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Phytochemical and Medicinal Aspects of Wheatgrass: A Comprehensive Review

Kamal Kishore[1], Vivek Sharma[2], H.S. Dhaliwal[3], Nandita Thakur[4*] and Dheeraj Vasu[5]
[1]Department of Chemistry and Biochemistry,
Eternal University Baru Sahib, Sirmaur (Himachal Pradesh) India.
[2]Department of Botany, Eternal University Baru Sahib, Sirmaur (Himachal Pradesh) India.
[3]Department of Genetics, Plant Breeding & Biotechnology,
Eternal University Baru Sahib, Sirmaur (Himachal Pradesh) India.
[4]Deparment of Sciences, Akal Academy, Baru Sahib, Sirmaur (Himachal Pradesh) India.
[5]Research Trend, Bilaspur (Himachal Pradesh) India.

(Corresponding author: Nandita Thakur)*
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ABSTRACT: Herbal medicines have been subjected to be more potable from ancient times because of its safeguard and inexpensive nature. One of the pioneer cereal crop wheat played an important role due to its nutritive and medicinal aspects. Various chemical constituents such as flavonoids, alkaloids, glycosides, terpenoids, saponins, enzymes, tannins, dietary fibres, and phenolic compounds have been analyzed in wheat seedlings which played important role in growth and maintenance of body tissues. The availability of bizarre vitamins like vitamin A, B-complex, C, E and minerals such as phosphate, zinc, molybdenum, potassium, alkaline earth metals, selenium make it healthiest remedy. Antioxidant enzymes superoxide dismutase and cytochrome oxidase have the ability to neutralize free radicals and changed them to desirable forms. Wheatgrass has recognized as green blood due to higher chlorophyll content that behaved as shield for cells to protect them from reactive oxygen species and supplied oxygen to enhance body's immunitic potential. The presence of secondary metabolites and minerals in seedlings possessed anticancer, antiulcer, antioxidant, antiarthritic, iron chelating and blood building properties.

Keywords: Wheatgrass, chlorophyll, secondary metabolites, oxidative stress, antioxidant, anticancer.

INTRODUCTION

Plants have played significant role in medicinal world for thousands of years. World Health Organization reported that up to 80% people believed in traditional remedies concerned with health activities, knowledge, therapies, medicines and techniques which aided to inhibit various illnesses for well beings. Traditional medicines categorized into three classes i.e. codified medicine, folk medicine and allied health knowledge. The professionalized system of codified medicine has comprised of Siddha, Unani and Ayurveda medicines which held the unusual perceptive of physiological and pharmacological processes. Folk medicineshad comprised of traditional knowledge imparted orally which created by communities and transferred from one generation to other. Such kind of knowledge was familiarized between local peoples. The diversity and adaptability of folk medicines have depended on changing circumstances not on formalization. The third type of traditional medicine included allied kinds of health knowledge which carried some techniques like yoga, percussion, tai-chi and various meditations (Payyappallimana, 2010). World Health Organization (WHO) compiled a kind of atlas at global level which gathered all the necessary information regarding traditional medicines by taking under consideration rules, regulations, policy terms, funding, knowledge acquisition, investigation, research and applications. The use of traditional medicines has increased worldwide because of its easier availability, adaptability, affordable price, technological input at lower level and favourableconsequences (Bodeker and Ong 2005). Wheat seedlings held amazing medicinal properties in spite of their nutritive values and more popularized due to bioavailability of phytoconstituents.

In 2010, survey of 1000 plants had been carried out in which 356 plants clinically reported for their therapeutic and pharmacological applications including wheatgrass. Wheat seedlings have contained chlorophyll pigments, amino acids, vitamins like A, C, E, B-complex, B_{12} and multiple minerals such as calcium, phosphorus, magnesium, potassium, iron, zinc and molybdenum (Meyerowitz, 2006; Rana *et al.,* 2011). The phytochemical constituents such as flavonoids, hydrocarbons, saponins, triterpenes, phytosterols, alkaloids, tannins, glycosides and proteinogenic compounds also found in it. Some indole compounds like amygdalin (vitamin B_{17}), choline and glycoside have been isolated from wheatgrass, acquired potency to

impede DNA oxidative damage (Padalia *et al.,* 2010; Falcioni *et al.,* 2002). Wheatgrass has ability to reduce inflammation, scavenge free radicals, counteract the effects of a carcinogen, reduce the harmful effects of bacteria, delay aging, immune-modulation, blood pH maintenance and reduced colon inflammation, renal impairment, atherosclerosis, wound infection and vitiated state of kapha and pitta. Its extract has also improved the digestion problems and more nutritious than the green vegetables like spinach and broccoli (Ferruzzia and Blakesleeb 2007). Intake of synthetic drugs, chemotherapy and radiation therapy caused serious health hazards. But the wheatgrass remedy helped to overcome these problems by targeting only on tumor cells and shielded healthy ones. Its chlorophyll enriched diet enhanced haemopoiesis and anti-mutagenic potential of hydrolyzed product chlorophyllin helped to combat discomfiture ill effects of chemotherapy (Lai, 1979; Te *et al.,* 1997). Alkaline nature of wheat sprouts assisted to reduce the increasing number of infectious microbes and cancerous cells. It has been control the altered physiological mechanisms of body and help to boost up the immunity (Liu and Wang 2008; Durairaj *et al.,* 2014a). Wheatgrass aqueous extract has normalized the uncotrolable growth of cancerous cells in oral squamous cell carcinoma (KB cell line) (Gore *et al.,* 2017). The apoptotic and anti-proliferative effects of wheat seedlings were also determined in case of K_{562} (CML i.e. chronic myeloid leukemia), Vero and HeLa cell lines (kidney cells and human cervical cancer cell line) (Aydos *et al.,* 2011; Patel, 2016). Excessive intake of red meat induced accumulation of haem content resulted to colon cancer. Wheatgrass based supplements helped to ward off its cytotoxic effects and maintain magnesium level in the body to accelerate biochemical reactions (de Vogel *et al.,* 2005; Balder *et al.,* 2006). It is an efficient, cost-effective and safer phytomedicine without any side effect.

HISTORICAL BACKGROUND

We are familiar with cereal grasses from ancient time due to its sacred, nutritive and medicinal values. The use of wheatgrass had been traced back in history over 5000 years to ancient Egypt and early Mesopotamian civilizations. Egyptians had used wheat seedlings for their religious purposes and health benefits. Charles F. Schnabel, a food scientist had started the use of wheatgrass in 1930s by performing experiments on chickens. He had mixed it with grains in different proportions and found that chickens taking higher amount of wheatgrass grew healthier with good egg production. He had got same results when these experiments carried out on other animals. Animals that took wheatgrass enriched diets were certainly healthier than others (Murphy and Sean 2006). Schnabel had used wheatgrass powder as an accessory nutritive diet for his family also. Wheatgrass had come into limelight and its production started at broad scale by large corporations like Quaker Oats and American Dairies Inc. Ann Wigmore wrote 'The Wheatgrass Book' which included lots of applications and he was the one who used 'Wheatgrass Therapy' for the first time. Wheatgrass based treatments were started by Ann Wigmore in her Hippocrates centre. Another book 'Wheatgrass: Natures Finest Medicine' was published in 1983 by 'Sproutman' Steve Meyerowitz. One of the colleagues of Ann Wigmore, Dr. Earp-Thomas had estimated 4700 grass varieties throughout the world which were beneficial for mankind. Ann had done lots of experiments on her pets and interpreted that wheatgrass really a best medicinal remedy. All these findings of past are carried valuable information for the present studies.

CULTIVATION TO PRODUCT FORMULATION

Wheat is used as a major staple food and consumed by many people as daily energy resource. Levant region has recognized as its origin place but now cultivated worldwide. Approximately 95% of the wheat production included the hexaploid species, particularly*Triticum aestivum* (common bread wheat) (Mayer, 2014). *Triticum* is a genus of annual and biennial grasses which yield various wheat species indigenous to Mediterranean regions and South-West-Asia. Itbelongs to the family Poaceaeand cultivated worldwide with 15-20 recognizable species. The bread wheat (*T. aestivum*) is the major source of wheatgrass and other species such as *Eremopyrum*, *Elytrigia*, *Pascopyrum*and some species of *Agropyron*like *A. spicatum*, *A. critatum* and *A. trachycaulum* belong to the temperate regions also used. Various developmental scales such as Zadoks, Haun and Feekes have been used to study the growth and developmental stages of wheat from seed sowing to harvesting in the form of numerical code. The growth stages are germination, seedling growth, tillering, jointing, booting, heading, anthesis, milk development, dough development and ripening. Seedling stage is the best one for wheatgrass harvesting that starts with the emergence of first leaf and ends at first tiller appearance. The 3-4 leaves and 5-6 seminal roots are found at this stage (Barber *et al.,* 2015). Wheatgrass consumed at seedling stage because maximum beneficial nutrients store in tender green blades. It can be grown in different mediums such as soil, sand and water. Its leaves have simple, flat, narrow and lanceolate shape with obtuse to acuminate leaf apex, acute rounded base and glabrous surface. Leaf surface has parenchymatous and thin-walled epidermal cells with graminaceous and oval stomata arranged in parallel rows. Abundance of chloroplasts occursin reniform shaped guard cells (Lae and Oo 2014).

Wheatgrass and its extracted juice further lyophilized to retain heat-labile components shown in Fig. 1. Lyophilization process has three phases freezing, primary and secondary drying that assisted to acquire final formulation with lesser moisture content (Wang, 2000; Jeff, 2009). Wheatgrass products commercialized in national and international market as fresh juice, frozen juice, tablets, powders, soap and cream. Their compositionis varying according to production processing as well as growing conditions. Different environmental parameters such as heat, temperature, light and humidity affect the quality of wheatgrass during processing. Variations in light intensity cause alteration in anti-oxidant properties. Sirvinta 1' and

'Ada' wheat varieties were recognized as more efficient than 'Tauras' 'Milda' and 'Alma' due to their higher oxidative stress releasing ability and presence of phenolic compounds, vitamin C, carotenoids like violaxanthin and zeaxanthin (Urbonaviciute *et al*., 2009). The wheat beverages of different flavors are also found in market with anti-ageing, anti-cancerous and hepatoprotective properties.

Chemical composition. Wheatgrass contained various phytopigments such as minerals, enzymes, vitamins, phenols and flavonoids in varying proportions.

Chlorophyll. Chlorophyll recognized as the most important constituent structurally similar to human hemoglobin. Magnesium is centrally located in chlorophyll, accelerated many enzymatic functions in our body. Chlorophyll actas an anti-bacterial agent to neutralize infection, heal lesions, reduce inflammation or pain and ward off parasitosis (Chernomorsky, 1988; Meyerowitz, 2006). The most effective water-soluble inducer chlorophyllin is derived from chlorophyll that carried defensive mechanism against harmful diseases and abundantly present in wheatgrass (Fahey *et al*., 2005).

Proteins and amino acids. Proteins play an indispensable role in enhancing the physical and muscular status of body. Immunoglobin, the major class of proteinshaseffective against pathogenic infections and accelerated metabolic processes such as haemopoiesis, heart strengthening and digestive activities. Amino acids like leucine, isoleucine, threonine, valine, methionine, phenylalanine, tryptophan, lysine, asparagines, arginine, aspartic and glutamic acid, alanine, histidine, serine and tyrosine have been found in wheatgrass (Bar-Sella, 1998). Higher proportion of arginine, glutamic acid, threonine, GABA (Gamma-aminobutyric acid), histidine, leucine and citrulline was determined in wheatgrass juice powder. The decarboxylation of glutamic acid resulted in synthesizing GABA i.e., directly proportional to the glutamic acid content. On the nutritional basis wheatgrass juice powder was analyzed as more effective than the shoot powder (Lee *et al.*, 2007; Ghumman *et al.*, 2017). Amino acids and enzymatic contents behaved as natural cleanser which brought out toxic substances from liver, blood and other body organs and control aging (Wheat and Currie 2008).

Vitamins. Vitamins are important part of nutritious diet for healthy body helped to regulate cell functioning and metabolic activities (Glavinic *et al.*, 2017; Khayat *et al.*, 2017). Human nutrition contained thirteen vitamins that classify on the basis of their solubility (Eggersdorfer *et al.*, 2012). Wheat seedlings are vitamin enriched resource contain retinol, riboflavin, thiamine, pantothenic acid, pyridoxine, inositol, cobalamin, ascorbic acid, tocopherol and phylloquinone. Vitamin-A assisted to improve eyesight and B-complex relieved the digestive problems, mental stress, premature aging, insomniac and anorexic disorders. Ascorbic acid and tocopherol enhanced the anti-oxidant potential and help to treat male sterility, maintain blood sugar level, control abnormal cell growth, cardiac disorders, dysmenorrheal and vitamin-K for blood clotting and age resisting (Mujoriya, 2011).

Minerals. Some of the significant minerals like magnesium, phosphorus, calcium, iron, zinc, copper, sulphur, boron, molybdenum and iodine have beendetected in wheat seedlings (Thakur *et al.*, 2019). Dietary intake of iron has acknowledged better than the inorganic iron help in haematopoiesis. Calcium strengthens the teeth, bones, regulate heartbeat and equilibrate blood pH. Potassium has recognized as a youth mineral which helped to reduce hypertension, physical and mental exertion, maintain fluid balance and improve renal dysfunctions. Presence of zinc has enhanced the enzymatic and defensive mechanism of body and sodium maintains fluid balance, regulate nerve and muscle functions. One of the vital constituent of cereal seedlings, magnesium assisted to strengthen the muscles, intensify immunity potential, improved heart and liver functioning (Mujoriya, 2011). It is acted as an activating agent for various systems of plant enzymes (Cu–Zn SOD) (Powell, 2000). Iron and zinc deficiency becamea major nutritional problem at universal level and approximately 30% of the world population has suffering from it (Maret and Sandstead 2006; Zimmermann and Hurrell 2007). According to a survey of NDNS (National Diet and Nutrition Survey) the cereal products primarily wheat carried adequate amount of minerals *i.e.*, essential for healthy well being (Bates *et al*., 2016).

Phenolic Compounds. Phenolic compounds are secondary metabolites formulated in plants against stress conditions which found as free soluble and bound insoluble forms in cereal grains (Beckman, 2000). Phenolic acids maintain equilibrium between oxidants and anti-oxidants that assisted to release oxidative stress in human body (Temple, 2000). They arecategorized as derivatives of benzoic acid and hydroxylcinnamic acid familiarized for their analgesic, tranquilizer, anti-pyretic, and anti-biosis characteristics (Waksmundzka-Hajnos *et al.*, 2007). Wheatgrass juice powder has higher amount of ferulic acid, sinapic acid and p-coumaric acid as compare to pulses powder (Mattila *et al.*, 2005). Their composition has varied according to genotype, growth, harvesting and environmental conditions (Mpofu *et al.*, 2006).

Enzymes. Enzymes have imperative role to sustain life by regulating metabolic mechanisms such as absorption of nutrients, digestion, detoxification, signal transduction and muscle contraction. Wheat seedlings have various enzymes like protease, amylase, lipase, cytochrome oxidase and superoxide dismutase. Lipase has hydrolyzed the fats in body tissues and preventsartherogenic risks. It is recognized as a flexible enzyme played significant role in pharmaceutical, cosmetics and food industries (Houde *et al.*, 2004). Protease and amylase have facilitated digestion and malic dehydrogenase fight against parasitic infection; regulate oxidative and metabolic pathways (Mujoriya, 2011). Superoxide dismutase and catalase were distinguished as antioxidant enzymes that converted superoxide anion into desirable forms (Samad *et al.*, 2014).

Flavonoids. Flavonoids are plant derived compounds assistance to intensify anti-oxidant and anti-cancerous

potential. Flavonoids such as apigenin, quercetin, rutin, epicatechin and luteolin were identified in wheat seedlings. Quercetin and luteolin have potential to revert carcinogenesis and apigenin used as an antineoplastic and inflammation reducing agent. Agropyrenehas anti-inflammatory properties and indole assisted in enzyme synthesis and carcinogen deactivation (Ben-Arye, 2002). Rutin and epicatechin have free radical scavenging potential. The most familiar flavonoids like quercetin and apigenin have possessed anti-bacterial potency (Wu *et al.,* 2008). Flavonoids helped to cease lipid peroxidation that responsible for several illnesses like arteriosclerosis, hepatic toxicity, diabetes and inflammation (Halliwell *et al.,* 1992).

PHYTOCHEMICAL SCREENING

Qualitative analysis. Qualitative analysis of wheat seedlings has revealed the presence of alkaloids, steroids, cardiac glycosides, tannins and flavonoids in hexane and chloroform extracts. Carbohydrates determined in methanolic extract while aqueous extract contain starch, α-amino acids, reducing sugars and saponins. Some chemical tests helped to detect the availability of phytoconstituents in seedlings represented in Table 1.

Chromatographic analysis. Wheatgrass has bizarre of phytoconstituents that determined by using advanced chromatographical techniques such as HPLC (High-performance liquid chromatography), HPTLC (High-performance thin-layer chromatography) and GC-MS (Gas chromatography-mass spectrometry) etc. The composition of phytopigments in wheatgrass has been detected by thin layer chromatography (TLC). Samples whether in powdered, juice and extract form have given the same TLC chromatogram of phaeophytin (Klimov, 2003), chlorophyll a, chlorophyll b (Willstater, 1928) and lutein compounds (Koushan *et al.,* 2013) (Figure 2). Reduced color intensity was observed in long term stored material due to chlorophyll loss (Lae and Oo 2014).

Research regarding quality control of herbal extracts is an area of great concern. The advanced chromatographic techniques like HPLCand HPTLC have implemented for qualitative and quantitative assessment of various plant extracts (Khan *et al.,* 2015a). Majority of the phenolic compounds in wheatgrasshave been existing as ester form which retrieved by alkaline hydrolysis. The monomeric phenolic acids such as gallic acid (Adefegha *et al.,* 2015), caffeic acid(Olthof *et al.,* 2001), ferulic acid(Gelinas and McKinnon 2006), p-hydroxy benzoic acid(Pugazhendhi *et al.,* 2005; Chong *et al.,* 2009), syringic acid (Kong *et al.,* 2008; Itoh *et al.,* 2010) ellagic acid (Narayanan *et al.,* 1999), quercetin, p-coumaric acid (Ferguson *et al.,* 2005), benzoic acid (Lillard, 1919) and bha (butylated hydroxyanisole) (Lam *et al.,* 1979) analyzed in wheat seedlings by using these techniques (Kardas and Durucasu 2014; Thakur *et al.,* 2020). Major bioactive constituents in seedling such as rutin(Metodiewa *et al.* 1997; Guardia *et al.,* 2001), tocopherol(Helzlsouer *et al.,* 2000), catechol (Fiege *et al.,* 2002), cinnamic acid (Liu *et al.,* 1995), quercetinand chlorogenic acid (Russo *et al.,* 2014; Johnston *et al.,* 2003)were separated by HPLC using specific solvent systems. The availability of these phytoconstituents in traditional medicines has significant potential against various pathogenic illnesses (Rajoria *et al*., 2015). Some of the important phenolic and flavonoid components are shown in Fig. 3.

The GC-MS has recognized as an analytical technique carried characteristics of gas chromatography and mass spectrometry helped to determine several plant constituents (Sparkman *et al.,* 2011). Saturated and unsaturated fatty acids like octadecatrienoic acid (Linolenic acid) and palmitic acid (Kumar *et al.,* 2010) alcohols like phytol and 2-methyl benzenediol (De Moraes *et al*., 2014; Boer and Jemec 2010) and terpenes for example amyrin, caryophyllene and caryophyllene oxide (Oliveira *et al.,* 2005; Gertsch *et al.,* 2008; Guimaraes-Santos, 2012; Yang *et al.,* 2000) were determined by Gas Chromatography-Mass Spectrometry (GC-MS) in wheat seedlings. Hexadecanoic acid (Palmitic acid) was identified as saturated fatty acid and octadecatrienoic acidas polyunsaturated fatty acid assisted to reduce harmful effects of toxic substances (Aparna *et al.,* 2012). Phytol, an acyclic diterpene was acted as an antecedent for contriving simulated forms of anti-oxidant vitamins and 2-methyl benzene diol used asa mediator in pharmaceutical formulations. Phytol as a cancer preventing agent has induced apoptosis and its potency determined in diverse cell lines such as osteosarcoma cells (MG63), human colon cancer (HT29), gastric cancer cells (AZ521) and Hep2 cells (Malek *et al.,* 2008).

Most of the extracted compounds belong to hydrocarbon group such as octadecene, nonadecene, 2-methyl octacosane, squalene, nonacosane and tricontane (Lee *et al.,* 2007; Mishra and Sree 2007; Durairaj *et al.,* 2014a; Dandekar *et al.,* 2015). Sitosterol, a plant steroid has overcome the metabolic disorders of diabetes mellitus and hypercholesterolemia that determined by liver and intestinal cell lines (Ho and Pal 2005; Balamurugan *et al.,* 2011). Squalene, a polyunsaturated hydrocarbon has halted the pathway of oxidative stress (Kabuto *et al.,* 2013). The anti-cancerous and free radical sequenzing potential of caryophyllene and its oxides have inhibited various pathways such as P13K, AKT, S6K1 and mTOR which persuade apoptosis (Park *et al.,* 2011). The bioactive compounds α and β amyrin have assistance to combat the ill effects of oxidative and inflammation stress (Pinto *et al*., 2008). The phytoconstituents of wheatgrass analyzed by GC-MS are shown in Fig. 4.

Liquefaction power of wheatgrass. Liquefaction, the enzymatic action of starch hydrolyzation has more efficiency and specificity comparative to acid hydrolyzation (Regassa and Wortmann 2014). Liquefaction hydrolyzed complex sugars into simple sugars by enzymatic action of α-amylase and amyl-glucosidase (Warren *et al.,* 2015). Amylase has hydrolyzed the starch molecules into glucose units which further examined by liquefaction power experiment. In liquefaction insoluble starch particles have dispersed into aqueous solution and partially hydrolyzed by thermostable amylases. Water revealed the consequential impact on enzymatic reactions by participating in solubilization, hydrolysis of

macromolecules like carbohydrates, proteins and lipids and metabolite transportation. Fresh wheatgrass juice has higher enzymatic potential as compare to shade dried powder which was examined by liquefaction (Lae and Oo 2014).

BIO-ACCESSIBILITY OF ESSENTIAL ELEMENTS

Some of the analytical techniques like AAS (Atomic Absorption Spectroscopy), ICP-AES (Inductively coupled plasma atomic emission spectroscopy), ICPMS ((inductively coupled plasma-mass-spectrometry) and INAA (instrumental neutron activation analysis) have used to identify the elements (Herber and Stoeppler, 1994; Zang *et al*., 1997; Rajurkar and Pardeshi 1997; Cao *et al*., 1998). The most popularized analytical technique, INAA was analysedeven the trace elements in different matrices. It has measured the elemental concentration more precisely because of proficient detecting limit and minimal matrix effect. Several biological samples such as leaves, roots of medicinal plants, cereals like wheat, barley, legumes and other food materials have subjected for elemental analysis. Growth conditions, developmental stages, food composition, chemical nature of components and elemental interactions have affected the bio-accessibility of essential elements (House, 1999). The fresh wheat seedlings have large proportion of bio-accessible materials as compared to grains and other commercialized products. It's due to the presence of elements such as Fe, Mn and Zn (Kulkarni *et al.,* 2007).

Medicinal aspects. Natural plant products are effectual and devoid of side effects. There is frequent need of suitable, safer and economic medicinal upshot which provide permanent cure from illnesses. Natural anti-oxidants have ceased the oxidative stress induced pathway and reduced the risk of disorders like diabetes, hypertension, cancer and ulcers (Zengin *et al.,* 2011).

Anti-oxidant activity. Plants have influential role in today's advanced and technological world. Innumerable plant species have been investigated for their medicinal importance, especially for the anti-oxidant properties. Anti-oxidants are acted as reducing agents like thiols, ascorbic acid and polyphenols which ceased the chain reactions by abolishing free radical intermediates (Sies, 1997). Human body has immanent anti-oxidative mechanism to fight against infection which could be enhanced by using natural formulations (Gulcin, 2012). The reactive oxygen species (ROS) have caused pathogenic conditions lead to many disorders like tumors, diabetes, inflammation, infertility, brain dysfunction and cancer. These disorders have impeded by anti-oxidant enzymes such as superoxide dismutase and peroxidase (Ben-Arye, 2002; Nunes, 2012). Phenolic and flavonoids enriched wheat seedlings have been used by many Ayurveda practitioners from ancient time because of their assistance to avert free radical oxidative stress pathways (Zendehbad *et al.,* 2014). Wheatgrass has potential to halt the oxidative damage of deoxyribonucleic acid (DNA), stimulation of gap junction communication, strive influence on cell transformation and differentiation, obstruct cell proliferation and ontogeny expression that accelerated tumors, and retarded endogenic emergence of carcinogens (Wheat and Currie, 2008). Antioxidant enriched wheatgrass potentialassisted to retrieve the free radical induced oxidative stress shown in Fig. 5.

Anti-cancerous activity. Cancer became the prime cause of increasing death rate throughout the world. Some of the commonly recognized cancers are lung cancer, colon cancer, blood cancer, breast cancer, bladder cancer, ovarian and prostate cancer. Radiotherapy, chemotherapy and chemotherapeutic drugs have adverse impact on human health. Polyphenol enriched wheatgrass has cancer preventive and apoptosis persuading potential that change the metabolic pathway of cancerous cells and inhibit TNF (Tumor Necrosis Factor) expression (Gupta *et al.,* 2014). Anti-proliferative and anti-oxidant potential of its extracts haveprotected normal cells from oxidative damage by ceasing tumor cell proliferation. Abscisic acids (ABA), one of the major constituent of wheatgrass has modulated calcium signaling and block chorionic gonadotropin hormone released by cancerous cells. Patients suffering from lung and breast cancers have increased level of lipid peroxidation and impairment in anti-oxidant defense mechanisms. Malondialdehyde (MDA), the product of lipid peroxidation has induced changes in DNA, proteins and other bimolecular functioning (Gonenc *et al.,* 2005). The presence of SOD (super oxide dismutase) and cytochrome oxidase enzymes in wheat seedlings have facilitated the reduction of MDA content during colon cancer by converting oxygen free radicals to hydrogen peroxide and water (Lakshmi *et al*., 2014). Wheatgrass has used to normalize and restore the increased level of Gamma GT during malignant tumors (Seth *et al.,* 2003). Quantitative analysis of LDH (lactate dehydrogenase) assured the level of pathological conditions in colon cancer persuades mice. Its increased level signified cell destruction that normalized by wheatgrass (Kher *et al.,* 1997; Lakshmi *et al.,* 2014). Carcino embryonic antigen (CEA) is released only during pregnancy and their increased level in non-pregnant women determined as cervical and gastrointestinal tumor indicator that normalized by wheatgrass therapy (Dearnaley *et al.,* 1981). Chlorophyllin, the constituent in wheat seedlings assisted to control the risks of cancer by ceasing activities of cytochrome P-450 liver enzyme (Guengerich *et al.,* 1991). Alkaline diet is helpful in cancer chemotherapy and reduction of microbial count in diet. Wheat seedling juice has pH around 7.4 so, it is considered as a reliable option for cancerous approach and the presence of chlorophyll advantageous for inhibiting metabolic activation of carcinogens (Pokhrel, 1999).

Anti-leukemic activity. Epigenetic defects have inhibited differentiation of blood stem cells cause hematological cancer, leukemia. The elevating WBC count has specified the symptoms of leukemia which imbalance platelet production and affect hemoglobin content. The chief phytoconstituents of wheat seedlings such as flavonoids and polyphenols have assistance to enhance neutrophil attractions and exhibited anti-

leukemic potency to promote apoptosis. Quercetin, genistein, flavopiridol, matairesinol, honokiol and arctigenin flavonoids have prevented fatal consequences of leukemia cells (Faderl and Estrov 2003; Liesveld *et al*., 2003; Khan *et al*., 2015b).

Anti-diabetic activity. Mostly all forms of diabetes have characterized by insulin deficiency but one of the persistent endocrine disorder i.e., diabetes mellitus (DM) responsible for metabolic disorders of carbohydrate, fat and protein (Boyle, 2001). On the basis of a report of World Health Organization (WHO) in 2010, 285 million people were suffered with diabetes and the number presumed to be exceeding in future because of changing dietary habits and physical indolence (Snehalatha and Ramachandaran 2009). Various medicinal plants have been reported to cure diabetes worldwide and according to WHO the treatment of diabetic patients with traditional plant remedies efficacious to normalize the blood sugar level and pancreatic tissues. The presence of secondary metabolites such as terpenoids, flavonoids, carotenoids, alkaloids and phenolic constituents in wheatgrass have potential to retard the intestinal absorption of blood sugar (Malviya *et al.,* 2010). Chlorophyll pharmacologically active component in wheat seedlings exhibited anti-cancerous and anti-diabetic properties (Shirude, 2011). It has induced anti-hyperlipidemic, hypoglycemic and free radical scavenging properties that controlled disintegration of DNA and pancreatic β-cells in streptozotocin treated diabetic rats. Wheatgrass has ability to decline the exalted level of triglycerides, cholesterol, VLDL and LDL (very low and low density lipoproteins). It has restored the potentiality of enzyme liver hexokinase, SGPT (serum glutamic pyruvic transaminase) and SGOT (serum glutamic oxaloacetic transaminase) (Mohan *et al.,* 2013). Amyrin, one of the important component of wheatgrass reported anti-hyperglycemic and hypolipidemic potency (Santos et al. 2012).

Haemopoetic activity. The presences of ABA (Abscisic acid) in wheat seedlings has nullified the adverse consequences of Human chorionic gonadotropin hormone (HCG) and facilitatedimmunitic potential against cancerous cells. The HCG has released by human placenta which balance the hormonal secretions of corpus luteum and safeguard for embryo against mother's immunity system (Singhal et al. 2012). Glutathione (GSH) reduction in cancer induced mice affect the hemoglobin level caused bone marrow malfunctioning. Glutathione as an anti-oxidant has halted cellular component deformities induced by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals (Naveena and Selvasubramanian 2011). The structural similarities of chlorophyll with hemoglobin have probability to fight against blood related complications and played major role in restoration of RBCs and WBCs count in colon cancer. The centralized magnesium in chlorophyll has imperative role in enzymatic synthesis and functioning (Jagatheesh *et al.,* 2010). Maltoheptaose has recognized as an immune modulatory bioactive compound of oligosaccharide fractions WG-PS3 (derived from wheatgrass) possessed systematic immunity-activating potential such as phagocytosis, antigen presentation and cytokine production. The maltoheptaose has revealed as a dynamic ligand of TLR-2 (Toll like receptor 2) activated the monocytes and increasedimmunitic potential (Lee *et al.,* 1998; Tsai *et al.,* 2013). The young seedlings of wheat helped to normalize hemoglobin level in thalassemic patients by overcoming the side effects of drug hydroxyurea (Fibach *et al*., 1993). The chlorophyll and its synthetic derivative chlorophyllin have significant role in intensifying the anti-oxidant potential of red blood cells (RBCs) and increased the life span of newly synthesized cells and transfused red blood cells (Fernandes and Donovan 2005). Daily doses of wheatgrass juice have helped to reduce the blood transfusion requirements in thalassemia patients without affecting their hemoglobin level (Marwaha *et al*., 2004). Chlorophyllin has induced the erythrocytes production, increase hemoglobin content in anemic animals, and contains expeditious therapeutic potential due to activation of connective tissues like granulation tissue and keratinocytes in epithelization (Gahan *et al.,* 1943).

Chelating activity. The polyphenol enriched wheatgrass has iron chelating potential assisted to minimize serum ferritin (Singhal *et al.,* 2012). The phenolic and flavonoid compounds have played influential role as membrane defender by scavenging free hydroxyl, peroxy radicals and quenched ferrozine-Fe^{2+} complex formulation (Ebrahimzadeh *et al*., 2008; Zendehbad *et al.,* 2014). The malfunctioning and disintegration of transfused RBCs have liberated iron in thalassemic patients which accumulated on spleen, liver, myocardium and endocrine organs as hemosiderin and ferritin (Taher *et al.,* 2006). Excessive deposition of iron has caused tissue destruction, heart failure, endocrine abnormalities, hypothyroidism, liver cirrhosis and even sudden death (Loukopoulos, 2005). Thalassemic patients have needed regular blood transfusion throughout their lifetime which caused iron overload that leads to liver cirrhosis, heart problem and faulty functioning of the body organs. Wheat seedlings have desirable potential for metal chelation because of higher availability of chlorophyll and magnesium that helped to overcome thalassemic deformities. The wheat seedlings as iron chelator hasassistance to reduce iron overloading (Tirgar *et al.,* 2011).

Hepatoprotective activity. Liver functioning immensely concerned with individual welfare because it expelled harmful and undesirable substances from body. The invigorating and regenerative potential of chlorophyll, therapeutic or healing properties of choline and higher mineral content have prime role as hepatoprotective agents to cease excessive accumulation of fats in liver. Choline has identified as a lipotropic compound helped in fat metabolism by changing fatty acids into transferrable forms in plasma. The hepatoprotective agents of wheatgrass such as choline helped to prevent fat accumulation, magnesium removed the excess fats, magnesium sulfate (Epsom salts) used to expel pus from infection and potassium behaved as a stimulant (Wigmore, 1985). Other compounds such as indole-3-carbinole and ascorbigen have increased the activity of phase I and phase II xenobiotic metabolic enzymes in

intestine and liver resulted to inactivation of carcinogens (Padalia *et al.,* 2010). Carbon tetrachloride (CCl_4) toxicity has produced consequential reduction in liver enzymes resulted to malfunctioning of plasma membrane and hepatic tissues that restored by using wheat seedlings (Jain and Argal 2014). Anti-oxidants like tocopherol, β-carotene and ascorbic acid have controlled ethanol induced oxidative damage of hepatic tissues (Datta *et al.,* 2012). The wheat seedlings have various hepatoprotective constituents shown in Fig. 6.

Anti-hyperlipidemic activity. Excessive intake of alcohol and oil rich PUFA (polyunsaturated fatty acid) have activated hepatic glycerol-3-phosphate acyl transferase, malic enzyme and fatty acid synthase that resulted to hypercholesterolemia (You and Crabb 2004). It ensued to obliterate cell membrane's major components like phospholipids and affected the structural stability and composition of bio-membranes that leads to liver dysfunctions. Wheatgrass is a rich source of ascorbic acid, tocopherol, phenolic acids such as ferulic acid, vanillic acid and flavonoids. Toxicity induced by alcohol and repeatedly heated polyunsaturated fatty acids (PUFA) imbalanced the LDL and HDL level which restored by ferulic acid (Kulkarni *et al.,* 2006; Nechifor and Dinu 2010). So, the presence of such potent anti-oxidants in wheat seedlings has ability to effectively reduce the lipid profile (Durairaj *et al.,* 2014b).

Anti-inflammatory activity. Wheatgrass juice has used to heal the wounds, reduce inflammation and odour. Inflammatory Bowel Disease (IBD) has caused intestine inflammation, ulcerative colitis and Crohn's disease. The disease IBD is mostly concerned with the urban areas due to increasing concentration of micro particles like titanium dioxide and aluminosilicates in diet that alter intestinal functioning. These antigens have activated the inflammatory cascade to liberate various inflammation mediators such as cytokines and tumour necrosis factors (TNF). Apigenin has identified as a chief bioflavonoid in wheatgrass that took part in TNF induced transactivation and balanced lipid peroxidation and anti-oxidant level. It also significantly controlled nephrolithiasis and cardiac deformities by modulating increased oxidative strain (Singh *et al*., 2004; Shah, 2007; Zhang *et al.,* 2020). Inflammatory disease rheumatoid arthritis has mostly found in females than males. Inflammation, laceration and reddening of joints initial indicators of rheumatoid arthritis caused deformities wart and lumps formulation. The regular intake of wheatgrass has minimized the ritchie index (articular measurement of joint tenderness) and exhibited consequential improvement in stiffness. Wheatgrass have multiple vitamins, minerals like magnesium, calcium, selenium, zinc, anti-oxidant enzymes such as superoxide dismutase, mucopolysaccharides and chlorophyll which helped to reduce the steroid dosage in patients. Inflammation reducing potential of wheatgrass has strived significant impact on rheumatoid arthritis treatment due to abundance of lactobacilli (Rana *et al.,* 2011).

Anti-ulcerative activity. Bioflavonoid enriched wheatgrass juice has knownas perfect remedy for ulcerative colitis due to its anti-inflammatory and anti-oxidant properties. It could be used as a promising medicine for plantar ulcers and the reasonable price might be favorable substitute in leprosy endemic areas of developing countries (Reynolds *et al.,* 2014). According to Dr. Wigmore, the wheatgrass constituents such as abscisic acid and laetrile have anti-cancerous and anti-ulcerative properties.

Anti-microbial activity. The bioactive components of wheatgrass like alkaloids, flavonoids, terpenoids, glycosides, tannins and saponins have anti-microbial potential. Wheatgrass has showed anti-microbial potential against gram-positive *Streptococcus mutans* and *Lactobacillus* spp. (Rajpurohit *et al.,* 2015). Anti-microbial potentiality of wheatgrass has depended upon the time of harvesting and solvent extraction. Hexane extract of seven day old wheatgrass has revealed higher anti-bacterial activity against *Salmonella paratyphi* and *Yersinia enterocolitica* than fourteen and twenty one day's old seedlings (Sundaresan *et al*., 2015). Lyophilized wheatgrass powder has considerable amount of polyphenolic compounds that efficient against microorganisms of all strains (Das *et al.,* 2011). The young seedlings of wheatgrass exhibited anti-microbial potential against gram-positive and gram-negative bacteria such as *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* and *Shigella flexneri* (Das *et al.,* 2012).

Anti-septic properties. In American Journal of Surgery (1940) Benjamin Gruskin, M.D. had supported wheatgrass for its antiseptic properties. It has used to reduce foul odors, neutralize strep infections and tonsillitis, wound healing, cure chronic rhino-sinusitis, reduce ear inflammation, lower varicose veins infection, leg ulcers treatments, eliminate contagious skin infection, cure rectal prolapsed, minimize gum infection, reduce uterine inflammation and parasitic vaginal infections. "Dr. Wheatgrass Skin Recovery Cream" has inflammation reductant, immunotherapeutic, hemorrhage reductant and wound healing properties. Chlorophyllin, the chief derivative of chlorophyll in wheat seedlings has triggered harmful bacterial growth. It is used as a wound healer to rehabilitate skin laceration, ulceration and burns (Gahan *et al*., 1943; Wheat *et al.,* 2006).

Anti-ageing properties. Superoxide dismutase (SOD) is naturally occurring substantial anti-oxidant enzyme that delays the aging process by decomposing superoxide radicals. It has known as foremost safeguard against oxidative stress mediated deformities (Landis and Tower 2005). The uncontrolled oxidative deterioration by free radical induction has affected the biomolecular functioning of cell organelles and resulted to aging (Hekimi and Guarente 2003). Wheat seedlings are used as natural stimulant to reinstate the SOD level and assisted to extend life span (Levin, 2005).

Anti-allergic potential. Wheatgrass has pivot role as anti-allergic agent due to active minerals and vitamins. Zinc has probability to minimize pro-inflammatory cytokines activity and magnesium acted as bronchodilator that dilated bronchi and controlled respiratory infections (Prasad, 2009). Tocopherols minimized IgE associated allergic effects by triggering

the pathway of inflammativecells (Fogarty *et al.*, 2000). Luteolin and quercetin bioflavonoids have reverted the effects of allergic mediators leukotriene, histamine, prostaglandin and GM-CSF (granulocyte macrophage colony stimulating factor) (Kimata *et al.*, 2000).Wheatgrass flavonoid, Isoorientin has inhibited the histamine and leukotrienes secretion in lung mast cells of guinea pig (Kim *et al.*, 2010).The allergic immunological responses have inhibited by wheat sprouts in OVA (ovalbumin) sensitized mice by suppressing Th2 differentiation mediated by transcription factor (GATA-3) and Interleukin-4 (IL-4) activation (Ki *et al.*, 2017).

Coating agent. Wheatgrass juice has also used as coating agent. Edible coatings help to delay ripening process and act as barrier between fruits and external environment. The wheat seedling juice based edible coating was applied on bananas and its effectiveness checked by taste, odor, peel coloration, weight loss and acidity parameters. It has observed as reducer of high acidic proportion of coated fruits and enhanced storage capacity (Ratra *et al.*, 2016).

Other activities

Wheatgrass increased enzyme synthesis that balance sex hormone and assisted to restore fertility (Eissa *et al.*, 2020). It strengthens cardiac and pulmonary potential, lower septicemia level and minimized adverse effects of carbon dioxide. These young seedlings have used as perfect therapy for digestive dysfunctions such as constipation, dyspepsia, nausea, acid reflex, gastric ulcers and intestinal malfunctioning (Jacob *et al*., 1998).

Table 1: Chemical tests for qualitative analysis of wheatgrass constituents.

Components	Chemical Test	Indication	References
Alkaloids	Mayer's reagent test	Cream color	(Harborne, 1973); (Trease and Evans, 1983)
	Wagner's reagent	Reddish brown coloration	
	Hager's test	Yellow color precipitates	
	Dragendroff's test	Reddish brown precipitates	
Glycosides	Sodium hydroxide test	Yellow color	(Harborne, 1973); (Sofowora, 1993)
Steroids	Salkowski's test	Pinkish red color	(Kumar *et al.*, 2012)
Saponins	Foam test	Froth formation	(Kumar *et al.*, 2012)
Phenols	Ferric chloride test	Bluish green color	(Harborne, 1973)
Tannin	Lead acetate test	Blue green color	(Trease and Evans 1989)
Flavonoids	Shinoda test	Red to pink color	Prabhavathi *et al.*, 2016
	Alkaline reagent test	Yellowish precipitates	
Terpenoids	Salkowiski Test	Reddish brown color	(Edeoga *et al*., 2005)
Carbohydrate	Fehling test	Brick red precipitates	(Harborne, 1973; (Trease and Evans1989)
	Benedict 's test	Red precipitates	
	Molisch's test	Violet ring	
Proteins and Amino acids	Xantho protein test	Orange color	(Kokate *et al.*, 2003; (Khandelwal, 2010)
	Millons test	Red precipitates	
	Ninhydrin test	Bluish color	

Fig. 1. Wheatgrass juice extraction and lyophilization.

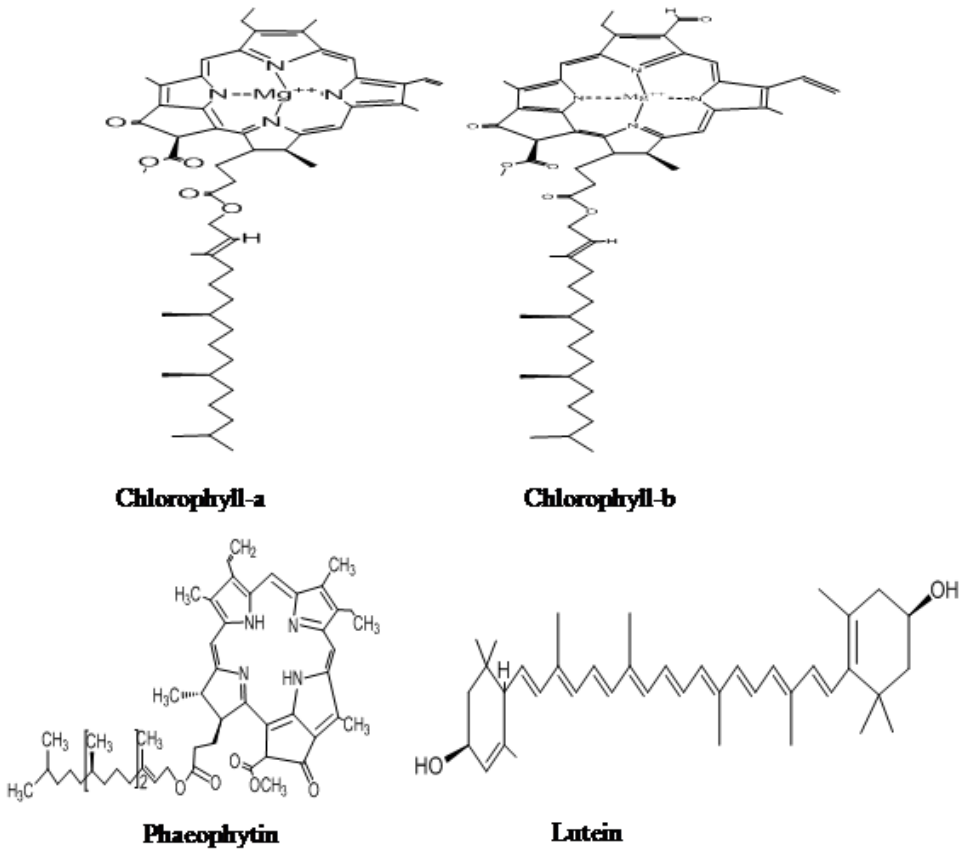


Fig. 2. Wheatgrass constituents detected by thin layer chromatography (TLC).

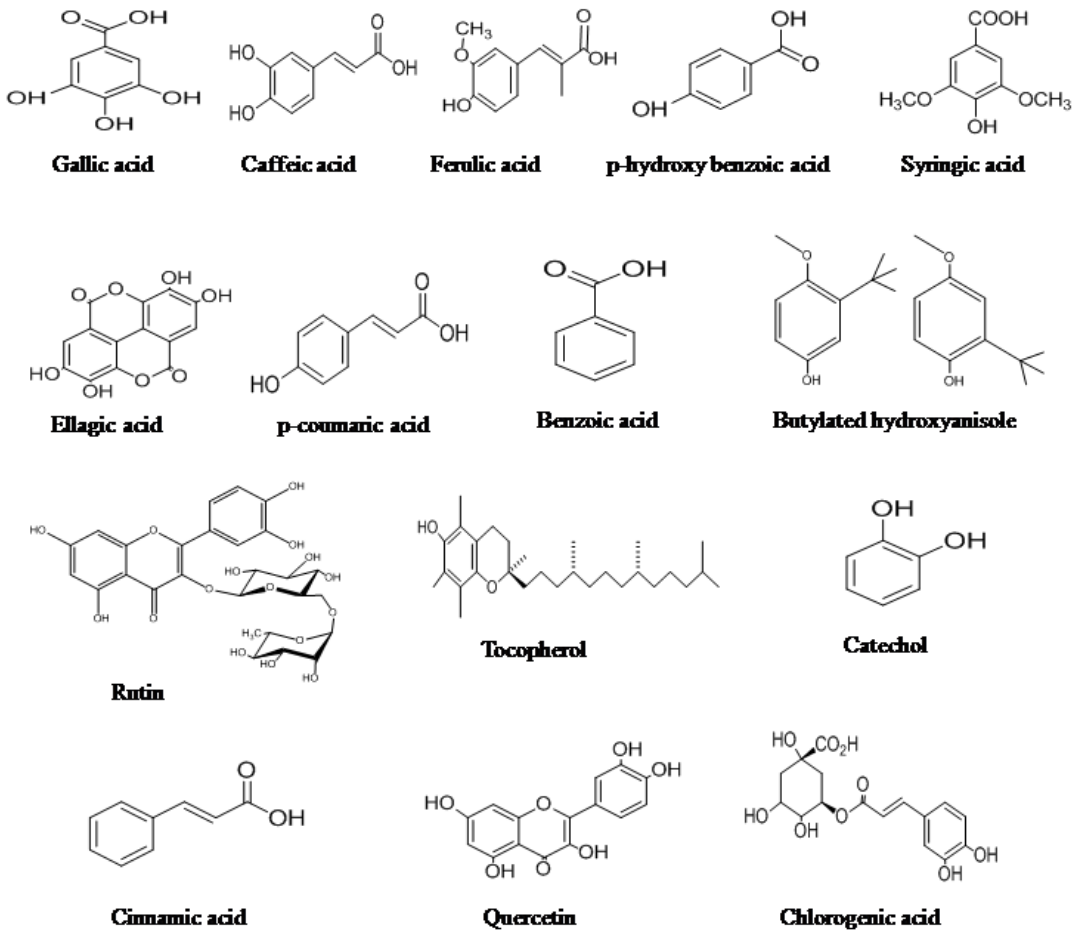


Fig. 3. Phenolic and flavonoid constituents present in wheatgrass.

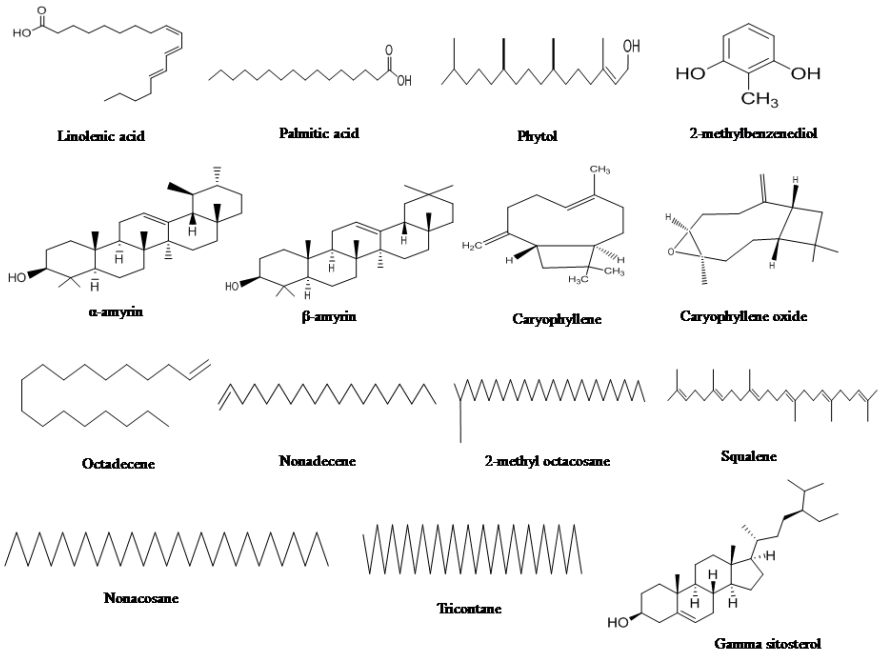
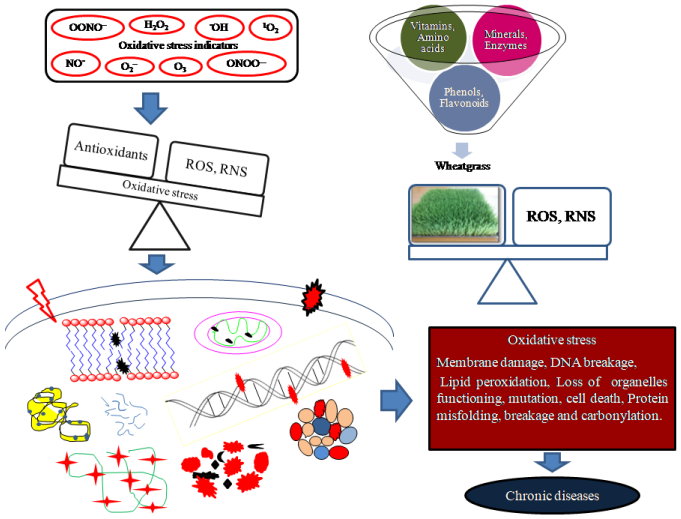


Fig. 4. Wheatgrass componentsdetermine by gas chromatography-mass spectrometry.



ROS – Reactive Oxygen Species, RNS – Reactive Nitrogen Species.

Fig. 5. Free radical induced oxidative stress retrieve by antioxidant enriched wheatgrass.

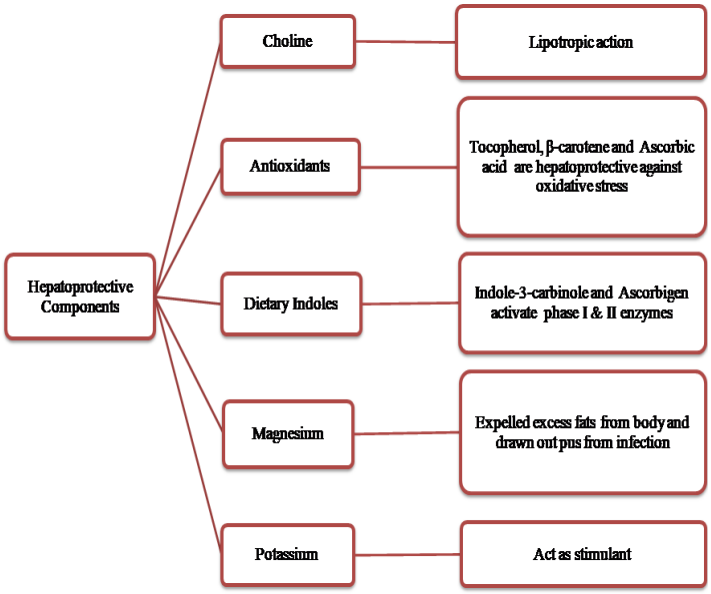


Fig. 6. Hepatoprotective constituents in wheatgrass.

CONCLUSIONS

India has biodiversity richness and the medicinal plants availed approximately 80% raw material for drug formulation. Medicinal plants demand is increasing day by day due to acute product formulation in pharmaceutical, food and cosmetic industries. The young shoots of wheat have beneficiary role in human life and it must be added into human diet chart to improve nutrient balance. Wheatgrass enriched with life saving chlorophyll, vitamins, minerals, polyphenols, amino acids, saponins and fibres. It has used to heal various illnesses sch as tumour, diabeties, heart problems, inflammatory and ulcerative diseases etc. Various wheatgrass basedproducts launched in market with some new innovative prospectives by claiming their effectiveness. Wheatgrass based products have natural anti-oxidants and bioactive constituents which are capable to avert the track of noxious illnesses. It has much pharmacological diversity in addition to its nutritional values which yet to be explored furthermore.

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