EFFECT OF GROWTH DAYS ON BIOACTIVE COMPOUNDS OF WHEATGRASS POWDER (*Triticum aestivum*) AND SENSORY PARAMETERS

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Effect of Growth Days on Bioactive Compounds of Wheatgrass Powder (*Triticum aestivum*) and Sensory Parameters

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Approval Letter

This *dissertation* entitled *Effect of Growth Days on Bioactive Compounds of Wheatgrass Powder (Triticum Aestivum) and Sensory Parameters* presented by Sarju Baral has been accepted as the partial fulfillment of the requirements for the B.Sc. degree in Nutrition and Dietetics.

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……………………………….

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Abstract

Bioactive compounds of wheatgrass powder *(Triticum aestivum)* was determined at different stages of growth. Wheatgrass was grown by planting germinated wheat seeds and harvesting on 6th, 7th, 8th, 9th and 10th days manually. Wheatgrass was then subjected to cabinet drying (50°C, 5hr) and powdered separately. The crude extracts of samples were prepared using 80% of methanol using maceration technique for analysis of bioactive compounds. Sensory analysis of samples was carried out by group of semi-trained panelists. Experimental data was analyzed using software Genstat 12th Edition.

Growth of wheatgrass had significant impact on bioactive compounds and sensory characteristics. Total phenol, flavonoid and antioxidant activity of wheatgrass was found to increase with growth from 318.07±0.65 mgGAE/g, 105.7±33.29 mgQE/g, 80.48±1.27% on 6th day to 454.7±2.84 mgGAE/g, 429.4±66.06 mgQE/g, 93.09±0.63% on 10th day respectively. Tannin content of wheatgrass decreased significantly with growth from 10.24±1.04 mgGAE/g on 6th day to 2.077±0.01 mgGAE/g on 10th day. Chlorophyll content first increased with growth from 10.86±0.09 mg/g on 6th day to 14.27±0.06 mg/g on 8th day and decreased as 9.39±0.10 mg/g and 7.12±0.07 mg/g on 9th and 10th day respectively. Analysis of bioactive compounds and sensory attributes concluded wheatgrass of 10th day as superior sample among other samples.

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List of Abbreviations

Abbreviation	Full Form
NSA	Non-starch polysaccharides
SFE	Supercritical fluid extraction
ROS	Reactive oxygen species
TPC	Total phenol content
TFC	Total flavonoid content
GAE	Gallic acid equivalent
QE	Quercetin Equivalent
L.S.D	Least Significant Difference

PART I

Introduction

1.1 General Introduction

Herbal medicine is still the mainstay of about 75% of the world population, especially in the under developed and developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, in the last few years there has been a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs (Desai, 2005). In the developing countries, many low-income families rely on a simple diet which mainly consist of staple food crops such as wheat, maize and rice that are poor sources of various nutrients and minerals. To provide a wholesome nutrition for a healthy and rejuvenating body, green foods could be very useful in providing nutrient like vitamin, proteins, minerals and antioxidants which are researched for numerous health benefits in USA, East Asian countries and Central Europe (Singhlal *et al.*, 2012). Medicinal plants have a promising future because there are about half million plants around the world and most of their medicinal activities have not been investigate yet and their medical activities could be decisive in the treatment of present or future studies (Singh, 2015).

Cereal grasses such as wheat, oat, barley and brown rice are most familiar cereal grasses and also one of the "functional foods" that is gaining recognition as a potential nutritional product which has medical and health benefits (Chaturvedi *et al.*, 2013). Wheat is the third important cereal crop of Nepal after rice and maize both in area and production (Thapa, 2010). Wheat (*Triticum aestivum*) germinated over a period of time is called as a wheatgrass, belonging to family: *Gramineae* (*Poaceae*). *Triticum* is a genus of annual and biennial grasses, yielding various types of wheat, native to south west Asia and the Mediterranean region (Verma *et al.*, 2012).

Wheatgrass is a humble weed that is a power house of nutrients and vitamins for the body. During germination, vitamins, minerals, and phenolic compounds including flavonoids are synthesized in wheat sprouts reach the maximum antioxidant potential (Aydos *et al.*, 2011). Wheatgrass supplement is available commercially in liquid, powdered or concentrated forms, depending on the supplier and can be consumed on its own, or mixed with fruit juices (Chauhan, 2014). Availability of wheatgrass in powder form has proven to be very

convenient and effective. Wheat grass powder retains all important nutrients of wheatgrass and having higher quantity of dietary fiber, cost effective and higher shelf life than wheat grass juice. Wheat grass powder can be easily included in the daily life of average age group which can definitely increase their vitamins, minerals and antioxidants intake and in return it will protect the body from degenerative diseases (Singh and Singh, 2016).

1.2 Statement of the problem

Wheatgrass is a food prepared from the cotyledons of the common wheat plant, *Triticum aestivum.* It is sold either as juice or powder concentrate. Diet occupies an important place during sickness and healthy condition. Our body has the inbuilt ability to heal itself if provided proper nutrition, environment and exercise. Wheatgrass is renowned for its therapeutic value since ancient times. The beneficial nutrients naturally obtained from wheatgrass helps to promote health and healing (Jain and Jain, 2014).

Cereal sprouts and microgreens (known as wheatgrass) may represent a valid alternative in human feeding. Normally sprouts are used as components of salads, although it has been proposed that dried sprout power could be blended in certain proportion with conventional flours in order to increase the nutritional value of flours and derivatives without changing people's feeding behaviors. On the other hand, wheatgrass is often used to extract drinking juice with several expected benefits (Benincasa *et al.*, 2014).

The modern medicines are very efficient in curing diseases but they produce a number of side effects. On the contrary, the crude drugs are somewhat less efficient than modern medicines, but they are relatively free from side effects which made the traditional system of medicine to create the competition to modern day medicine (Ashok, 2011). Wheatgrass is easy to cultivate at home level but health benefits of wheatgrass is still unknown to mankind. Growing wheatgrass for daily availability to consume in fresh form is difficult and tedious task, thus most of the people do not prefer to use the wheatgrass. So, in order to ease its adaptation, the enmass growing and drying can be done to make available the wheatgrass to the common people. Wheatgrass in powder form is a method for preserving the wheatgrass nutrients with long shelf life and use of wheatgrass powder is more convenient than wheatgrass juice. Therefore, it is necessary to learn about its functional value and to use it as a medicinal drug.

1.3 Objectives of the study

1.3.1 General objective

The general objective of the work was to study the effect of growth days on bioactive compounds of wheatgrass powder *(Triticum aestivum)* and their sensory attributes.

1.3.2 Specific objective

a) To grow wheatgrass.
b) To prepare wheatgrass powder.
c) To prepare the methanolic extract of wheatgrass powder.
d) To study the effect of growth days on bioactive compounds of wheatgrass powder.
e) To evaluate the antioxidant activity of the extract of sample.
f) To evaluate the sensory parameters of samples.

1.4 Significance of the study

Wheat (*Triticum aestivum*) has a crucial role on human diet in most countries. Many epidemiological studies have proposed that adding whole grain and whole grain products to the diet has a protective contribution against chronic diseases. Following germination of wheat for 6-10 days, sprouts formed are called wheatgrass. Changes during germination and sprouting lead the plants to synthesize compounds like vitamins, antioxidants or phenolics. The most important active component in wheatgrass is chlorophyll that is known to have an active role for the inhibition of the metabolic activation of carcinogens (Akbas *et al.*, 2017).

At present, wheatgrass is quickly becoming one of the most widely used supplemental health foods and is available in many health food stores as fresh produce, tablets, supplemental health foods, frozen juice and powder. In developing countries like Nepal, introduction of wheatgrass can be very effective as people here mostly rely on herbal medicine rather than modern drugs due to their higher cost and limited access. Therefore, this study is preliminary effort towards finding the bioactive compounds present in wheatgrass powder to enhance its consumption.

1.5 Limitations of the study

a) Single extraction technique was used to prepare the extract.

PART II

Literature review

2.1 Wheatgrass

Wheatgrass, (*Triticum* speices) a cereal grass of the *Gramineae* (*Poaceae*) family, is the world's largest edible grain cereal-grass crop. Wheatgrass refers to the young grass of the common wheat plant (Mujoriya, 2011).Wheat has been a food crop for mankind since the beginning of agriculture. Wheat has been appreciated as a source of carbohydrates and proteins (albeit poor in some essential amino acids, especially lysine) but also contributes a significant proportion of fibre, minerals and antioxidant compounds such as phenolic acids and tocols, to the human diet (Hidalgo *et al.*, 2016; Ward *et al.*, 2008). For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in the levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant. The young germinated plant is a factory of enzyme and growth activity. In the early stages of growth they store large amounts of vitamins and proteins in the young blades. After jointing stage, the nutrition level in the leaves drops rapidly while the fiber content increases rapidly. Agriculturally, important species of *Triticum* include – *Triticum asetivum, Triticum durum* and *Triticum dicoccum* (Desai, 2005).

2.2 History on consumption of wheatgrass

Wheatgrass can be traced back in history over 5000 years, to ancient Egypt and perhaps even early Mesopotamian civilizations. It is purported that ancient Egyptians found sacred the young leafy blades of wheat and prized them for their positive affect on their health and vitality.

More recently, the consumption of wheatgrass in the Western world began in the 1930s as a result of experiments by Charles F. Schnabel and his attempts to popularize the plant. Schnabel, an agricultural chemist, conducted his first experiments with young grasses in 1930, when he used fresh cut grass in an attempt to nurse dying chickens back to health. The hen not only recovered, but they produced eggs at a higher rate than healthy hens. Encouraged by his results, he began drying and powdering grass for his family and neighbors to supplement their diets. The following year, Schnabel reproduced his experiment and achieved the same results. Hens consuming rations supplemented with grass doubled their

egg production. Schnabel started promoting his discovery to feed mills, chemist and the food industry. Two large corporations, Quaker Oats and American Diaries Inc., invested millions of dollars in further research, development and production of products for animals and humans. By 1940s, cans of Schnabel's powdered grass were on sale in major drug stores throughout the United States and Canada. Sometime during the 1940's a lady by the name of Ann Wigmore healed herself of cancer from the weeds she found in vacant lots in Boston. She began a study of natural healing modalities and with the help of a friend, Dr. Earp Thomas, she found that there are 4700 varieties of grass in the world and all are good for man. With the help of her pets, she arrived at the conclusion that wheatgrass was the best, or the medicinal grass (Seymour, 2015).

At present, the wheatgrass is available in the form of products such as health supplements (powders, extracts) and medicines (tablets). These are not so much popular since they are consumed only by people in poor health conditions (Singhlal *et al.*, 2012). Nowadays, wheatgrass juice is consumed by people in different areas of Nepal.

2.3 Nutritional aspect of wheatgrass

Wheatgrass is a humble weed that is a powerhouse of nutrients and vitamins for the human body. *Triticum aestivum*, freshly juiced or dried into powder are fit both for animal and human consumption. Both provide chlorophyll, amino acids, minerals, vitamins and enzymes. In the form of fresh juice, it has high concentrations of chlorophyll, active enzymes, vitamins and other nutrients.

Wheatgrass juice has chlorophyll that neutralizes infections, heals wounds, overcomes inflammations and gets rid of parasitic infections. The three most important effects of wheatgrass on the human body are: blood purification, liver detoxification and colon cleansing. This is because wheat grass is the richest source of vitamins A, B, C, E and K, calcium, potassium, iron, magnesium, sodium, sulphur and 17 forms of amino acids. Wheatgrass is high in oxygen like all green plants that contain chlorophyll. The brain and all body tissues function at an optimal level in high-oxygenated environment. The pH (hydrogen molecules) value of human blood and wheatgrass both is about 7.4 (alkaline) and therefore quickly absorbed in the blood and is highly beneficial. Wheatgrass juice is found to be great for blood disorders of all kinds (Mujoriya, 2011).

2.4 Germination

In plant life cycle, seed germination is an important development phase, playing a critical role in seedling establishment and overall adaptation. The ability of a species to germinate rapidly under a broad range of environmental conditions has been regarded as a critical traits for invasive species (Ye and Wen, 2017). Germination is a complex process causing physical, chemical and structural changes in grains, has been identified as an inexpensive and effective technology for improving cereal quality. The germination process is characterized by the growth of the embryo of the grain, manifested by the rootlets growth and increase in the length of the shoot (acrospire), with the concomitant modification of the contents of the endosperm (Senhofa *et al.*, 2016).

2.4.1 Effect of germination on nutrient content

Germination of cereals has been used for centuries to soften kernel structure, to increase nutritional content and availability, to decrease the content of antinutritive compounds, and to add new flavours without knowing the biochemistry behind these phenomena (Norja *et al.*, 2004).

Germination of grain commences with the uptake of water. Once germination is initiated, the predominant endosperm reserves, starch, cell wall, and storage proteins, are mobilized by the action of hydrolytic enzymes, which are synthesized in the aleurone layer and in the scutellum and secreted into the starchy endosperm of germinating grains (Lu *et al.*, 1998; Shaik *et al.*, 2014). During germination, endogenous enzymes of cereal grains are activated and some major substances such as starch and protein undergo degradation to small molecules. For example, a significant decrease in starch content is found during germination of rice. Furthermore, some functional compounds can be enriched, meanwhile some anti-nutrition factors, such as phytic acid, is degraded during germination. Current studies indicated germination enriched β-aminobutyric acid in brown rice (*Oryza sativa)*, wheat and foxtail millet (*Setaria italic*). Germinated cereal grains show higher total phenolic content and antioxidant activity than those of un-germinated rice, wheat and oat. The germination process improves the nutritional quality of cereal. During the process of germination, significant changes in the biochemical, nutritional, and sensory characteristics of cereals occur due to degradation of reserve materials as used for respiration and synthesis of new cell constituents for developing embryo in the seed (Danisova *et al.*, 1995; Sharma *et al.*, 2016).

Germination is reported to improve the nutritive value of pulses, particularly in the level of vitamins such as riboflavin, niacin, choline, biotin and ascorbic acid. Besides, it brings biochemical changes such as converting more indigestible carbohydrates into digestible and absorbable forms. Furthermore, the germination process reduces or eliminates most of the antinutrients, such as phytates, tannins, oxalates, etc., present in the legumes and also renders the sprouted legumes comparatively safe and delicious. The activities of enzymes such as amylase, protease, phytase, etc., are increased during germination that helps in digestion of complex biomacromolecules into simpler absorbable forms such as individual sugars, simple peptides and amino acids (Chaudhary *et al.*, 2014).

As compared to un-germinated seed, germinated seeds contain high protein, low unsaturated fatty acids, low carbohydrate and mineral content (Narsih, 2012; Sharma *et al.*, 2016). Alpha-amylase enzyme plays an important role in native starch granule degradation, and its expression is controlled by both gibberellin and sugar demand/ starvation. Sugar on carbon starvation activates alpha-amylase promoter (Lu *et al.*, 1998; Shaik *et al.*, 2014). As a result, during germination amylases are produced and partial breakdown of starch into simple sugars occurs (Chesworth *et al.*, 1998). Intense biochemical processes during the grain activation (the first stage of germination); as a result, grain biological value increases. The content of vitamins B_2, E and niacin, total sugar, dietary fibre and glucosamine increase; vitamin C is synthesized, and the content of irreplaceable amino acids is increased during the process of protein hydrolysis (Senhofa *et al.*, 2016).

2.5 Drying

Drying is an ancient technique of food preservation and for extension of shelf-life of foods. Drying or dehydration is, by definition, the heat and mass transfer process for removal of water by application of heat, from a solid or liquid food, with the purpose of obtaining a solid product sufficiently low in water content. Where removal of water takes place by virtue of a difference in osmotic pressure and not by evaporation. The main objectives of dehydration are: Preservation as a result of lowering of water activity; low transport and storage cost as a reduction in weight and volume; transformation of food to a form more convenient to store, package, transport and use, e.g. transformation of liquids such as milk or coffee extract, to a dry powder that can be reconstituted to the original form by addition of water (Kumar *et al.*, 2015). Drying rate depends on external parameters like solar radiations, ambient temperature,

wind velocity, relative humidity and internal parameters like initial moisture content, type of crop, crop absorptivity, and mass of product per unit exposed area (Shahdev, 2014).

Drying removes the moisture from the food so bacteria, yeast and mold cannot grow and spoil the food. Drying also slows down the action of enzymes (naturally occurring substances which cause foods to ripen), but does not inactivate them (Ramya *et al.*, 2018). In today food market dried foods play an important role in the food supply chain. Dried foods are tasty, nutritious, light weight, easy to prepare, easy to store and use. The energy input is less than what is needed to freeze or can, and the storage space is minimal compared with that needed for canning jars and freeze containers. Morever, the nutritional value of food is minimally affected by drying.

2.5.1 Cabinet drying

Cabinet drier consist of an insulated cabinet fitted with shallow mesh or perforated trays, each of which carries a thin layer of food. Hot air is circulated through the cabinet tray. A system of duct and baffles is used to direct air, over and/or through each tray, to promote uniform air distribution either horizontally between the trays of food materials or vertically through the trays and food. Air heaters may be direct gas burners, steam coil exchangers or electrical resistance heaters. The air is blown past the heaters and thus heated air is used for drying. It is relatively cheap to build and maintain, flexible in design, and produces variable product quality due to relatively poor control. It is used singly or in groups, mainly for small-scale production (1-20 ton/day) of dried fruits and vegetables (Fellows, 2000).

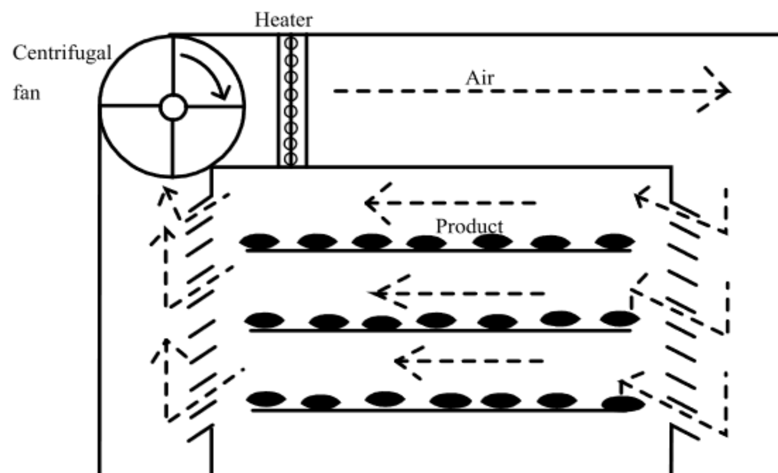


Fig. 2.1 Cabinet Dryer

2.6 Chlorophyll

Chlorophyll is a green pigment found in most plants, and its name is derived from the Greek *chloros* (green) and *phyllon* (leaf) (Levent, 2011). Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green are of leaves, stems, flowers and roots. However the chlorophyll production is mainly depended on penetration of sun light and it is the main source of energy for plant (Kamble *et al.*, 2015).

There are few different forms of chlorophyll. Chlorophyll a, greenish- yellow in solution, is the primary photosynthetic pigment in green plants for the transfer of light energy to a chemical acceptor. Chlorophyll b, blue- green in solution, found in higher plants and green algae with Chlorophyll a. Chlorophyll c, is also an accessory pigment found with Chlorophyll a in brown algae and diatoms. Chlorophyll d, together with Chlorophyll a, is in some red algae. All forms of chlorophyll are oil- soluble (Levent, 2011).

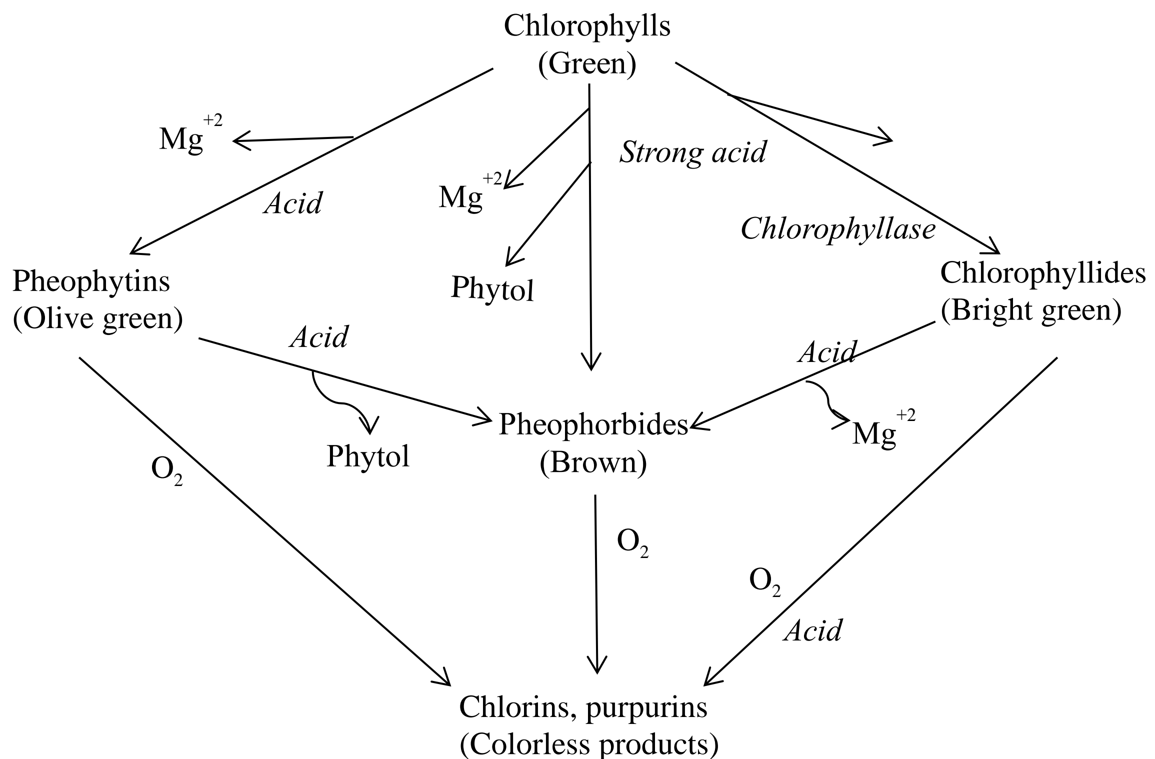


Fig. 2.2 Pathways for the degradation of chlorophyll

2.6.1 Chlorophyll and wheatgrass

The wheatgrass juice is a source of the alkaline elements, prophylactic and curative elements. It also supplements proteins, carbohydrates and fat. The lacteal substances found it in are considered to be an effective element and the only remedy for curing cancer. Chlorophyll, the most important element of the wheatgrass is contained in the cells called chloroplasts. Chloroplasts produce nutritious elements with the help of sunshine. The chemical formation of the wheatgrass juice has a striking similarity with the chemical formation of the human blood. The pH factor of the human blood is 7.4. The pH factor of the wheatgrass juice is also 7.4 that is the reason why it is quickly absorbed in the blood (Mujoriya, 2011). Wheatgrass juice contains crude chlorophyll and can be taken orally and as colon implant without side effects. Science has proven that chlorophyll arrests growth and development of unfriendly bacteria. Chlorophyll is antibacterial and can be used inside and outside the body as a natural healer. Chlorophyll can be extracted from many plants, but wheatgrass is superior because it has been found to have over 100 elements needed by man (Sowjanya *et al.*, 2015).

2.7 Phytochemicals

The "phyto" of the word phytochemicals is derived from the Greek word phyto, which means plant. Therefore, phytochemicals are plant chemicals. Phytochemicals are defined as bioactive non nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked to reducing the major chronic diseases. It is estimated that >5000 individual phytochemicals have been identified in fruits, vegetables, and grains, but a large percentage still remain unknown and need to be identified (Liu, 2018).

These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. It is well known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Altiok, 2010). Bioactive and disease preventing phytochemicals present in plant are shown below in Table 2.1.

Table 2.1 Bioactive Phytochemicals in Medicinal Plants

Classification	Main Group of Elements	Biological Function
Non-starch polysaccharides	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids.
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection.
Antioxidants	Polyphenolic compounds,flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols,curcumine, flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti-oxidants, cancer chemoprevention

Source: (Saxena *et al.*, 2013)

2.7.1 Classification of phytochemicals

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In recent year phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidine of nucleic acids,

chlorophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, steroids, curcumines, saponins, phenolic, flavonoids and glucoside (Saxena *et al.*, 2013).

2.7.1.1 Phenolic

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group (Altiok, 2010).Based on the numbers of carbon atoms present in its structure, phenolic are categorized as Table 2.2

Table 2.2 Major classes of phenolic compounds in plants

S.N.	No of carbon atom	Basic skeleton	Class
1	6	C_6	Simple phenols Benzoquinones
2	7	C_6-C_1	Phenolic acids
3	8	C_6-C_2	Acetophenones Tyrosine derivatives
4	9	C_6-C_3	Hydroxycinnamic acid, Coumarins
5	10	C_6-C_4	Naphthoquinones
6	13	C_6-C_1-C_6	Xanthones
7	14	C_6-C_2-C_6	Stilbenes
8	15	C_6-C_3-C_6	Flavonoids
9	18	$(C_6$-$C_3)_2$	Lignans
10	30	$(C_6$-C_3-$C_6)_2$	Bioflavonoids
11	N	$(C_6$-C_3-$C_6)_n$	Condensed tannins

Source: (Altiok, 2010)

2.7.1.1.1 Activity of phenolic acids

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical and medicinal studies. Phenolic compounds in many plants are polymerized into larger molecules such as the proanthcyanidins (PA; condensed tannins) and lignins. Moreover, phenolic acids may arise in food plants as glycosides or esters with other natural compounds such as sterols, alcohols, glucosides and hydroxyfatty acids. Varied biological activities of phenolic acids were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as staphylococcus aureus are some of biological activities of phenolic acids. Phenolic acid possesses diverse biological activities, for instance, antiulcer, anti- inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic and antidepressant activities (Saxena *et al.*, 2013).

2.7.1.2 Flavonoids

Flavonoids are secondary metabolites abundantly widespread throughout the plant kingdom. The major sources of flavonoids are fruit products (e.g. citrus fruits, rosehip, apricot, cherry, grapes, black currant, bilberry, apple), vegetables (e.g. onion, green pepper, broccoli, tomato, spinach), beverages (red wine, coffee, tea), cocoa bean, soy products and herbs. They are found in all plant tissues, where they are present inside the cells or on the surfaces of different plant organs (Viskupicova *et al.*, 2008). They have several important functions in plants, such as providing protection against harmful UV radiation or plant pigmentation. In addition they have antioxidant, antiviral and antibacterial properties (Kozłowska and Węgierek, 2014).

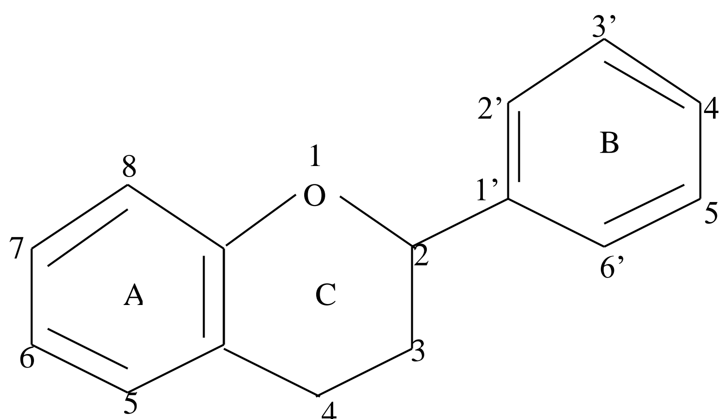


Fig. 2.3 Basic structure of flavonoid

They can occur both in free form (aglycones) and as glycosides, and differ in their substituents (type, number and positions) and in their insaturation. The most common classes are the flavones, flavonols, flavanones, catechins, isoflavones and anthocyanidins, which accounts for around 80% of flavonoids. All flavonoids share a basic C6-C3-C6 phenyl-benzopyran backbone. The position of the phenyl ring relative to the benzopyran moiety allows a broad separation of these compounds into flavonoids (2-phenyl benzopyrans), isoflavonoids (3-phenyl-benzopyrans) and neoflavonoids (4-phenyl-benzopyrans). Division into further groups is made on the basis of the central ring oxidation and on the presence of specific hydroxyl groups. Most common flavonoids are flavones (with C2-C3 double bond and C4-oxo function), flavonols (flavones with a 3-OH group) and flavonnes (flavone analogues but with a C2-C3 single bond), and abundant isoflavonoids include isoflavones (the analogue of flavones). 4-arylcoumarin (a neoflavonoid with a C3-C4 double bond) and its reduced form, 3,4-dihydro-4-arylcoumarin, are the major neoflavonoids. Other natural compounds, such as chalcones and aurones also possess the C6-C3-C6 backbone, and are henceforth included in the general group of flavonoids (Pinheiro and Justino, 2012).

2.7.1.2.1 Biological activity of flavonoids

Flavonoids have gained recent attention because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand flavonoids such as luteolin and cathechins, are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Tapas *et al.*, 2008). Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani *et al.*, 2009).

2.7.1.3 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The term tannin refers to the use of tannins in tanning animal hides into leather. However, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000. Tannins are found shapeless yellowish or light brown masses like powder, flakes or sponge. Tannins are found in all plants and in all climates all over the world. Lower plants such as algae, fungi and mosses do not contain much tannin. The percentage of tannins present in the plants, however varies. Tannins are usually found in large quantities in bark of trees where they act as a barrier for micro-organisms and protect the tree (Upadhyaya and Ashok, 2012).

Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce Gallic acid and ellagic acid and depending on the type of acid produced, the hydrolysable tannins are called Gallo tannins or egallitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein (Doughari, 2012).

2.7.1.3.1 Activity of tannins

The tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors (Bruyne *et al.*, 1999) and as anti-inflammatory, antiseptic, antioxidant and hemostatic pharmaceuticals (Dolara *et al.*, 2005). In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals. Tannins are used in the dyestuff industry as caustic for cationic dyes (tannin dyes), and also in the production of inks, and also in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer and wine industries, and as coagulants in rubber production. Recently tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers. The

search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Saxena *et al.*, 2013).

2.7.2 Antioxidant and its activity

Antioxidants may be defined as substances that, when present in food, delay, control, or inhibit oxidation and deterioration of food quality. In the body, antioxidants reduce the risk of degenerative diseases arising from oxidative stress (Halliwell, 1999). Antioxidants are compound capable to either delay or inhibit the oxidation process which under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals. Antioxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals (Pisoschi and Negulescu, 2011). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, superoxide anion, peroxyl radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage. Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Doughari *et al.*, 2009).

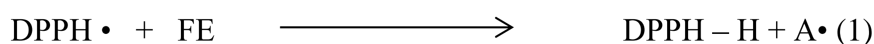
Antioxidants exert their activity by scavenging the ‘free-oxygen radicals’ thereby giving rise to a fairly ‘stable radical’. Free radicals generated in the body can be removed by the body’s own natural antioxidant defenses such as glutathione or catalases. Therefore, this deficiency had to be compensated by making use of natural exogenous antioxidants, such as vitamin C, flavones, beta-carotene and natural products in plants. Plants contain a wide variety of free radicals scavenging molecules including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity. Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which possess the ability to scavenge the free radicals in human body. Significant antioxidant properties have been recorded in phytochemicals that are necessary for the reduction in occurrence of many diseases (Omojate *et al.*, 2014).

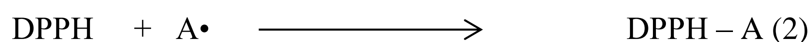
Antioxidant activity is the total capacity of antioxidants for eliminating free radicals in the cell and in food. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.*, 1987).

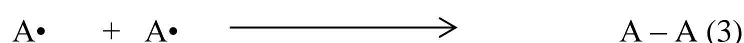
Polyphenols are excellent antioxidants due to 3,4 dihydroxy group in their B ring and the galloyl ester in the C ring of flavonoids (Chen and Chu, 2006). Tocopherols have their own antioxidant activity, including hydrogen atom transfer at 6- hydroxyl group on the chroman ring and scavenging of singlet oxygen and other reactive species (Korus and Lisiewska, 2011). The antioxidant activity of ascorbic acid is based on hydrogen atom donation to lipid radicals, quenching of singlet oxygen and the removal of molecular oxygen (Lee *et al.*, 2004). Chen and Chu (2006) reported that flavonoids have the most potent antioxidant activity because their chemical structure contains an O-diphenolic group, a 2-3 double bond conjugated with 4-oxo function and hydroxyl groups in the position 3 and 5. Flavonoids effectively scavange hydroxyl and peroxyl radicals, form complexes with metals and inhibit metal initiating lipid oxidation. The antioxidant activity of phenolic acid also depend on the number of orientation of hydroxyl groups relative to the electron withdrawing CO_2H, CH_2CO_2H, or $(CH)_2CO_2CH$ functional groups (Rice-Evans and Miller, 1996).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract.

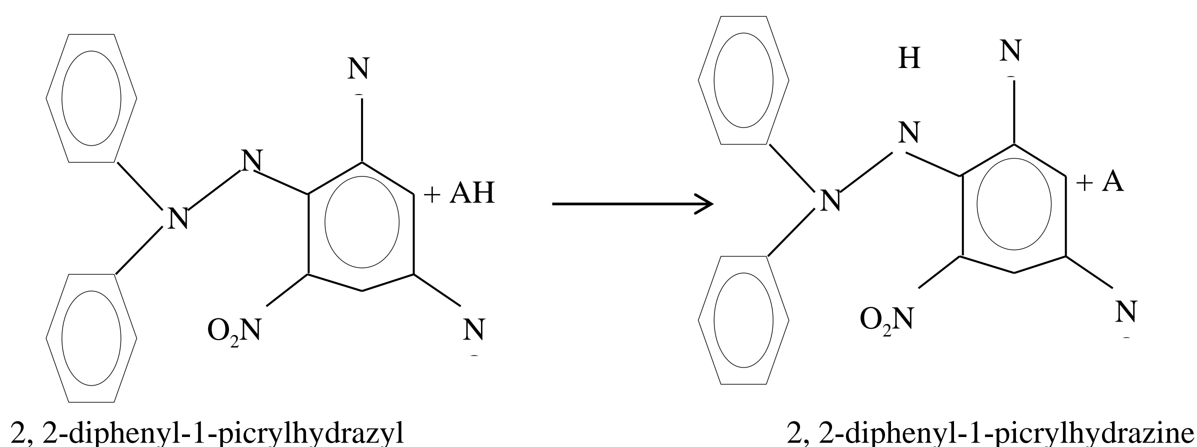
The scavenging of DPPH by radical scavengers can be summarized as:







Where FE is a scavenger of the extract and A• is a radical. The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Chandra and Goyal, 2014).



2, 2-diphenyl-1-picrylhydrazyl 2, 2-diphenyl-1-picrylhydrazine

Fig. 2.4 Reaction of DPPH-free radical with an antioxidant

2.7.3 Extraction of phytochemicals

Extraction is the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa, 2008). The general techniques of medicinal plant extraction include maceration, infusion, percolation, digestion decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic

extraction by fermentation, countercurrent extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction (Tiwari *et al.*, 2011).

- Maceration

Maceration is a technique used in wine making and has been adopted and widely used in medicinal plants research. It involves soaking plant materials (coarse or powdered) in a stoppered container with a polar solvent and allowed to stand at chilled temperature for a period of two to three days (Handa, 2008). This process is intended to soften and break the plant cell wall to release the soluble phytochemicals. After two to three days, the mixture is pressed or strained by filtration. The choice of solvents will determine the type of compound extracted from the samples (Azwanida, 2015). However, maceration have been suggested by (Vongsak *et al.*, 2013) as more applicable, convenient and less costly method compared to other modern extraction methods since all these extraction methods resulted in crude extracts containing a mixture of metabolites having almost similar recovery of phytochemicals. This particular fact suggests that preparation of crude extract through modern technology, which is rather complex and time consuming is not necessary if proper preparation and extraction are done (Azwanida, 2015).

- Supercritical fluid extraction (SFE)

Supercritical Fluid Extraction (SFE) involves use of gases, usually CO_2, and compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas and the gas is recovered for re-use. Solvent properties of CO_2 can be manipulated and adjusted by varying the pressure and temperature. The advantages of SFE are, no solvent residues left in it as CO_2 evaporates completely (Patil and Shettigar, 2010).

- Infusion

It is a dilute solution of the readily soluble components of the crude drugs. Fresh infusions are prepared by macerating the solids for a short period of time with either cold or boiling water (Cathrine and Banu, 2015).

- Digestion

This is a kind of maceration in which gentle heat is applied during the maceration extraction process. It is used when moderately elevated temperature is not objectionable and the solvent efficiency of the menstrum is thereby increased (Cathrine and Banu, 2015).

- Soxlet extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of compounds (Nikhal *et al.*, 2010).

2.7.4 Factors affecting phytochemicals

Production of phytochemicals in plants is affected by many pre- and post-harvest factors including farming practices, environmental factors (microclimate, location, growing season, soil type and nutrients), plant maturity, post-harvest storage and processing, but genetics is the primary factor among all (Li *et al.*, 2012).

- Genetic and environment

Genetics has the greatest effect on the production of plant secondary metabolites (Tsao *et al.*, 2006) . Mineral composition, soil type, temperature, light and water content are among the frequently reported factors that affect the total phytochemical contents in plants (Rao and Rao, 2007) (Hansen *et al.*, 2010).

- Post-harvest Storage and Processing Conditions

Changes in both the quality and phytochemical composition of plants can occur rapidly depending on postharvest handling such as storage and processing conditions (Rodriguez-Amaya, 2003). The two major chemical changes causing the deterioration are lipid oxidation and non-enzymatic browning during storage and food processing, which can lead to altered color and flavor. Lipid oxidation is influenced by light, oxygen, temperature, the presence of catalysts, such as transition metals iron and copper, and water activity. Non-enzymatic

browning occurs easily during the storage of dried and concentrated foods. Different phytochemicals are affected by these factors differently. Carotenoids are very sensitive to heat, and can incur significant losses during different vegetable processing steps (Zhang and Hamauzu, 2004).

The main cause of carotenoid degradation in foods is oxidation. Flavonoids and other phenolic compounds are relatively stable at high temperature and over long storage (Vallejo *et al.*, 2003). Phenolic in plants exist in both free and conjugated forms. Post-harvest loss of phenolic is mainly due to enzymatic oxidation by polyphenol oxidase and peroxidases (Jones, 2007).

- Post -harvest Storage Conditions

Post-harvest storage may affect the composition of some phytochemicals in plants; however, the degree of the effect depends largely on the storage conditions. Metabolism of the phytochemicals begins right after harvest, and it can involve complex biochemical reactions during transportation and post-harvest storage. These reactions can lead to significant changes in plant attributes (taste, smell, appearance and texture), and the health promoting phytochemicals, such as those with strong antioxidant activities (Michalczyk *et al.*, 2009). Storage temperature, atmosphere gas composition and use of chemicals are major factors that influence the quantity and quality of phytochemicals (Li *et al.*, 2012).

Lower temperature can slow the deterioration of phytochemicals. Opposed to lower temperature, high temperature also brings a significant change in total phenolic, flavonoid, tannin content and antioxidant activity compared with its fresh form. However their concentration may vary according to the drying methods used and the duration of exposure to hot air (Michalczyk *et al.*, 2009). Exposure to high temperature leads to discharge of phenolic compounds through disintegration of cellular constituents which then results in the migration of components, leading to losses by leakage or breakdown by various chemical reactions involving enzymes, light and oxygen as well as (Davey *et al.*, 2002)

- Food Processing Conditions

Plant foods are processed to meet different consumer requirements. Generally, processed products have lower nutritional values than their respective fresh commodities mainly due to the loss of nutrients during processing (Muftugil, 1986). Industrial processing e.g., blanching,

canning, sterilizing and freezing, as well as various cooking methods e.g., boiling, steaming and microwaving, of fruits and vegetables can affect the content and composition of nutrients including phytochemicals, normally leading to reduced availability of these compounds (Podsedek, 2007; Podsedek *et al.*, 2008). Studies have shown that blanching leads to reduced content of vitamins, carotenoids and phenolic compounds, which are relatively labile to heat treatments (Prochaska *et al.*, 2000).

PART III

Materials and methods

3.1 Raw materials

The plant under study during the research was wheatgrass (*Triticum aestivum*).

3.2 Identification of seed

Wheat seeds to be germinated were identified as *Triticum aestivum* in Botanical lab at Central Campus of Technology, Dharan.

3.3 Collection and preparation of sample

Collection and preparation of sample for analysis includes following steps:

- Analysis of germination capacity

Wheat seeds *(Triticum aestivum)* were brought from the local market of Dharan. Random samples of seeds were taken and placed in moist filter paper in covered petri dish. A temperature of 30°c for 16 hours and 20°c for 8 hours was maintained and germination capacity of wheat seeds was calculated as below (Ffolliot and Thames, 1983):

Germination capacity (%) = $(N_0/N)*100$

Where,

N_0= No. of healthy, well developed full seeds

N= Total number of seeds in sample

- Germination of wheat

Germination of wheat seeds was done in the month of November/ December at Dharan. Growing of wheatgrass included soaking wheat seed in water overnight and germinating them for 24 hours, by tying in muslin cloth and spreading them over the soil, avoiding overlapping of the seeds. The growing area was covered with 50% green shade net so as to provide natural air flow and avoid direct sunlight. Regular watering was done and when required during the growing period (Pardeshi *et al.*, 2013). pH of soil was determined by

method as described by (Jackson, 1958). Wheatgrass was successfully grown at temperature of 24°C to 26°C with average temperature of 24.8°C and relative humidity of 61% to 71% with average of 64.9% as shown in Appendix C.5.

- Drying and grinding of tender wheatgrass

The wheatgrass of 6th, 7th, 8th, 9th and 10th were harvested manually on respective days for drying. Those wheatgrass were subjected to cabinet drying (50°C, 5hr). Dried wheatgrass was powdered in grinder and thus five different samples were prepared for analysis.

- Preparation of plant extracts

Plant materials were extracted as per (Ahmad *et al.*, 2014) with slight modifications. Briefly, 10g of powdered plant materials were steeped in 80% methanol (100 ml) for 12 hours at room temperature. They were then filtered through Whatman No. 41 filter paper. Finally, extracts were transferred to brown colored glass bottles, sealed by using bottle caps and stored at 4 ± 2°C until analysis. The extract concentration was determined by evaporating 5 ml of extract (at 80°C) to dryness and measuring the weight as shown in Appendix C.2. The basic flow diagram of methodology is shown in Fig 3.1

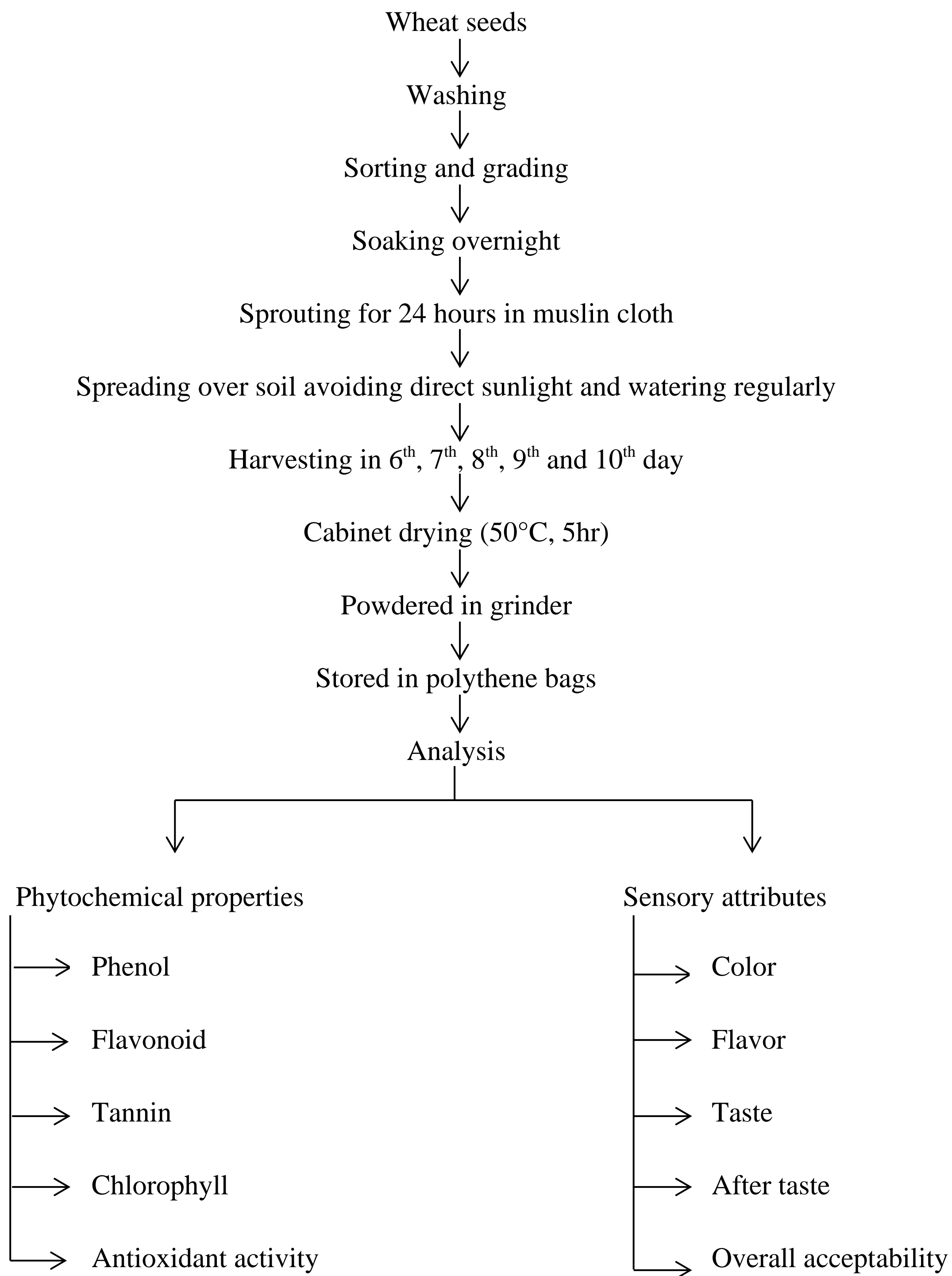


Fig 3.1 Flow diagram of wheatgrass production and analysis

3.4 Qualitative analysis for Phytochemical

The phytochemical contents of extracts were analyzed by using the following standard methods (Jaradat *et al.*, 2015).

3.4.1 Phenols and tannins

Two milliliter of 2% solution of $FeCl_3$ mixed with crude extract. Black or blue-green color indicated the presence of tannins and phenols.

3.4.2 Flavonoids

- Shinoda test: Pieces of magnesium ribbon and HCl concentrated were mixed with crude plant extract after few minutes pink colored scarlet appeared that indicated the presence of flavonoids.
- Alkaline reagent test: 2 ml of 2% NaOH solution was mixed with plant crude extract, intensive yellow color was formed, which turned into colorless when added 2 drops of dilute HCl to solution, this result indicated the presence of flavonoids.

3.4.3 Saponins

Five milliliter of distilled water was added to crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

3.4.4 Glycosides

- Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform mixed with entire plant crude extract. The mixture was then cooled and added H_2SO_4 concentrated, green color indicated the entity of aglycone steroidal part of glycosides.
- Salkowski's test: H_2SO_4 concentrated (about 2 ml) was added to the entire plant crude extract. A reddish brown color produced indicated the entity of steroidal aglycone part of the glycoside.
- Keller-kilani test: A mixture of acetic acid glacial (2 ml) with 2 drops of 2% $FeCl_3$ solution was added to the plant extract and H_2SO_4 concentrated. A brown ring produced between the layers which indicated the entity of cardiac steroidal glycosides.

3.4.5 Steroid

Two milliliter of chloroform and concentrated H_2SO_4 were mixed with the entire plant crude extract. In the lower chloroform layer produced red color that indicated the presence of steroids. Another test was performed by mixing 2 ml of each of acetic acid with H_2SO_4 concentrated and crude extract with 2 ml of chloroform. Green color indicated the entity of steroids.

3.4.6 Terpenoids

Two milliliter of chloroform was mixed with the plant extract and evaporated on the water path then boiled with 2 ml of H_2SO_4 concentrated. A grey color produced indicated the entity of terpenoids.

3.5 Quantitative analysis of phytochemicals

3.5.1 Determination of total phenol

Total phenolic content (TPC) in the plant methanolic and ethanolic extracts was determined using spectrophotometric method (Jaradat *et al.*, 2015)with some modifications. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of Na_2CO_3 aqueous solution. The samples were thereafter incubated in a thermostat at 45 ºC for 45 min. The absorbance was determined using spectrophotometer at wave length = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of gallic acid equivalent expressed in terms of (mg of GA/g of extract).

3.5.2 Determination of flavonoid

Total flavonoid content was determined using a modified aluminium chloride assay method as described by (Barek and Hasmadi, 2015).2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 minutes. 0.2 ml Aluminium Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 minute. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 minutes at 510nm against a reagent blank. The test result was correlated with standard curve of Quercetin (20,

40, 60, 80, 100µg/ml) and the total flavonoid content is expressed as mg quercetin equivalents(QE) (Barek and Hasmadi, 2015).

3.5.3 Determination of tannins

Tannin was determined by Folin-ciocalteu method. About 0.1 ml of the sample extracts added to volumetric flask(10ml) containing 7.5 ml distilled water and 0.5 ml folin-ciocalteu reagent,1 ml 35% Na_2Co_3 solution and dilute to 10 ml distilled water. The mixture is shaken well and kept at room temperature for 30min. A set of reference standard solution of Gallic acid (20, 40, 60, 80, 100µg/ml) are prepared in same manner as described earlier. Absorbance for test and standard solution are measured against blank at 725nm with an UV/visible spectrophotometer. The tannin content is expressed in terms of mg of GAE/g of extract (Mythili *et al.*, 2014).

3.5.4 Determination of chlorophyll

Total chlorophyll content in wheatgrass sample is determined as per (Rai, 2006).

Chl a, mg/g tissue = $12.7(A_{663}) - 2.69(A_{645}) \times V/1000 \times W$

Chl b, mg/g tissue = $22.9(A_{663}) - 4.68(A_{645}) \times V/1000 \times W$

Total chlorophyll, mg/g tissue = Chl a + Chl b (calculated above)

Where, A = absorbance at specific wavelengths

V = final volume of chlorophyll extract

W = fresh weight of tissue extracted

3.5.5 Determination of DPPH radical scavenging activity

DPPH free radical scavenging activities (antioxidant activities) of extracts were determined by method described by (Vignoli *et al.*, 2011)with slight variation. Different dilutions of the extracts were made using 80% methanol. Then 1 ml of the extract was mixed with 2 ml of 0.1 mM DPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, percentage scavenging activity was determined using following equation:

% scavenging activity = $(Ac-As) \times 100 / Ac$

3.5.6 Sensory analysis

Sensory analysis of the wheatgrass powder were analysed as similar to tea testing procedure developed by TRA (Tea Research Association), Assam. 2.8 gm of tea samples were weighed and they were kept in a cup. 140 ml hot water was added and covered with lid. It was left for 5 minutes. The brew was then transferred to the bowl and the infusion was taken out in the lid. Brew was evaluated for sensory attributes (color, flavor, taste, after taste and overall acceptability) by semi-trained panelists using 9 point hedonic scale.

3.5.7 Optimization of wheatgrass powder

Wheatgrass harvested on 6 to 10 days were powdered and sensory evaluation was carried out in Central Campus of Technology, Dharan by 10 semi trained panelists as per 9 point hedonic ratings to optimize color, flavor, taste, after taste and overall acceptability. Criteria for optimization of wheatgrass powder are shown in Table 3.3 and Table 3.4

Table 3.3 Phytochemicals parameters

Parameters	Goals
TPC	Maximize
TFC	Maximize
Tannins	Minimize
Antioxidant activity	Maximize
Chlorophyll	Maximize

Table 3.4 Sensory parameters

Parameters	Goals
Color	Maximize
Flavor	Maximize
Taste	Maximize
After taste	Maximize
Overall acceptance	Maximize

3.5.8 Statistical analysis

Analysis was carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2010. All the data obtained in this experiment were analyzed for significance by Analysis of Variance (ANOVA) using statistical software Genstat Release 12.1 (Discovery Edition 12 developed by VSN International Limited). From this, means were compared using Fischer's protected LSD (Least Significant Difference) at 5% level of significance.

Part IV

Results and discussion

A common variety of wheat *(Triticum aestivum)* was collected from the local market of Dharan for phytochemical analysis. Fresh wheatgrass of 6th, 7th, 8th, 9th and 10th day were harvested respectively and subjected to drying to analyse their phytochemical composition.

4.1 Determination of germination capacity and pH value

Eighteen among twenty wheat seeds were germinated during analysis. So, germination capacity of wheat seeds was found to be 90%. pH value of soil was found to be 7.3.

4.2 Qualitative analysis for phytochemical

During the experimental work, the methanolic extract of wheatgrass shown to have the total phenol, total flavonoid, tannins and antioxidant activity as shown in Table 4.1

Table 4.1 Qualitative analysis for phytochemicals

Test	Result
Phenols	+ve
Tannins	+ve
Flavonoids	+ve
Glycosides	+ve
Terpenoids	+ve
Steroids	+ve
Saponins	-ve

Plus (+) =positive test; minus (-) =negative test

Similar result for phytochemical analysis of wheatgrass was obtained by (Suriyavathana *et al.*, 2016). The qualitative phytochemical analysis results explored the presence of a wide range of phytochemical constituents which signifies the wheatgrass as a valuable therapeutic natural source to compact dreadful infectious diseases.

4.3 Quantitative analysis of phytochemicals

Qualitative analysis of phytochemicals was carried out for total phenol content, flavonoids, tannins, DPPH radical scavenging activity and chlorophyll. The statistically analysis showed significant difference among all five samples at 5% level of significance. The result of analysis of quantitative phytochemical analysis is shown in Appendix C.6.

4.3.1 Effect of growth days on total phenol content

The statistical analysis showed significant difference ($p<0.05$) in all five samples. Total phenol content in wheatgrass powder of 6th day was found to be 318.07 mg/g which increased as 367.42 mg/g, 385.55 mg/g, 409.49 mg/g, 454.68 mg/g on 7th, 8th, 9th and 10th days respectively. Similar observation made by (Kulkarni *et al.*, 2006) also showed increase in phenol content of wheatgrass with growth. The effect of growth days on total phenol content of wheatgrass is shown below in Fig. 4.1

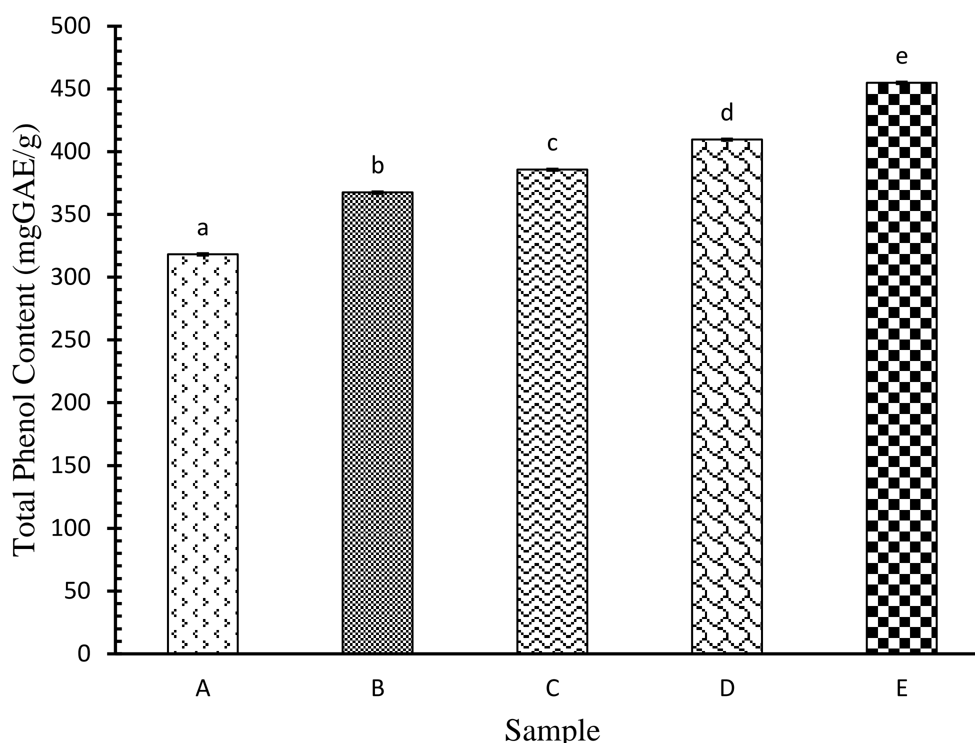


Fig. 4.1 Effect of growth days on total phenol content

The majority of phenolic acids in wheat is represented by bound phenolic compounds, as either hydroxycinnamic acid or hydrobenzoic acid derivatives, all involved in the

composition of the cell wall. The common phenolic acids found in whole grains include ferulic acid, vanillic acid, caffeic acid, syringic acid and p-coumaric acid. Among these, ferulic acid appears to be predominant, especially in its bound form, and is stated to be a major contributor to antioxidant activity. The amount of phenolic compounds in whole grains is influenced by cultivar and environmental conditions during the filling, post-harvest storage and germination (Benincasa *et al.*, 2014). During the germination process, the ferulic acid accumulates due to the phenolic biosynthesis and polyphenolic compounds bound to cell walls (Hung *et al.*, 2011). (Rattanapon *et al.*, 2017) reports increase in bound phenolic content at seedling stage due to enzymes involved in the phenylpropanoid pathway and degradation of the cell wall polysaccharides and proteins leading to release of bound phenolics. Moreover, (Randhir *et al.*, 2004) also reported that the total phenolic content of green mung decreased during germination. It pointed that an increasing and decreasing of phenolics depended on plant variety, germination or seedling time, priming condition and planting condition and so on.

4.3.2 Effect of growth days on flavonoid content

Flavonoid content of wheatgrass was also found to be increased with growth as 105.72 mg/g, 192.04 mg/g. 285.04 mg/g, 353.68 mg/g and 429.42 mg/g on 6th, 7th, 8th, 9th and 10th days respectively. Statistical analysis showed significant difference ($p<0.05$) between wheatgrass of 6th, 7th and 8th day but no significant difference ($p>0.05$) in 8th, 9th and 10th day sample. (Kulkarni *et al.*, 2006) also showed increase in flavonoid content of wheatgrass with growth. The effect of growth days on flavonoid content of wheatgrass is shown below in Fig. 4.2

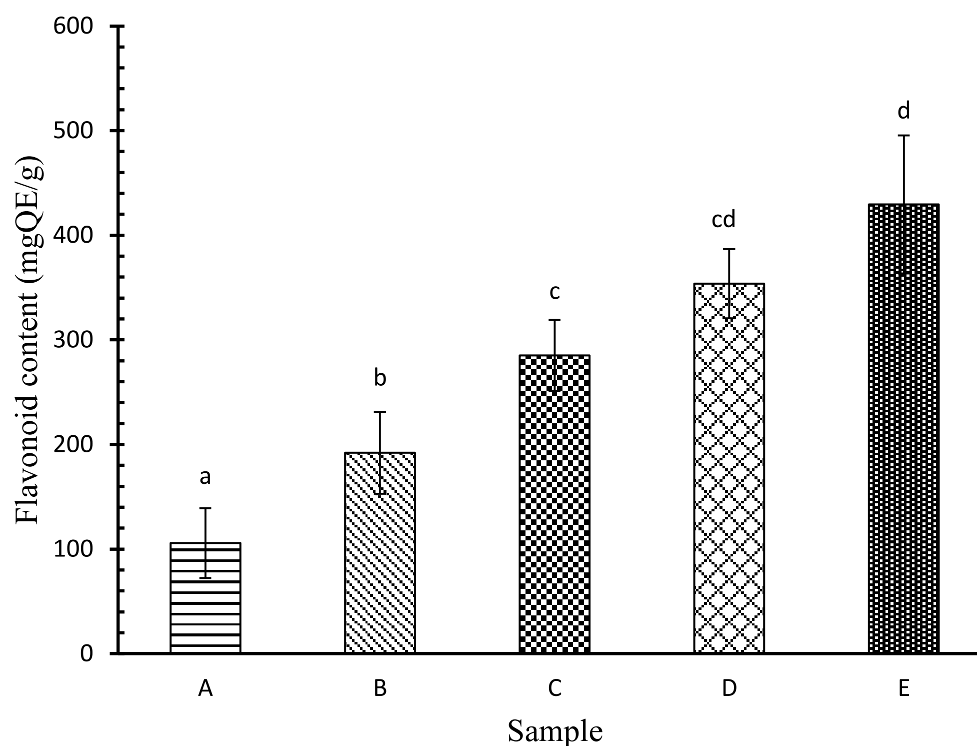


Fig. 4.2 Effect of growth days on flavonoid content

During germination, vitamins, minerals and phenolic compounds including flavonoids are synthesized in wheat sprouts, and wheat sprouts reach the maximum antioxidant potential (Suriyavathana *et al.*, 2016). Moreover, during seedling growth, L-phenylalanine is transformed to cinnamic acid under the catalysis of phenylalanine ammonialyase (PAL). Thereafter, many phenolic compounds are synthesized to be flavonoids, tannins, lignins, and other compounds (Xu *et al.*, 2015). Moreover, phenylalanine, ammonialyase (PAL) could be enhanced during germination. Increase on flavonoid content on germination was attributed to the biochemical metabolism of seeds during germination, which might produce some secondary plant metabolites such as anthocyanins and flavonoids (Uchegbu and Ndidi, 2015).

4.3.3 Effect of growth days on tannin content

Tannin content of wheatgrass decreased with growth. Statistical analysis showed significant difference ($p<0.05$) between wheatgrass of 7th, 8th, 9th and 10th days but wheatgrass of 6th and 7th day were not significantly different ($p>0.05$). Tannin content of 6th day was found to be high with value 10.24 mg/g. Likewise, tannin content of 7th, 8th, 9th and

10th day were found to be 9.20 mg/g, 6.77 mg/g, 4.79 mg/g and 2.07 mg/g respectively. (de Almeida *et al.*, 2014) also reported decrease in tannin content on germination of sorghum subjected drying. The effect of growth days on tannin content of wheatgrass is shown below in Fig. 4.3

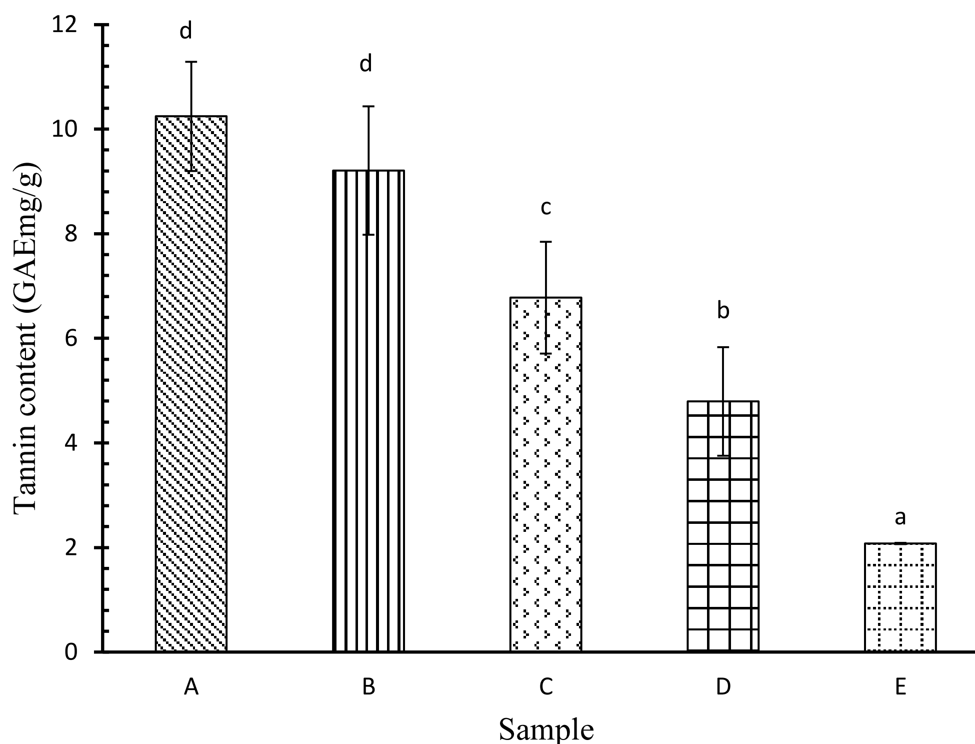


Fig. 4.3 Effect of growth days on tannin content

(Megat Rusydi and Azrina, 2012) found that non-germinated peanut and soybean had the highest tannin content compared to germinated peanut and soybean. The observed reduction in tannin content after germination was a result of formation of hydrophobic association of tannins with seed proteins and enzymes. In addition loss of tannins during germination also may be due to leaching of tannins into the water (Shimelis and Rakshit, 2007) and binding of polyphenols with other organic substances such as carbohydrate or protein(Saharan *et al.*, 2002).

4.3.4 Effect of growth days on DPPH radical scavenging activity

Antioxidant activity of wheatgrass also increased with growth. Statistical analysis also showed significant difference ($p<0.05$) on antioxidant activity of five different sample. Antioxidant capacity increased from 80.48% on 6th day to 84.38%, 87.38%, 90.39% and

93.09% on 7th, 8th, 9th and 10th days respectively. (Kulkarni *et al.*, 2006) reported increase in antioxidant activity as a function of growth under different conditions.(Agrawal *et al.*, 2015) observed 9-10 inches of wheatgrass has richest nutrients and antioxidants and is best to obtain for consumption. The effect of growth days on DPPH radical scavenging activity of wheatgrass is shown below in Fig. 4.4

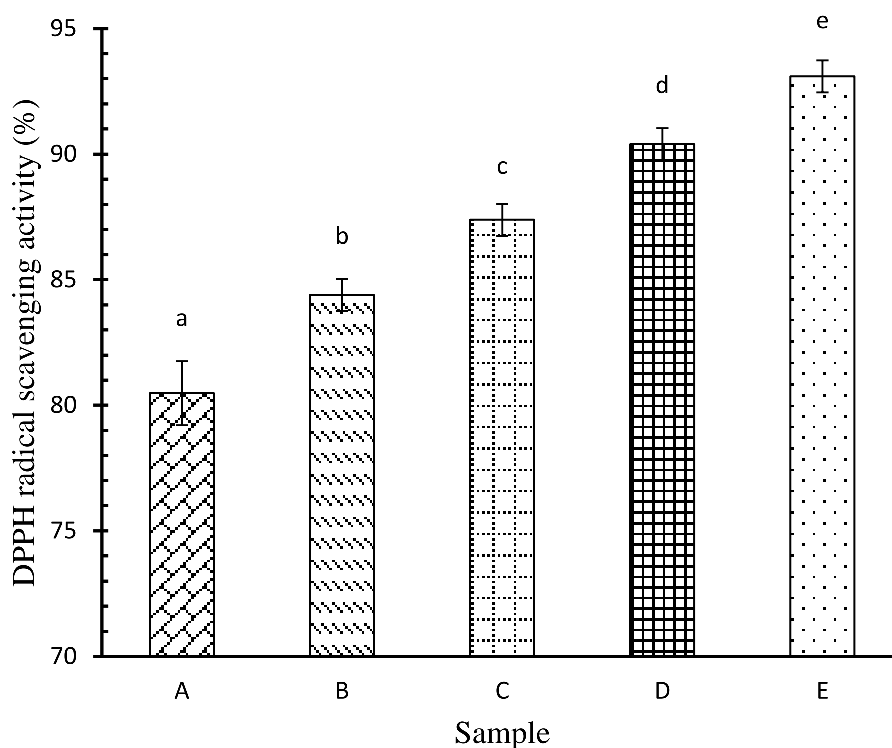


Fig. 4.4 Effect of growth days on DPPH radical scavenging activity

Recently it was shown that during germination, some biologically active compounds were synthesized in the wheat sprouts (Calzuola *et al.*, 2004; Mancinelli *et al.*, 1998). (Yang *et al.*, 2001)also concluded that wheat sprouts reached the maximum potential after 7 days of plant growth. Increase in vitamins, ferulic acid and vanillic acid content with increase in germination period results in increase in antioxidant potential (Dey *et al.*, 2006). (Ghumman *et al.*, 2017) concluded higher radical scavenging capacity of wheat may be attributed to its high chlorophyll content. Study in lead tree seeds by (Suryanti *et al.*, 2016) suggests that germination is a good way to enhance the antioxidant properties and increase the phenolic compounds, β-carotene, ascorbic acid and α-tocopherol contents.

4.3.5 Effect of growth days on chlorophyll content

With growth, chlorophyll content of wheatgrass increased significantly upto day 8 and then consequently decreased on day 9 and day 10 respectively. The obtained chlorophyll content from this study was 10.86 mg/g, 11.49 mg/g, 14.72 mg/g, 9.39 mg/g and 7.12 mg/g for day 6th, 7th, 8th, 9th and 10th respectively. Statistical analysis showed significant difference ($p<0.05$) between all these samples.(Murali *et al.*, 2016) concluded that the 8th day wheatgrass harvest had high chlorophyll content and hence is the appropriate day for wheatgrass consumption due to its close structural similarity of Hemoglobin of blood. Value obtained for 10th day wheatgrass was similar to observation made by (Jain and Jain, 2014) on wheatgrass of same day. Chlorophyll content of wheatgrass powder was 10.27mg/2g. The effect of growth days on chlorophyll content of wheatgrass powder is shown in Fig. 4.5

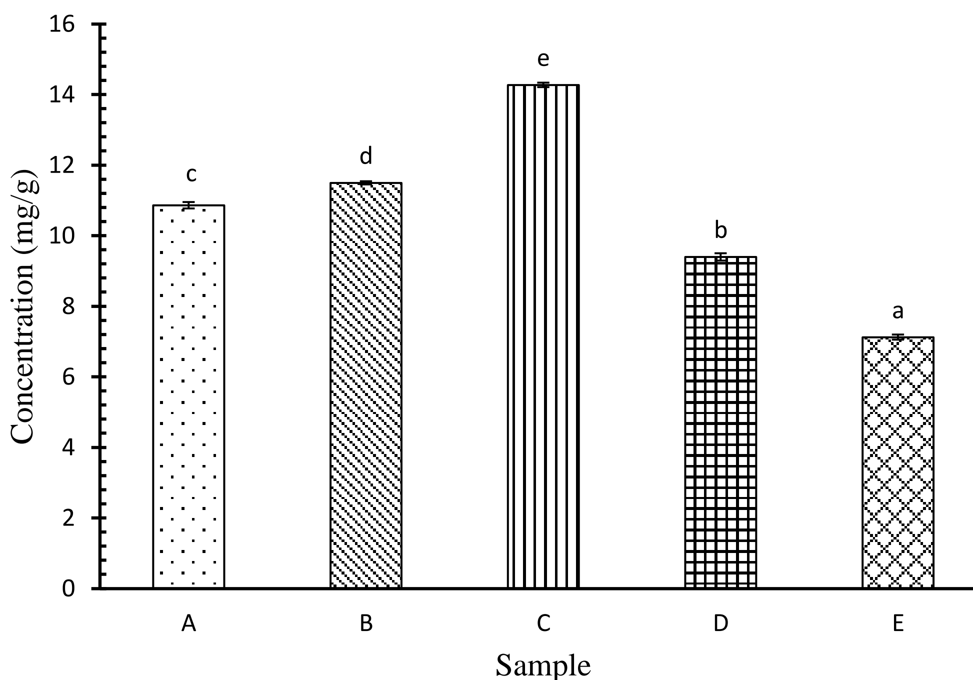


Fig. 4.5 Effect of growth days on chlorophyll content

Aging of leaves involves degradation of proteins, chlorophylls, nucleic acids, membranes and subsequent transport of some of the degradation products to other parts of the plant. Yellowing of leaves due to chlorophyll degradation is the most obvious symptom (Fang *et al.*, 1998). (Kamble *et al.*, 2015) carried out research in chlorophyll content of young

leaves and adult leaves. Some plants showed high chlorophyll content in young whereas some showed high chlorophyll content in adult plants. Reason for high chlorophyll content in adult plants was attributed to maturity of plants and for young plants was attributed to micronutrient deficiency of soil e.g. Fe deficiency or less penetration of sunlight. Changes in chlorophyll content can occur as a result of nutrient deficiencies, exposure to environmental stresses and certain herbicides, and differences in irradiance during plant growth.

4.4 Sensory evaluation

Sensory evaluation was carried out for color, flavor, taste, mouth feel and overall acceptability by semi trained panelists using 9 point hedonic scale. The statistical analysis (two way ANOVA no blocking) was done. ANOVA was carried out using LSD at 5% level of significance. There was significant difference for most of the sensory attributes viz., color, flavor, taste, after taste and overall acceptability at $P<0.05$. The result of the sensory evaluation is shown in Appendix C.7.

4.4.1 Color

Mean sensory score for color of sample A, B, C, D and E were found to be 7.5, 7.5, 8.1, 6.2 and 5.4 respectively. LSD showed that sample A and B were not significantly different while other sample while other sample B and C, C and D, D and E were significantly different from each other at 5% level of significance. Sample E had lowest mean score whereas sample C had highest mean score. So, sample C was superior than other on the basis of color from statistical analysis.

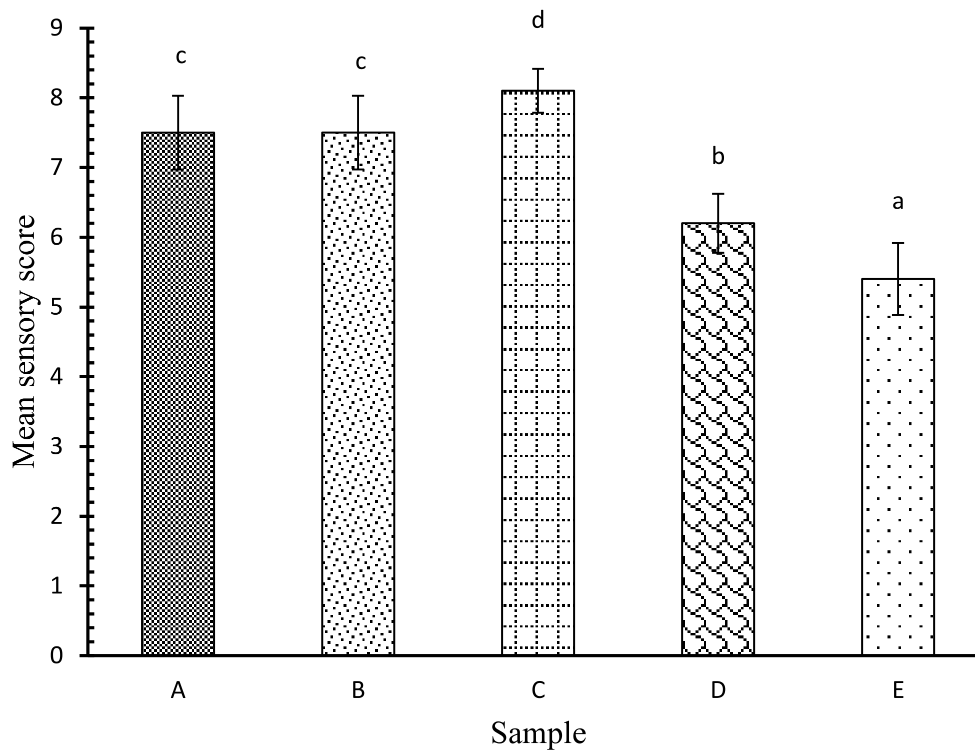


Fig.4.6 Mean sensory score for color

The values in the figure are the mean sensory score for color. Values on top of the bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

The primary pigments imparting color in fruits and vegetables are fat soluble chlorophylls (green) and carotenoids (yellow, orange and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow) and betalains (red) (Barrett *et al.*, 2010). (Hortensteiner and Krautler, 2011) says chlorophyll degradation massively occurs during leaf senescence or fruit ripening as a response of many biotic and abiotic responses. This reason may be attributed to inferiority in color of sample D and E.

4.4.2 Flavor

Mean sensory score for flavor for sample A, B, C, D and E were found to be 5.9, 6.6, 6.7, 8.0 and 7.4 respectively. LSD showed that sample A and B, C and D, D and E were significantly different whereas sample B and C were not significantly different at 5% level of significance. Sample D had highest mean score and sample A had lowest. From all among samples, sample D was found to be superior in terms of flavor.

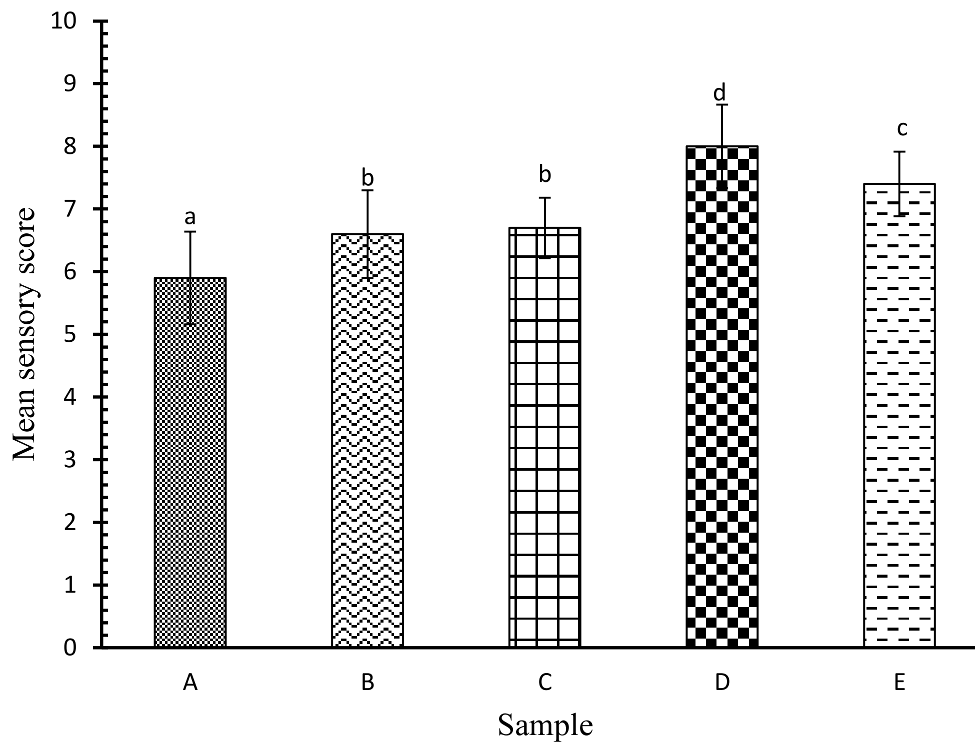


Fig.4.7 Mean sensory score for flavor

The values in the figure are the mean sensory score for flavor. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

The germination process is well known to intensify both color and flavor of grain products. The kilning (drying) step is especially important in the formation of different kinds of flavors and color of germinated grain products(Fayle and Gerrard, 2002).

4.4.3 Taste

Mean sensory score for taste for sample A, B, C, D and E were found to be 5.9, 6.3, 6.8, 8.2 and 8 respectively. LSD showed no significant difference between sample A and B and significant difference between B and C, C and D, D and E but no significant difference between sample B and C at 5% level of significance. Sample D was found to be superior in terms of taste compared to other samples.

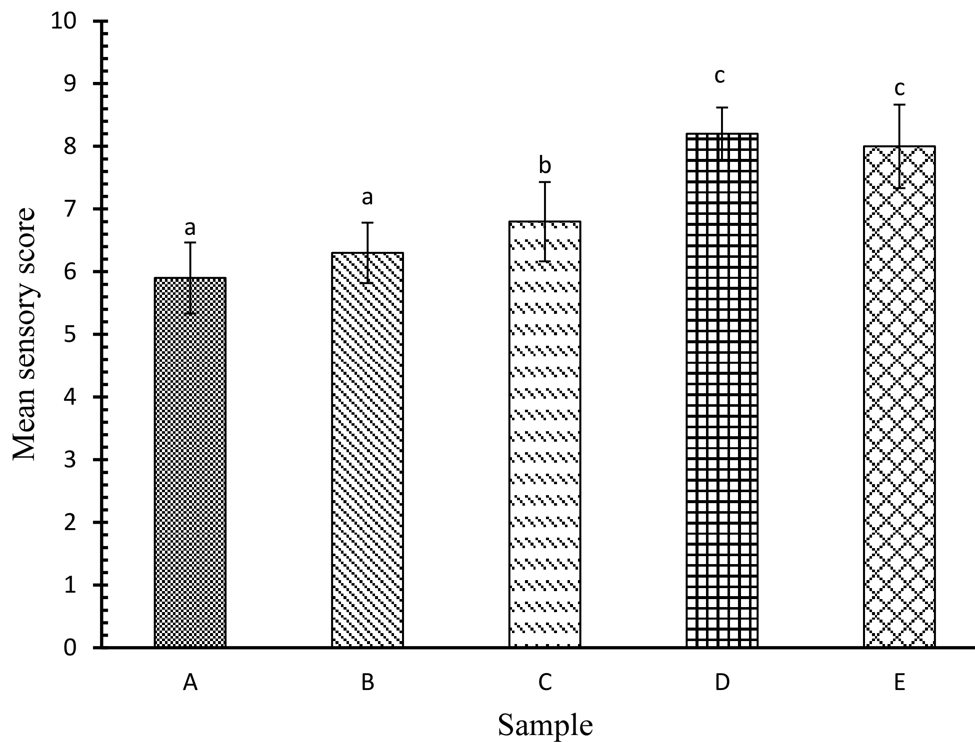


Fig.4.8 Mean sensory score for taste

The values in the figure are the mean sensory score for taste. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores. The reason for sample D to be superior may be its maturity.

4.4.4 After taste

Mean sensory score for after taste for sample A, B, C, D and E were found to be 5.7, 6.3, 6.9, 7.2 and 8.2 respectively. LSD showed significant difference between A and B, B and C, D and E where no significant difference was found between sample C and D at 5% level of significance. Sample E was found to be superior in terms of taste while sample A was found to be inferior.

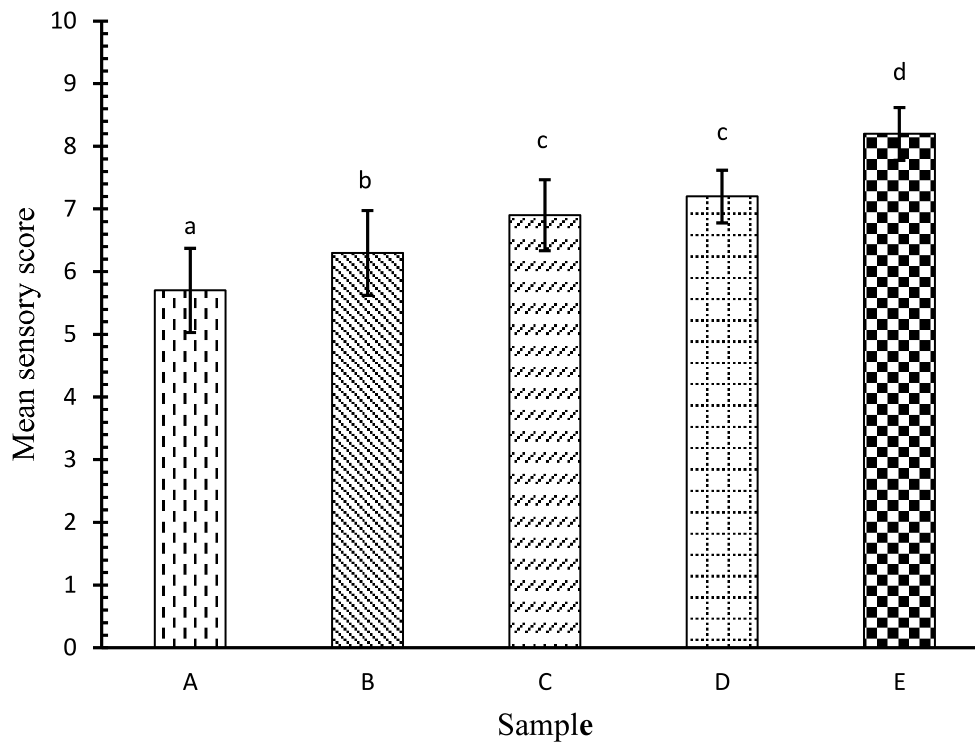


Fig.4.9 Mean sensory score for after taste

The values in the figure are the mean sensory score for after taste. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

4.4.5 Overall acceptability

Mean sensory score for overall acceptability sample A, B, C, D and E were found to be 6, 6.4, 7.1, 7.4 and 8.2 respectively. LSD showed significant difference between samples B and C, D and E but no significant difference between samples A and B, C and D. Samples E was found to be superior considering overall acceptability.

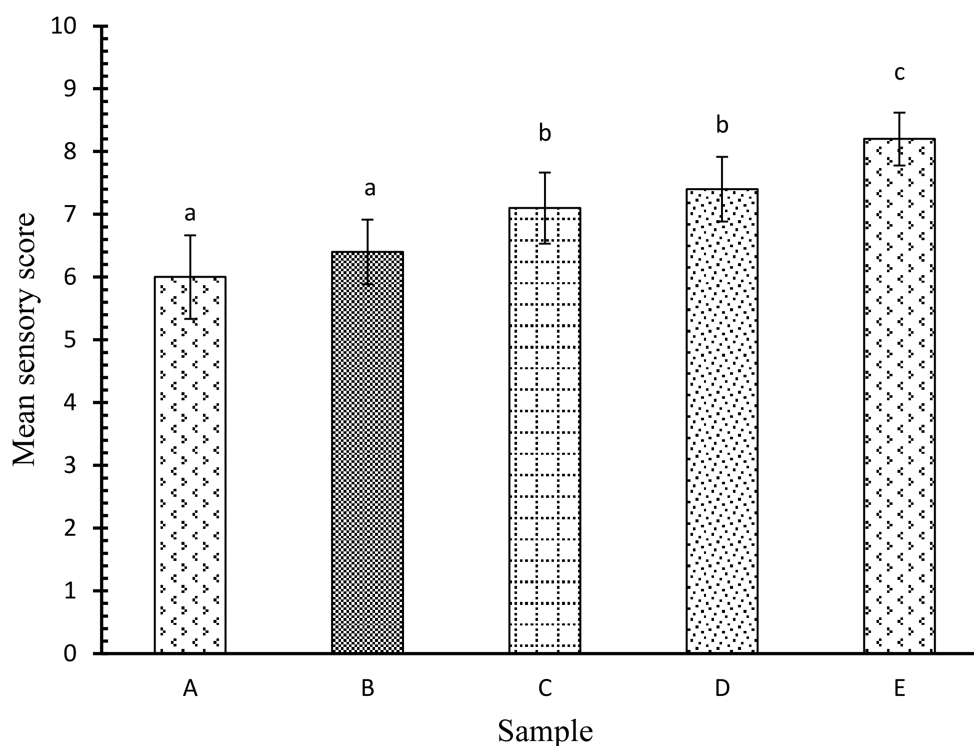


Fig.4.10 Mean sensory score for overall acceptability

The values in the figure are the mean sensory score for overall acceptability. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represent standard deviation of scores.

Overall acceptability is the reflection of other sensory attributes. On the basis of color, flavor, taste, after taste sample D and E were most liked by panelists. Statistical analysis showed higher acceptability for sample E.

4.4.6 Optimization of study

As regard with the phytochemicals analysis and sensory scores, the optimization study is carried out as given below.

Table 4.2 Optimized goals for treatment (phytochemical analysis)

Parameters	Optimized treatment
TPC	E
TFC	D=E
Tannins	E
Antioxidant activity	E
Chlorophyll	C

Table 4.3 Optimized goals for treatment (sensory)

Parameters	Optimized treatment
Color	C
Flavor	D
Taste	D=E
After taste	E
Overall acceptance	E

From the study of different parameter, sample E had superior sensory characteristics. Similarly, in terms of phytochemicals analysis sample E was found to be superior. So it can be concluded that sample E i.e. wheatgrass of day 10 is superior than any other samples in terms of both sensory and phytochemicals analysis.

Part V

Conclusions and recommendations

5.1 Conclusion

The effect of growth days on bioactive compounds of wheatgrass powder *(Triticum aestivum)* and their sensory attributes was studied. Based on this research following conclusion can be drawn.

a) Methanolic extract of wheatgrass powder showed presence of total phenols, flavonoids, tannins, antioxidant activity and chlorophyll
b) Total phenol content of wheatgrass powder increased from 318.1 GAEmg/g on 6th day to 454.7 GAEmg/g on 10th day.
c) Flavonoid content of wheatgrass powder increased from 105.7 QEmg/g to 429.4 mg/g 10th day.
d) Tannin content of wheatgrass powder decreased with growth from 10.24 mg/g on 6th day to 2.077 mg/g on 10th day.
e) Antioxidant activity of wheatgrass powder was found to be 80.48% on 6th day which increased respectively to 93.09% on 10th day.
f) Chlorophyll content of wheatgrass powder increased from 10.86 mg/g on 6th day to 14.27mg/g on 8th day and then decreased to 7.12 mg/g on 10th day.
g) Growth of wheatgrass showed significant impact on phytochemical and sensory attributes.
h) Wheatgrass of 10th day was found to be superior in terms of phytochemical and sensory attributes among other samples.

5.2 Recommendations

The plant studied in this work is on high demand because of its nutritional value and traditional uses. Thus, following suggestions are recommended for future work in the field of wheatgrass.

a) Effect of processing on phytochemical composition of wheatgrass can be studied.
b) Response of wheatgrass powder on blood sugar level of human body can be studied.

c) As phytochemical composition was increasing, further research on wheatgrass above 10th day can be done.
d) Preparation and quality evaluation of wheatgrass incorporated product can be performed.

Part VI

Summary

Wheatgrass, (*Triticum* speices) a cereal grass of the *Gramineae* (*Poaceae*) family, is the world's largest edible grain cereal-grass crop. Wheat (*Triticum aestivum*) germinated over a period of time is called as a wheat grass (Nepalese tongue – Jamara). *Triticum* is a genus of annual and biennial grasses, yielding various types of wheat, native to south west Asia and the Mediterranean region. Agriculturally, important species of *Triticum* include – *Triticum asetivum, Triticum durum* and *Triticum dicoccum*. Among them, *Triticum aestivum* was brought from the local market of Dharan.

Wheat (*Triticum aestivum*) seeds were soaked overnight, germinated for 12 hours on muslin cloth and spreaded over the soil avoiding direct sunlight. After plantation, wheatgrass was harvested manually on 6th, 7th, 8th, 9th and 10th day separately. Harvested wheatgrass was subjected to cabinet drying (50°C, 5hr) and then powdered in a grinder to get the required sample. Crude extract of samples were prepared using 80% methanol through maceration technique. Phytochemicals screening of the methanol extract of the sample showed the presence of total phenol content, flavonoid, tannin, glycosides, terpenoids, steroids and saponins. Phytochemicals and sensory analysis of wheatgrass powder of 6th, 7th, 8th, 9th and 10th days was carried out.

Total phenol content, flavonoid and DPPH radical scavenging activity of wheatgrass increased significantly ($p<0.05$) with growth to 454.7 mgGAE/g, 429.4 mgQE/g and 93.09% on 10thday. Tannin content decreased significantly ($p<0.05$) to 2.077 mgGAE/g. Chlorophyll content increased with growth upto 8th day to 14.27 mg/g and then decreased on day 9 and day 10 respectively. Wheatgrass harvested on day 10 was found superior in terms of both phytochemicals and sensory attributes. The composition of phytochemicals varies widely with several factors like the stage of maturity, variety, climatic conditions, part of the plant analyzed, post-harvest handling, processing, and storage.

Wheatgrass is known as complete nourishment and is promoted to treat a number of conditions. So, it is better to include wheatgrass in regular diet in any form like juice, powder, etc. to prevent chronic diseases.

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Appendices

Appendix A

SPECIMEN CARD FOR SENSORY EVALUTION

Hedonic rating test

Name of the panelist ………………….

Date ………………………..

Name of the product : Wheatgrass powder

Dear panelist, you have 5 sample of wheatgrass powder. Please taste the sample and score how much you prefer the each one. Please give point for your degree of preference for each parameter as shown below using the scale given.

Parameter	A	B	C	D
Color				
Flavor				
Taste				
After taste				
Overall acceptability				

Give points as follows:

Like extremely 9

Like very much 8

Like moderately 7

Like slightly 6

Neither like nor dislike 5

Dislike slightly 4

Dislike moderately 3

Dislike very much 2

Dislike extremely 1

Comment(if any)………………………………………………………

Signature…………………

Appendix B

Table B.1 List of chemicals used

Chemicals	Supplier/Manufacturer	Other specifications
Sodium hydroxide (NaOH)	Thermo fisher Scientific India Pvt. Ltd.	Pellets, AR grade, 98%
Hydrochloric acid (HCl)	Thermo Electron LLS India Pvt. Ltd.	36%, LR grade
Sulphuric acid (H_2SO_4)	Thermo fisher Scientific India Pvt. Ltd.	97%, LR grade
Petroleum ether	Merck life Pvt. Ltd.	B.P. 60°C-80°C
Sodium Carbonate (Na_2CO_3)	Qualigens fine chemicals	99.5%, LR grade
Methanol	Merck life science Pvt. Ltd	99% Liquid
Sodium nitrate($NaNO_3$)	Thermo Fischer scientific India, Pvt. Ltd	98%
Aluminium chloride($AlCl_3$)	S.D fine-chem Ltd	98% hygroscopic
Ferric chloride($FeCl_3$)	Thermo Fischer scientific India, Pvt. Ltd	96% anhydrous
Chloroform	Merck life science Pvt. Ltd	99% Liquid
Folin-ciocalteu's reagent	Thermo Fischer scientific India, Pvt. Ltd	Liquid
Acetic acid	Thermo Fischer scientific India, Pvt. Ltd	99% Liquid
Gallic acid	-	99.5%
DPPH	Himedia laboratories(India) Pvt. Ltd	Amorphous

Table B.2 List of equipment used

S.N	Physical apparatus	Specification
1.	Electric balance	Phoenix instrument, 620g
2.	Spectrophotometer	Labtronics, India
3.	Hot air oven	Victolab, India
4.	Incubator	Victolab, India

5. Knives: Stainless steel knives were used for the purpose of cutting

6. Micropipette, pipette

7. Thermometer

8. Measuring cylinder

9. Refrigerator

10. Grinder

11. Glasswares (Beaker, Volumetric flask, Conical flask, Burette, Petri dish, Porcelain basin, etc.)

Appendix C

Table C.1 Nutritional composition of wheatgrass (*Triticum aestivum*)

Basic Nutrients	Minerals	Vitamins
Calories: 21.0cal	Iron: 0.61 mg	Vitamin C: 3.64 mg
Water: 95 g	Magnesium: 24 mg	Vitamin A: 427 mg
Fat: 0.06 g	Potassium: 147 mg	Vitamin B1: 0.08mg
Carbohydrates: 2.0 g	Zinc: 0.33 mg	Vitamin B2: 0.13 mg
Dietary fiber: <0.1g	Calcium: 24.2 mg	Vitamin B3: 0.011 mg
Choline: 92.4 mg	Selenium: <1ppm	Vitamin B5:6.0 mg
Glucose: 0.80 g	Phosphorous: 75.2 mg	Vitamin B6:0.2 mg
Chlorophyll: 42.2 mg	Sodium: 10.3 mg	Vitamin B12: <1mcg

Source: (Sowjanya *et al.*, 2015)

Table C.2 Dry amount of extract

Samples	Day 6	Day 7	Day 8	Day 9	Day 10
Dry matter in 10ml (mg)	0.181	0.154	0.177	0.182	0.158

Table C.3 Absorbance reading (100 fold dilution)

Samples	Abs for TPC	Abs for TFC	Abs for tannins
Day 6	0.464, 0.466. 0.465	0.002, 0.001, 0.001	0.009, 0.009, 0.008
Day 7	0.456, 0.458, 0.456	0.003, 0.002, 0.002	0.008, 0.007, 0.007
Day 8	0.556, 0.554, 0.555	0.004, 0.005, 0.004	0.007, 0.007, 0.006
Day 9	0.609, 0.606, 0.609	0.005, 0.006, 0.006	0.006, 0.005, 0.006
Day 10	0.581, 0.588, 0.587	0.005, 0.006, 0.007	0.005, 0.004, 0.004

Table C.4 Absorbance reading (100 fold dilution)

Samples	Abs for AOA	Abs for chl at 645 nm	Abs for chl at 663 nm
Day 6	0.022, 0.02, 0.023	0.253, 0.254, 0.256	0.401, 0.404, 0.410
Day 7	0.017, 0.018, 0.017	0.280, 0.283, 0.282	0.410, 0.406, 0.401
Day 8	0.015, 0.013, 0.014	0.309, 0.311, 0.313	0.572, 0.577, 0.573
Day 9	0.010, 0.011, 0.011	0.238, 0233, 0.239	0.315, 0.320, 0.325
Day 10	0.009, 0.008, 0.006	0.151, 0.149, 0.157	0.294, 0.292, 0.289

Table C.5 Temperature and Relative Humidity during germination of wheat

Sample	Room temperature (°C)	Water temperature (°C)	Relative Humidity (%)
Day 1	24.5	24.4	71
Day 2	24.5	24.9	68
Day 3	23.5	24.3	65
Day 4	24.5	25.1	69
Day 5	25.5	25.6	62
Day 6	24.9	24.3	62
Day 7	25.5	25.2	62
Day 8	25.5	24.8	64
Day 9	24.9	24.9	61
Day 10	25.1	24.9	65

Table C.6 Quantitative analysis of phytochemicals in wheatgrass

Samples	TPC (mgGAE/g)	TFC (mgQE/g)	Tannins (mgGAE/g)	DPPH radical Scavenging activity (% inhibition)	Chlorophyll (mg/g)
A	318.1±0.65^{a}	105.7±33.29^{a}	10.24±1.04^{d}	80.48±1.27^{a}	10.86±0.09^{c}
B	367.4±0.88^{b}	192.0±39.13^{b}	9.20±1.22^{d}	84.38±0.63^{b}	11.50±0.04^{d}
C	385.6±0.66^{c}	285.0±34.04^{c}	6.77±1.06^{c}	87.38±0.63^{c}	14.27±0.06^{e}
D	409.5±1.12^{d}	353.7±33.11^{cd}	4.79±1.03^{b}	90.39±0.63^{d}	9.40±0.10^{b}
E	454.7±2.84^{e}	429.4±66.06^{d}	2.077±0.01^{a}	93.09±0.63^{e}	7.12±0.07^{a}
F pr.	<0.001	<0.001	<0.001	<0.001	<0.001
L.S.D	2.700	78.3	1.784	1.845	0.1414

Table C.7 Average sensory score

Sample	Color	Flavor	Taste	After taste	Overall acceptability
A	7.5±0.52^{c}	5.9±0.73^{a}	5.9±0.56^{a}	5.7±0.67^{a}	6.0±0.66^{a}
B	7.5±0.52^{c}	6.6±0.69^{b}	6.3±0.48^{a}	6.3±0.67^{b}	6.4±0.51^{a}
C	8.1±0.31^{d}	6.7±0.48^{b}	6.8±0.63^{b}	6.9±0.56^{c}	7.1±0.56^{b}
D	6.2±0.42^{b}	8.0±0.42^{d}	8.2±0.42^{c}	7.2±0.42^{c}	7.3±0.51^{b}
E	5.4±0.51^{a}	7.4±0.51^{c}	8±0.66^{c}	8.2±0.42^{d}	8.2±0.42^{c}
F. pr	<0.001	<0.001	<0.001	<0.001	<0.001
L.S.D	0.4454	0.4903	0.4243	0.4940	0.4828

Appendix D

Standard curve of Gallic acid for phenol

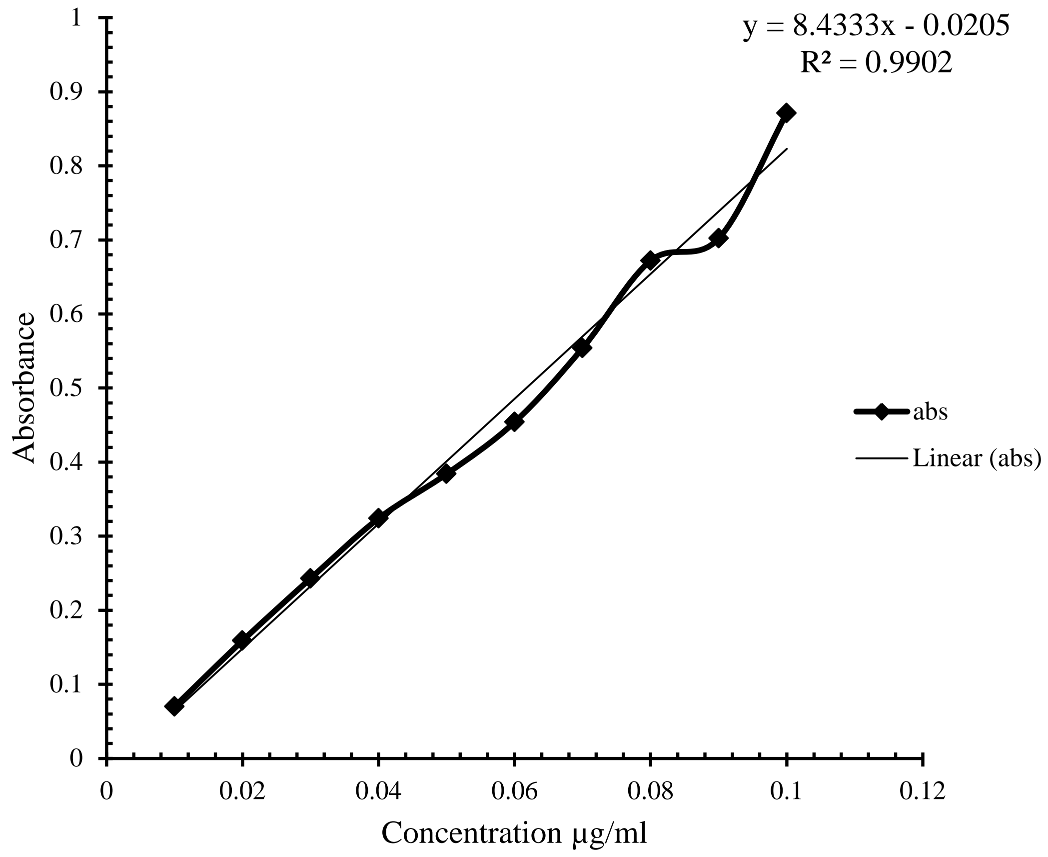

Fig D.1 Gallic acid standard curve

Standard curve of quercetin for flavonoid

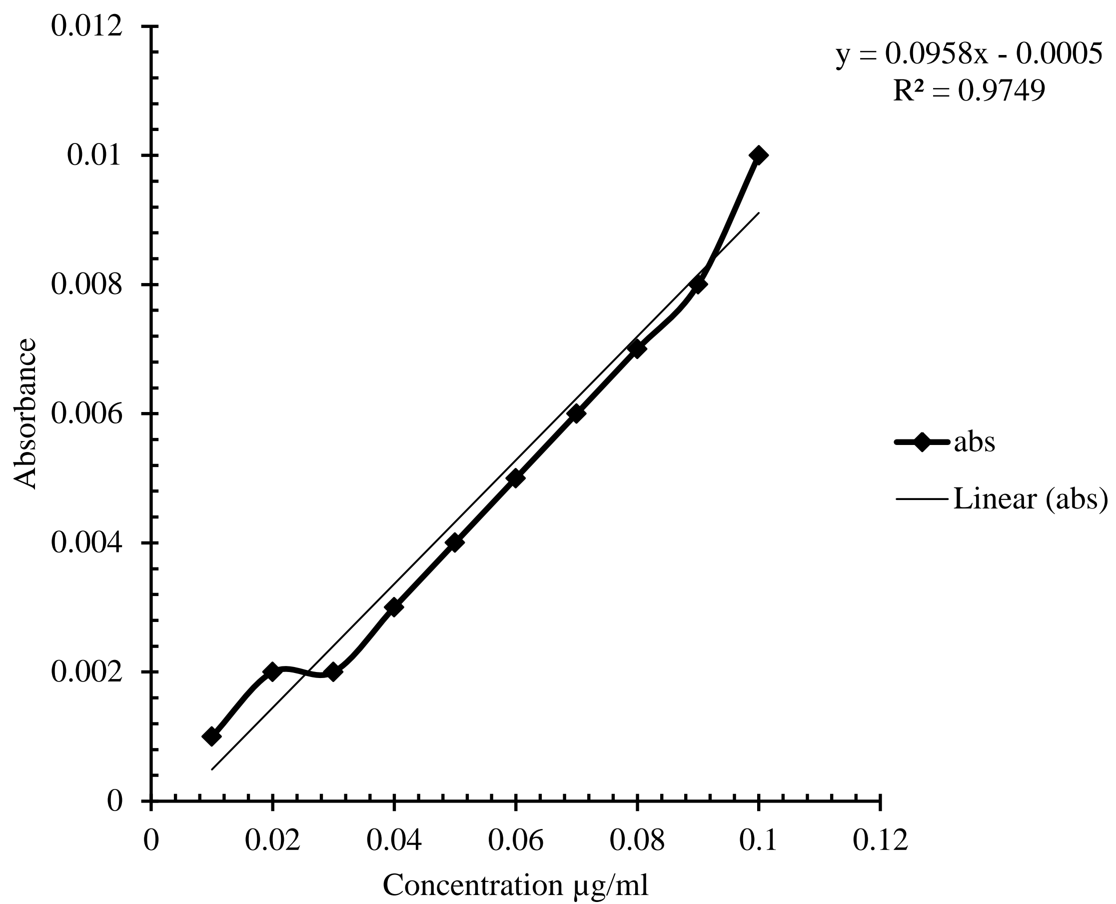

Fig D.2 Quercetin standard curve

Standard curve of Gallic acid for tannin

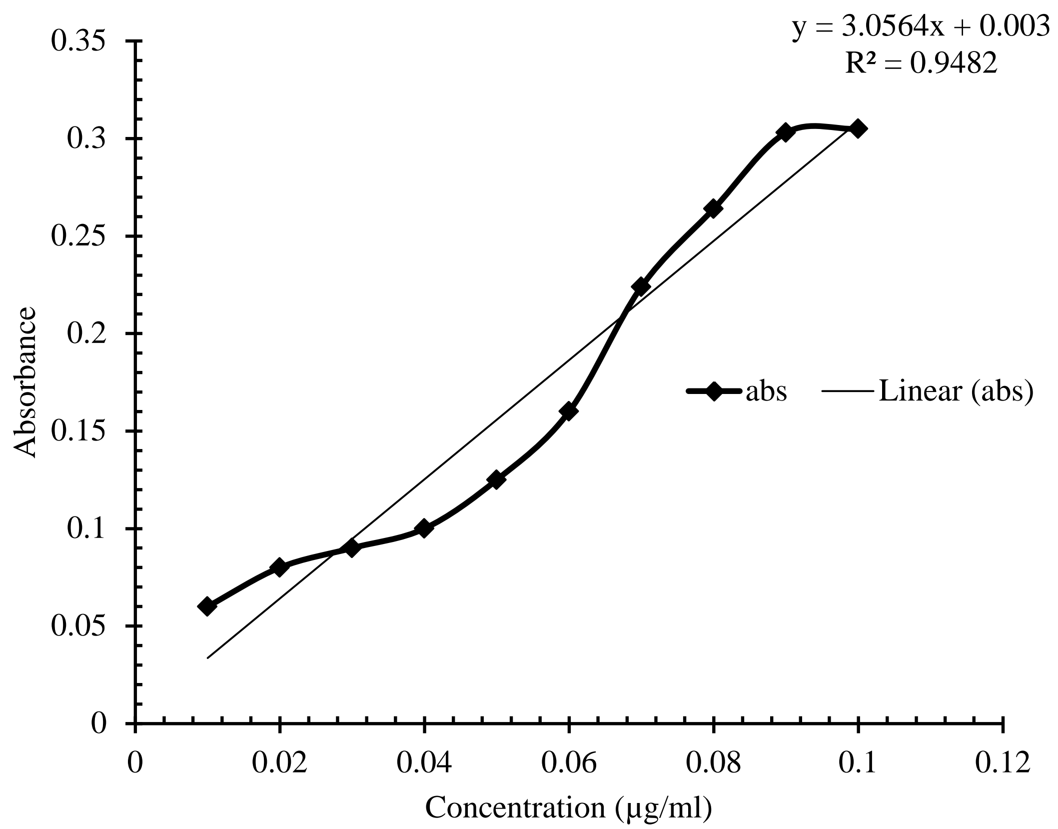

Fig D.3 Tannin standard curve

Appendix E

Table E.1 One way ANOVA(no blocking) for total phenol content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	30671.831	7667.958	3480.44	<.001
Residual	10	22.032	2.203		
Total	14	30693.862			

Table E.2 Least significant differences of means (5% level) for total phenol content

Table	Rep	d. f.	L.S.D.
Sample	3	10	2.7

Table E.3 One way ANOVA(no blocking) for Flavonoid content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	196970	49243	26.59	<.001
Residual	10	18521	1852		
Total	14	215492			

Table E.4 Least significant differences of means (5% level) for flavonoid content

Table	Rep	d. f.	L.S.D
Sample	3	10	78.3

Table E.5 One way ANOVA(no blocking) for Tannin content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	131.4563	32.8641	34.16	<.001
Residual	10	9.6202	0.9620		
Total	14	141.0765			

Table E.6 Least significant differences of means (5% level) for tannin content

Table	Rep	d. f.	L.S.D
Sample	3	10	1.784

Table E.7 One way ANOVA(no blocking) for DPPH radical scavenging activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	219.3545	54.8386	92.14	<.001
Residual	10	5.9519	0.5952		
Total	14	225.3064			

Table E.8 Least significant differences of means (5% level) for DPPH radical scavenging activity

Table	Rep	d. f.	L.S.D.
Sample	3	10	1.845

Table E.9 One way ANOVA(no blocking) for Chlorophyll

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	43.0240	10.7560	11.23	<.001
Residual	10	9.5767	0.9577		
Total	14	52.6008			

Table E.10 Least significant differences of means (5% level) for Chlorophyll

Table	Rep	d. f.	L.S.D.
Sample	3	10	0.1414

Table E.11 Two way ANOVA(no blocking) for Color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	48.9200	12.2300	50.72	<.001
Panelist	9	1.2200	0.1356	0.56	0.818
Residual	36	8.6800	0.2411		
Total	49	58.8200			

Table E.12 Least significant differences of means (5% level) for color

Table	Rep	d.f.	L.S.D
Sample	10	36	0.4454
Panelist	5	36	0.6298

Table E.13 Two way ANOVA(no blocking) for Flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	25.8800	6.4700	22.14	<.001
Panelist	9	7.2800	0.8089	2.77	0.014
Residual	36	10.5200	0.2922		
Total	49	43.6800			

Table E.14 Least significant differences of means (5% level) for flavor

Table	Rep	d.f.	L.S.D
Sample	10	36	0.4903
Panelist	5	36	0.6934

Table E.15 Two way ANOVA(no blocking) for Taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	41.7200	10.4300	47.65	<.001
Panelist	9	6.3200	0.7022	3.21	0.006
Residual	36	7.8800	0.2189		
Total	49	55.9200			

Table E.16 Least significant differences of means (5% level) for taste

Table	Rep	d.f.	L.S.D
Sample	10	36	0.4243
Panelist	5	36	0.6001

Table E.17 Two way ANOVA(no blocking) for After taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	35.7200	8.9300	30.10	<.001
Panelist	9	3.6200	0.4022	1.36	0.244
Residual	36	10.6800	0.2967		
Total	49	50.0200			

Table E.18 Least significant differences of means (5% level) for After taste

Table	Rep	d.f.	L.S.D
Sample	10	36	0.4940
Panelist	5	36	0.6986

Table E.19 Two way ANOVA (no blocking) for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	29.00	7.2500	25.59	<.001
Panelist	9	2.80	0.3111	1.10	0.389
Residual	36	10.20	0.2833		
Total	49	42.00			

Table E.20 Least significant differences of means (5% level) for overall acceptability

Table	Rep	d.f.	L.S.D
Sample	10	36	0.4940
Panelist	5	36	0.6986

Color plates

P1 Growing of wheatgrass

P2 Wheatgrass powder

P3: Absorbance reading

P4: Sensory analysis