Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 8 Issue 6 June 2024

Research Article

Phytochemical Investigation and Isolation of Active Constituent from Hydro-Alcoholic Extract of *Triticum aestivum* (Wheat Grass)

Paras Kumar Prajapati[1]*, Pankaj Mishra[2] and Pushpendra Kannojia[3]

[1]*Ph.D Research Scholar, BIU College of Pharmacy, Bareilly International University, Bareilly, UP, India*

[2]*Principal, Rohilkhand College of Pharmacy, Bareilly International University, Bareilly, UP, India*

[3]*Principal, BIU College of Pharmacy, Bareilly International University, Bareilly, UP, India*

***Corresponding Author:** Paras Kumar Prajapati, Ph.D Research Scholar, BIU College of Pharmacy, Bareilly International University, Bareilly, UP, India.

Email-Id: paras03py@gmail.com

DOI: 10.31080/ASPS.2024.08.1073

Received: May 09, 2024

Published: May 23, 2024

Abstract

The present study was based on the phytochemical investigation and isolation of active constituent from Hydro-alcoholic extract of *Triticum aestivum* (Wheat Grass). T. aestivum was grown under indoor conditions in Bareilly. The plant was authenticated by Dr. Alok Srivastava (Associate Professor) Plant Science. The authentication letter reference no. RU/PS/19/03 as issued from department of plant science MJP Rohilkhand University, Bareilly. Dried powdered leaves (50gm) and root (50gm) were extracted separately with Soxhlet apparatus using 500 ml of hydro-alcoholic solution (1:1) ethanol: water for 24 hrs. Using rotatory evaporator gthjeh(≤40°C) the extract was dried. The dried extract of leaves and root was subjected to phytochemical investigation. The compound was isolated and characterized for the physicochemical and spectroscopy analysis. In results, upon calculation, the % yield for *Triticum aestivum* leaves and root extract was obtained as 54% and 18%, respectively. Upon column chromatography, NMR, FTIR, UV and Mass spectroscopy analysis the isolated compound in leaves extract was reported as 2,27-diamino-3,26-dihydroxyoctacosan-12-one. The melting point, molecular weight, molecular and Rf value were obtained as 82.6ºC, 470.7872, $C_{28}H_{58}N_2O_3$ and 1.46, respectively. In conclusion, it was found as amorphous, yellow-whiteand crystalline in nature with fish like odor. In future prospective, it suggests to isolate diverse phytochemicals for the treatment of various human illnesses. The active constituents could be utilized in suitable dosage form for better bioavailability and pharmacological effect.

Keywords: *Triticum aestivum*; Active Constituents; Isolation; Column Chromatography; Mass Spectroscopy

Introduction

Triticum aestivum (wheatgrass) belongs to family-Poaceae (Gramineae) which has been a major nutritional source since the ancient era. Wheatgrass is widely recognised for its abundant chlorophyll content, making up 70% of its composition. Wheatgrass has been employed as a traditional herbal remedy and is highly valued for its therapeutic and nutritional characteristics [1]. The presence of bioactive elements such as phenolics, flavonoids, and other chemicals largely contributes to the effectiveness of plant extracts. During the process of germination, wheat sprouts incorporate nutrients, minerals, and phenolic compounds that contain flavonoids. This allows the wheat to reach its maximum antioxidant potential [2]. Flavonoids exert a diverse range of biological effects in various mammalian cell systems, as revealed by numerous in vitro and in vivo experiments. Rutin, also known as rutoside, quercetin-3-rutinoside, and sophorin, is a compound that is commonly found in various plants, notably *Triticum aestivum*. This flavonoid has a broad range of organic activities including antibac-

terial, anti-inflammatory, antioxidant, neuroprotective, antiviral, and antiulcerogenic properties. The thorough investigation of the anticarcinogenic efficacy of rutin is still pending [3,4]. Wheatgrass juice is abundant in Vitamins A, C, E, and B complex, which includes B12. The substance is rich in a wide variety of minerals, including calcium, phosphorus, magnesium, alkali earth metals, potassium, zinc, boron, and molybdenum [5]. The pharmacological actions of this substance are attributed to various proteins, including protease, amylase, lipase, cytochrome oxidase, transhydrogenase, and superoxide dismutase [6].

Figure 1: *Triticum aestivum* (Wheat Grass).

Another notable feature of wheatgrass is its abundance of amino acids, such as aspartic acid, glutamic acid, arginine, alanine, and serine. Wheat has been a crucial component of human diets across Europe, Anatolia, west Asia, and northern Africa for over 8000 years [7]. Wheat, as one of the three major cereal crops, is the main food crop cultivated globally, with an annual harvest of over 600 million tonnes. Bread wheat is a significant dietary source of plant sterols for humans. Turkey is one of the leading nations in the world when it comes to wheat production. Between 2011 and 2016, Turkey's wheat production exceeded 21.03 million tonnes [8]. Historically, it was commonly used to treat asthma, atherosclerosis, Parkinson's disease, joint aches, constipation, hypertension, diabetes, sleeplessness, bronchitis, sterility, haemorrhage, and obesity [9].

Taxonomy

Kingdom: Plantae
Division : Magnoliophyta
Class: Liliopsida
Order: Poales
Family: Poaceae
Genus: *Triticum*
Species: *Aestivum.*

It likewise has a high substance of bioflavonoids like apigenin, quercetin and luteolin. These compounds add to its cancer prevention agent movement. Different mixes present, which make this grass remedially successful, are the indole mixes, choline and laetrile (amygdalin) [10]. The present study was aimed tophytochemical investigation and isolation of active constituent of *Triticum aestivum* (Wheat Grass).

Materials and Methods

Cultivation and harvesting and of plant material

First of all, Wheat grass (*T. aestivum)* was grown under indoor conditions in Bareilly. Overnight soaked wheat grass seeds were used to cultivate. Little quantities of water were sprinkled evenly over soil and 3-4 hours of indirect sunlight projection was allowed daily for growth of grass, on tenth day wheat grass was harvested and used for further study.

Authentication of plant material

After complete process of dryness, prepared herbarium file of the plant and submitted for authentication in Department of Plant Science, MJP Rohilkhand University Bareilly. The plant was authenticated by Dr. Alok Srivastava (Associate Professor) Plant Science. The authentication letter reference no. RU/PS/19/03 as issued from department of plant science MJP Rohilkhand University, Bareilly.

Extraction of plant

Dried powdered leaves (50gm) and root (50gm) were extracted separately with Soxhlet apparatus using 500 ml of hydro-alcoholic solution (1:1) ethanol: water for 24 hrs. The solvent (500ml) added to each round bottom flask, which was attached to a Soxhlet extractor and condenser. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When, the Soxhlet chamber is gradually fill the level of solvent reaches the siphon it pours back into the distillation flask. This cycle was allowed to repeat many times for 24 hours then, the solution of extract placed on water bath for a few days at a 40-50 °C for the removal of solvents and obtained drug extract [11].

The % yield was calculating:
% Yield= Weight of extract (g)/Weight of dry powder (g) ×100

Phytochemical investigation (Qualitative Test)

In the second part of experiment, obtained dry extract of leaves and root were subjected to the process of phytochemical investigation for the identification and availability of active constituents. Following identification test were performed for the presence active constituents.

The hydroalcoholic dilution extracts were subjected to preliminary qualitative test for the presence of carbohydrates, proteins, amino acids, steroids, glycosides, saponins, alkaloids, tannins, phenolic compounds and flavonoids [12-15].

Test for carbohydrates

Molisch's test

To about 2 ml extracts, few drops of α-napthol (20% in ethyl alcohol) was added. Then about 1 ml of conc. H_2SO_4 was added along the side of test tube, reddish violet ring at junction of the two-layer appeared in the presence of carbohydrates.

Reduction of fehling's solution

10 ml of Fehling's solution (copper sulphate in alkaline condition) were added to conc. Extracts and heated on steam bath; brick red ppt. indicates the presence of carbohydrates.

Test for proteins

Biuret test

To 3 ml of extracts added 4% sodium hydroxide and few drop of 1% copper sulphate solution, violet or pink color appeared.

Million's test

Mixed 3 ml of extracts with 5 ml Million's reagent, white ppt., warm ppt. turn brick red or the ppt. dissolved to give color solution.

Test for amino acids

Ninhydrin test

Heated 3 ml of extracts added 2 ml chloroform and 2 ml of conc. sulphuric acid shake well, chloroform layer appears red and acid layer showed greenish yellow fluorescence.

Test for steroids

Salkowski test

To 2 ml of extracts added 2 ml chloroform and 2 ml of conc. sulphuric acid, shake well, chloroform layer showed greenish yellow fluorescence.

Liebermann-burchard reaction

The extracts evaporated to dryness and the residue was extracted with petroleum ether and acetone. The insoluble residue left after extraction were dissolved in chloroform and few drop acetic anhydride were added along with few drops of conc. sulphuric acid from the side the tube, the appearance of blue red color indicated the presence of sterols in the extract.

Test for glycosides

About 2 ml of extract were taken separately and subjected to the following test.

Killer-Killani test

1ml of glacial acetic acid containing traces of ferric chloride and 1ml of conc. sulphuric acid were added to extract carefully. A reddish-brown color is formed at the junction of two layer and upper layer turn bluish green in presence of glycosides.

Legal test

Concentrated extract was made alkaline with few drops of 10% sodium hydroxide and then freshly prepared sodium nitroprusside was added to the solution. Presence of blue colour solution indicated the presence of glycosides.

Test for saponins

The extracts evaporated to dryness and the residue was extracted with petroleum ether and acetone. To insoluble residue left after the extraction, a few ml of water was added and shaken well, and the residue gave a positive foam test in the presence of saponin.

Test for alkaloids

About 5 ml of alcoholic extract was evaporated to dryness and alcoholic residue was treated with 5 ml of 2% hydrochloric acid, saturated with sodium chloride and filtered. The filtrate was treated as following tests.

Dragendroff's test

To 2-3ml filtrate, added few drops Dragendroff's reagent, orange-brown ppt. was formed.

Wagner's test

To 2-3 ml filtrate, added few drops Wagner's reagent, reddish-brown ppt. was formed.

Test for tannins and phenolic compounds

Ferric chloride test

A few ml. of extract was evaporated to dryness and residue was further extracted with water then ferric chloride (5%) solution was added to it, blue-green color was formed in presence of phenolic compounds.

Vanillin-HCl test

In few ml of extract added Vanillin-HCl reagent [Vanillin (1g.) ethanol (10ml.) and Conc. HCl (10 ml.)]. A pink or red color is formed due to formation of phloroglucinol.

Test for flavonoids

Filter paper strips were dipped in the alcoholic solution of extract and ammoniated. The filter strips will turn yellow in the presence of flavonoid.

Isolation of active constituents by column chromatography

The dry leaves extract (20g) was first dissolved in 20 ml ethanol to prepare its solution form, this was later absorbed on 35g of Merc grade 60-120 mesh size silica gel for preparing the slurry. This slurry was then packed in to the column for isolation of the compounds. Three different solvent systems were used for isolation from non-polar to polar solvents. These solvents used were petroleum ether, 3% ethyl acetate in PET, 1:1 PET:DCM. A Borosilicate glass chromatography column with a fritted disc (30 mm x 600 mm) was used for the experiment. The isolated product from 1:1 PET:DCM was a white crystalline odorless compound whose melting point was 83.8ºC. TLC profiling was conducted to identify the appropriate solvent system to purify the extract. After using different combination of solvent system, a 30% Ethanol: 70% Dichloromethane solvent system was determined to be best system for purification of compound from extract. The compound is under characterization [16].

The dry root extract (5g) was first dissolved in 5 ml ethanol to prepare its solution form, this was later absorbed on 15g of Merc grade 60-120 mesh size silica gel for preparing the slurry. This slurry was then packed in to the column for isolation of the compounds. The same solvent systems (as in leaf extract isolation) were used for isolation of root extract, but no compound was found in root extract.

S. No.	Solvents used for Isolation	Polarity Index
	Hexane	0.01
	Petroleum Ether	0.1
	Toluene	2.4
	Dichloromethane	3.1
	Chloroform	4.1
	Ethyl Acetate	4.4
	Acetone	5.1
	Methanol	5.1
	Ethanol	5.2
	Water	10.2

Table 1

Identification of Physico-chemical properties

Melting point determination

Melting point determination: Thiel's melting point tube was used to determine the melting point of an organic compound (capillary tube method). The most important and straightforward means of distinguishing one compound from another is to determine its melting point.

Thin layer chromatography (Rf value)

TLC stands for thin layer chromatography and is used in synthetic chemistry to infer the production of a molecule based on its Rf value, which varies depending on the compound. It also aids in confirming the reaction's progress.

Infra-red spectroscopy

IR spectrum is considered as vibrational-rotational spectra. KBr pellet technique is used for solid compound, for liquid compound Nujol mull method is followed. It is very helpful record which would give information about functional group present in the organic compounds. Mechanism of bond stretching and bending is happened when electromagnetic radiation ranging from500cm-1 to 4000 cm-1 passed through sample [17].

1H NMR

Most commonly used NMR is Proton NMR, because of the sensitivity and wide range of characteristic information. Range of chemical 150 shift (δ) from 0 – 14 ppm. Chemical shift of the test unknown

compound was compared with TMS protons which are attributed at 0 ppm. But the shift extends for the organic compound range δ 0 – 14 for the component [18].

Mass spectroscopy

Mass spectrometry (MS) is an important physico-chemical tool applied for structural elucidation of compounds from natural products including medicinal plants. The fundamental principle of MS is the use of different physical means for sample ionization and separation of the ions generated based on their mass (m) to charge (z) ration (m/z). The ionization techniques available techniques include electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), electron ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), and matrix analysis laser desorption ionization (MALDI). Mass spectrometry has high sensitivity with detection limit of fentogram compared to NMR with sensitivity limit of nanogram range and above. The sensitivity and the flexibility for hypenation with other chromatographic technique made MS a versatile analytical instrument [19].

Results and Discussion

Percentage yield

Upon calculation, the % yield for *Triticum aestivum* leaves and root extract was obtained as 54% and 18%, respectively as shown in below tables.

Table 2: Yield of Leaves extract.

Powder drug (g)	Extract (g)	Yield (%)
50	27	54

Table 3: Yield of Root extract.

Powder drug (g)	Extract (g)	Yield (%)
50	9	18

Qualitative test

Triticum aestivum leaves and root extracts showed the presence of nature of different compounds on qualitative analysis. The result of qualitative test was summarized in the following table.

Table 3: Yield of Root extract.

Powder drug (g)	Extract (g)	Yield (%)
50	9	18

Table 4: Phytochemicals in *Triticum Aestivum L*eave extract.

Chemical classes	Tests	Result
Carbohydrates	Molisch's Test Fehling's Test	+
Protein	Biuret Test Millon's Test	+
Amino Acid	Ninhydrin Test	+
Steroids	Salkowski Leiber-mann-burchards Test	-
Alkaloids	Dragendroff's Test Wagner's Test	+
Glycosides	Legal's Test Killer-Killiani Test	-
Tannins	Lead acetate solution	+
Flavonoid	Ammonia Test	+
Saponin	Foam Test	+
Phenol	Ferric chloride Test	+

(+) = indicates presence of compounds, (-) = indicates absence of compounds.

Isolation of phytochemicals

In the present research, *Triticum aestivum* leaves successfully demonstrated the medicine value of wheat grass powder by studying its bio activities. Using column chromatography technique, it was successfully isolated one compound from wheat grass powder and were able to elucidate their complete structures by applying analytical techniques i.e., 1H NMR, IR, UV and Mass Spectrometry. On comparative and individual study of spectrum from these spectroscopy techniques the isolated compound was reported as 2,28-diamino-3,27-dihydroxynonacosan-12-one (shown below).

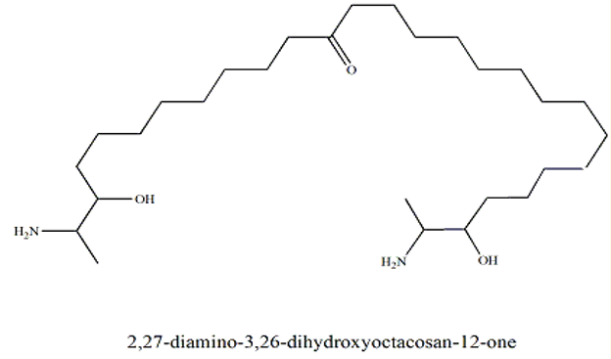

Figure 2: Structure of compound; IUPAC: 2,27-diamino-3,26-dihydroxyoctacosan-12-one.

Identification of physicochemical properties

Physical properties and Thin Layer Chromatography (Rf value)

The melting point, molecular weight, molecular and Rf value were obtained as 82.6ºC, 470.7872, $C_{28}H_{58}N_2O_3$ and 1.46, respectively. It was found as amorphous, yellow-white, and crystalline in nature, with fish like odor.

Table 6: Physicochemical properties of isolated compound.

Rf Value	Melting point	Molecular weight	Molecular formula
1.46	82.6ºC	470.7872	$C_{28}H_{58}N_2O_3$

FTIR

Fig. 3, 4, 5 and 6 represents the FTIR, UV, Mass and NMR Spectroscopy for the compound [2,27-diamino-3,26-dihydroxyoctacosan-12-one] as follows.

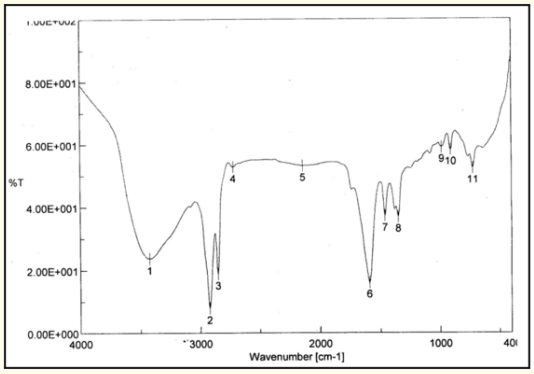

Figure 3: FTIR Spectrum of compound [2,27-diamino-3,26-dihydroxyoctacosan-12-one].

Interpretation

FTIR (KBr), cm -1): 3480.12 (O-H str), 2976.23 (1^0 N-H str), 1536.48 (C-O str).

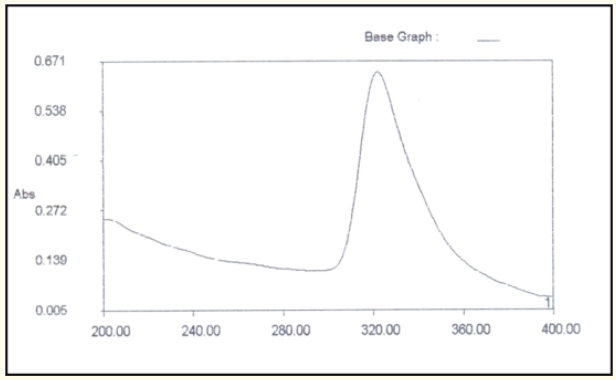

Figure 4: UV Spectrum of compound [2,28-diamino-3,27-dihydroxynonacosan-12-one].

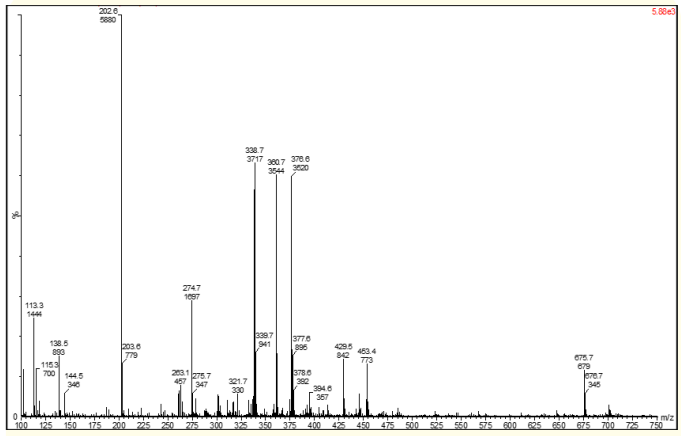

Figure 5: Mass spectrum of compound [2,28-diamino-3,27-dihydroxynonacosan-12-one].

Interpretation

MS (m/z): 676.7, 675.7, 453.4, 429.5, 394.6, 378.6, 377.6, 376.6, 360.7, 339.7, 338.7, 339.7, 321.7, 275.7, 274.7, 263.1, 203.6, 202.6, 144.5, 138.5, 115.3, 113.3.

Interpretation

1H NMR, δ ppm: 5.33-5.37 (DD,4H,NH2), 2.77-2.81(t,1H,CH), 2.04-2.31(M,2H,CH2), 1.59-1.69 (m,4H,CH2), 1.25-1.31 (M,2H,CH2), 0.86-0.99 (dt,6H,CH3).

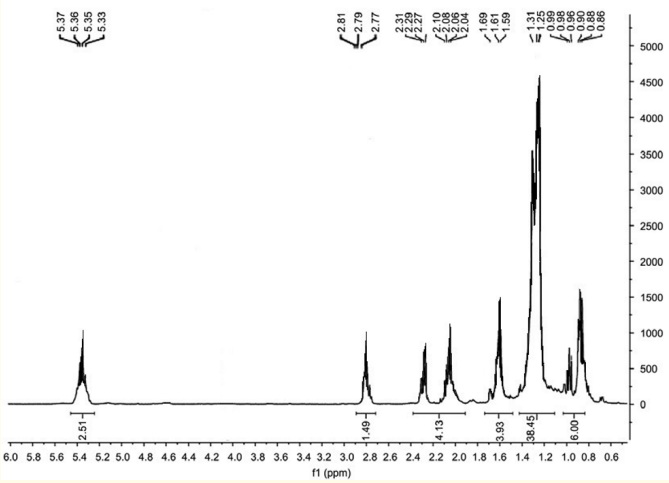

Figure 6: NMR spectrum of compound [2,27-diamino-3,26-dihydroxyoctacosan-12-one].

The previous investigation aimed to ascertain if the methanolic extract of *T. aestivum* hindered the production of nitric oxide. *Triticum aestivum* inhibited the generation of nitric oxide radicals from sodium nitroprusside at normal pH conditions. The study revealed a positive correlation between the quantities of the *T. aestivum* methanolic extract and its nitric oxide scavenging activities. The extracts demonstrate a comparable level of inhibition to that of ascorbic acid. The extract's ferric reducing activity varied between 0.06 and 0.15 at doses ranging from 200µg to 1000µg, whereas conventional ascorbic acid exhibited a range of 0.07 to 0.16. The FRAP values in the methanolic extract of *Triticum aestivum* were significantly lower when compared to ascorbic acid. The antioxidant chemicals can act as primary and secondary antioxidants by lowering the oxidised intermediates of the lipid peroxidation process. This is evident from their reducing power, which demonstrates their ability to donate electrons. FRAP tests are valuable for any laboratory or researcher studying oxidative stress and its effects. These tests offer a practical and informative measure of antioxidant defence that is easily accessible through technology. Figure 2.7 demonstrates a rise in absorbance from 0.06 at a concentration of 200 µg/ml to 0.15 at a concentration of 1000 µg/ml. This suggests that the ferric reducing ability of *T. aestivum's* methanolic extract is similar to that of synthetic antioxidants.

The presence of a significant amount of polyphenols and flavonoids in these herbs leads to a combined effect that enhances the anti-inflammatory capabilities of both herbs. According to a study, wheatgrass has been discovered to possess strong antioxidant capabilities at various concentrations during different stages of seed development [20]. According to a study conducted by Mat., *et al*. ascorbic acid found in coconut water has been found to prevent the oxidation of lipids in rats. Additionally, L-arginine, another component of coconut water, has been shown to reduce the generation of harmful free radicals [21].

Multiple studies have demonstrated that the coconut's capacity to eliminate free radicals is the primary reason for its heightened antioxidant characteristics [22]. The number 23 is enclosed in square brackets. The vitamins present in wheatgrass are plentiful and have the ability to eliminate free radicals. These vitamins are essential components of antioxidant defence systems. This may aid in regulating the amount of hydrogen peroxide that is emitted by the cells. Wheatgrass possesses potent anti-inflammatory effects and aids in the elimination of toxins from the body. Furthermore, it is rich in protein, amino acids, and exhibits antioxidant activity. A study conducted by Dasari., *et al*. demonstrated that wheatgrass had an anti-inflammatory impact on formalin-induced rat paw edoema [24].

The gel's overall improved efficacy may be attributed to the abundant presence of easily absorbed vitamins, enzymes, and other trace minerals in both phytomedicines. Furthermore, it has been emphasised that the antioxidant activity of these phytochemicals has a role in chemoprevention by reducing the oxidative stress that is responsible for the development of cancer [25].

Conclusion

Wheat grass (*Triticum aestivum*) is considered a highly adaptable plant in the field of medicine, since it has several sections that can be used for both human and animal health purposes. Wheat is a crucial component of the human diet, and the influence of bioactive compounds found in wheat on human health is substantial. The potential health-promoting components present in wheat grains and the complexity of studying their biological effects still require further investigation to fully understand their useful consequences. The wheat herb has been documented to possess several pharmacological properties, including anti-genotoxic, anticancer, anti-inflammatory, anti-diabetic, antacid, anti-hyperlipidemic, iron-chelating, fertility-enhancing, anti-hypertensive, antioxidant, and anti-hyperlipidemic effects. In conclusion, this plant has been

widely utilised for treating numerous disorders due to its distinct medicinal features and widespread availability.

In future prospective, it suggests to isolate diverse phytochemicals for the treatment of various human illnesses. The active constituents could be utilized in suitable dosage form for better bioavailability and pharmacological effect.

Funding

Nil.

Conflict of Interest

Authors have declared for none 'conflict of interest'.

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