.

PROCEDURE

- The concentration of the samples was prepared for the experiment.
- The methanol was used as a solvent.
- The sample is poured into the cuvette.
- Dried the outside of the cuvette, completely with a wipe.
- The sample cuvette was inserted into the cuvette holder and closed the sample compartment lid.
- Run on the control panel was pressed and the absorbance of the sample was recorded.
- The spectrum of the sample was obtained.[16]

IV) RESULT AND DISCUSSION

Extraction of active from wheatgrass was done using maceration method and preliminary phytochemical screening of wheatgrass extract was done for flavonoids, alkaloids, tannins and phenolic compounds, steroids, glycosides, carbohydrates, amino acids, and saponins, it was observed the presence of flavonoids, alkaloids, tannins, phenolic compounds, saponins, glycosides, amino acid, steroids.

i) Phytochemical testing of extract

Table 4: Phytochemical constituents found in methanolic extract.

Sr.no	Constituent /Test	Observation
1	Alkaloids	+
2	Glycosides	+
3	Tannins & Phenolic comp	+
4	Saponins	+
5	Flavonoids	+
6	Carbohydrates	-
7	Steroids	+
8	Amino acids	+

Abbreviations:- + sign indicates the presence and the – sign indicates absence.

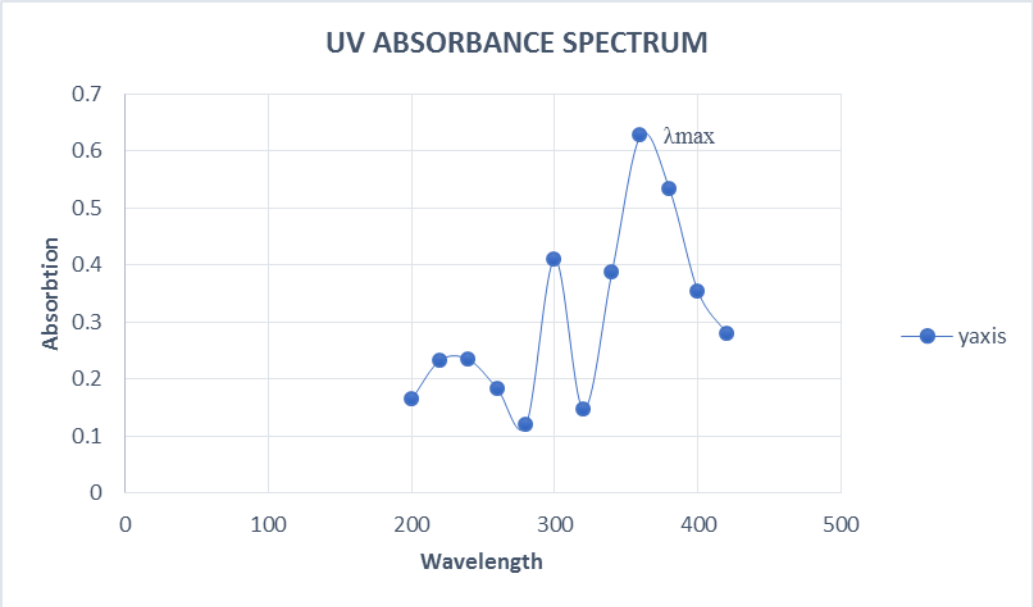
ii) Spectrophotometric analysis of methanolic wheatgrass extract with UV spectrophotometer.

UV spectrophotometric analysis of wheatgrass extract is done to determine the absorbance of quercetin in extract, it was observed that on wavelength 320 nm gives absorbance of 0.627 shows the highest peak (λ max) on the graph.

Table no 2: Absorbance of quercetin.

Sr. no	Wavelength in nm	Absorbance
1	360nm	0.627

The significance of this table no 2. shows the absorbance of actives at their specific wavelength.



Graph no 1: Spectrum graph of quercetin.

RESULT

When the extract of wheatgrass quercetin was evaluated from the spectrophotometric analysis, it was observed that it shows absorbance maxima at 360 nm. It confirms the presence of quercetin in table no.2 and graph no 1.

V) CONCLUSION

From the above study, it can be spontaneously stated that the methanolic extract of ten day's mature *T. Aestivum Linn*. shows positive results in phytochemical screening. Extraction of active from wheatgrass was done using maceration method and preliminary phytochemical screening of wheatgrass extract was done for flavonoids, alkaloids, tannins, phenolic compounds, steroids, glycosides, carbohydrates, amino acids saponins, it was observed the presence of flavonoids, alkaloids, tannins, phenolic compounds, saponins, glycosides, amino acid, steroids.

UV spectrophotometric analysis of wheatgrass extract is done to determine the absorbance of quercetin in extract, it was observed that on wavelength 320 nm gives absorbance of 0.627 shows the highest peak (λ max) on the graph.

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