"DESIGN AND DEVELOPMENT OF WHEATGRASS GRANULES AND ITS COMPARATIVE EVALUATION WITH MARKET SAMPLE "

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"Gratitude unlocks the fullness of life. It turns what we have into enough, and more. It turns denial into acceptance, chaos to order, confusion to clarity. It can turn a meal into a feast, a house into a home, a stranger into a friend. Gratitude makes sense of our past, brings peace for today, and creates a vision for tomorrow".

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Sr.No	Short form	Full form
1	%	Percentile
2	%W/V	Percentage weight by volume
3	%W/W	Percentage weight by weight
4	Cm	Centimetre
5	H_2SO_4	Sulphuric Acid
6	Kg	Kilogram
7	Mg	Magnesium
8	Min	Minute
9	Ml	Millilitre
10	Na_2CO_3	Sodium Carbonate
11	HCl	Hydrochloric Acid
12	R_f	Retention Factor
13	TLC	Thin Layer Chromatography
14	α	Alpha
15	β	Beta
16	µ	Micro
17	WHO	World Health Organisation
18	US	United States
19	mg	Milligram
20	mm	Millimetre
21	Std	Standard
22	IU	International Unit
23	IPA	Iso Propyl Alcohol
24	Rpm	Rotation Per Minute
25	Ppm	Parts Per Million
26	M	Molar
27	$NaHCO_3$	Sodium bicarbonate
29	$	Dollar
30	AE	Aqueous Extract
31	AYUSH	Ayurveda Yoga Unani Siddha Homeopathy

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ABSTRACT

ABSTRACT

The Human diet is enriched with young parts of plants (so called —green foods), which can improve nutrient balance intake in natural way. Wheatgrass (*Triticum aestivum*) refers to young grass of the common wheat plant, which belongs to Poaceae family. This is the most commonly found herb in India, although its nativity is currently unknown. This plant is believed to have many nutritional values; it has been shown to have anti-inflammatory, antioxidant, anti-carcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial. Wheatgrass juice helps in building red blood cells and stimulates healthy tissue cell growth. The wheatgrass was grown in laboratory in predefined conditions and the wheat grass extract was obtained and non-effervescent granules were formulated. Various nutritional properties were studied and compared with marketed sample and Formulation.

The stability of wheatgrass juice is very poor hence consuming it after few hours would not provide the same benefits as when consumed fresh. But, it is tedious for people to collect fresh grass daily and consume it, since the daily requirement of wheatgrass is 2-3 ounce only. Therefore our aim of the study was to formulate a safe, stable and effective formulation in form of oral nutritional herbal drink which would provide same benefits as the fresh wheatgrass.

The main objectives of the study were:

- Pharmacognostic evaluation of grass with the aim of establishing the diagnostic standards.
- Physicochemical evaluation and phytochemical analysis of grass using various chemical tests and quantitative estimations.
- Growing grass and preparation of extract from fresh wheat grass.
- Selection of appropriate solubilisation technique, optimization of various process variables and formulation of non-effervescent granules from wheat grass extract.
- Evaluations of various nutrients present in the formulation and its comparison with marketed sample and laboratory grass.

The characters were studied first for authentication of the grass of *Triticum Aestivum*. The morphological and microscopical evaluation was done for proper identification of the herbal drug. The morphology showed glabrous, auriculate, with blades narrowly to broadly linear; broad to narrow; 2-20 mm wide, flat, without cross venation. Transverse section of leaflet shows an Upper epidermis covered with cuticle. Only covering trichomes emerge from epidermal layer.Mesophyll is made up of uniform parenchyma cells, loosely arranged. Lower epidermis is very similar to upper epidermis. Midrib represents a flat ventral surface and convex dorsal surface. The epidermal layers are continuous over the midrib, collateral and conjoint vascular bundle is prominent occupying the central portion of the midrib. Vascular bundle is surrounded by sclerenchymatous tissues.same character were found for powder microscopy.

The wheat species *Triticum aestivum* were grown in plastic trays as per the standard procedure. After proper height was obtained by the grown grass, it was cut. This fresh mature wheat grass was taken and washed with fresh water in order to remove any earthy matter. This washed grass was then placed into an extracting solution of distilled water and methanol in a ratio of 7:3 respectively in order to obtain the proper extract and presence of methanol served as a preservative. This mixture was then sonicated for duration of 1-2 hours at room temperature. The resulting solution was filtered with wattman filter paper with a prepared cotton bed placed above it. During filtration, the extract was extensively and carefully squeezed in order to obtain almost all the filterate as well as to prevent any damage to the filter aid. The filterate was then placed in the rotary vacuum bath for solvent reduction for duration of 3-4 hours. The resulting concentrated solution was then centrifuged for 7 minutes at 5000rpm, room temperature. After centrifugation, the supernatant was decanted carefully and the semi-solid mass obtained below the supernatant was placed in a china dish and dried on water bath at a temperature below 40˚C for a duration it was completely dried. After drying, the product was scrapped from the china dish and slightly hard lumps of the extract were obtained. These hard lumps were properly grinded in a glass mortar pestle followed by sieving using a 100 mesh sieve until a powdered extract is obtained.The extract was prepared from fresh wheatgrass juice. The solubility was the main issue for the obtained extract found was very less soluble in water. So various techniques like complexation, particle size

reduction with β-cyclodextrin and evaporate to dryness were employed and compared to select the best approach to be used the formulation development.The combination of both complexation and particle size reduction with β-cyclodextrin showed an increase in solubility of the extract in water. The noneffervescent granules were formulated and the nutritional studies like estimation of Vitamin B1, Vitamin C, Vitamin E, Amino acid, Chlorophyll, Phenolics and Flavonoids in extract, marketed sample, laboratory grass and formulation.

The solubility of extract was found increased in water due to the combination approach of complexation and particle size reduction with β-cyclodextrin and the nutritional drink was formulated. The Phytochemical analysis combined with TLC studies showed many spots resolvedat different Rf values. The nutritional data obtained showed satisfactory result of Vitamin B1, Vitamin C, Vitamin E, Amino acid, Chlorophyll, Phenolics and Flavonoids in extract, marketed sample, laboratory grass and formulation.

Due to the various uses of wheatgrass, the extract was formulated so that it can be taken orally and can be useful during various disease conditions specifically blood related disorders and gastric problems.

Various phytochemical parameter were also evaluated for grass like Determination of acid in soluble ash, Determination of water soluble ash, Determination of alcohol soluble extractive, Determination of water soluble extractive, Determination of moisture contain.

Preliminary phytochemical screening showed the presence of various phytoconstituent like carbohydrates, sterols, seponins, phenolic compound and tennins, amino acid and proteins and flavonoids.

CHAPTER 1

INTRODUCTION

INTRODUCTION

Herbal drugs constitute a major share of all the officially recognised systems of health in India viz. Ayurveda ,Yoga, Unani, Siddha Homeopathy and Naturopathy, except Allopathy. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. However, there is a vast experiential-evidence base for many of the natural drugs.(Ashok et al.,2007).

There are 9493 manufacturing units, 22,635 dispensaries and 1355 hospitals of the Indian Systems of Medicine. Approximately 800 species of medicinal plants are in active trade and still there is a gap of 40,000 metric tonnes in the demand and supply of medicinal plants.(Anon,2006) With a view to strengthen the medicinal plants sector all over the country as well as to conserve the wild stock, the NMPB(National Medicinal Plants Board) was set-up by the Government of India on 24thNovember 2000.(Rawat,2003).

Herbal medicine is still the mainstay of about 75% of the world population,especially in the under developed and developing countries, for primary healthcare because of better cultural acceptability, better compatibility with thehuman body and lesser side effects. However, in the last few years there hasbeen a major increase in their use in the developed world. In Germany andFrance, many herbs and herbal extracts are used as prescription drugs. Theirsales in the countries of European Union were around $ 6 billion in 1991 andmay be over $ 20 billion now. About 25% of modern medicines are descendedfrom plants used traditionally.(Desai et al,2005).

Medicinal plants are not only a major resource for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. The domestic trade of the AYUSH (Ayurveda,Yoga,Unani,Siddha Homeopathy) industry is of the order of Rs. 80 to 90 billion.The Indian medicinal plants and their products also account of exports in the range of Rs. 10 billion. According to WHO(World Health Organisation) Annual revenues in Western Europe reached US$ 5 billion in 2003-2004. In China, sales of products totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007.(Traditional medicine: http://www.who.int/mediacentre/ factsheets/fs134/en).

According to The National Medicinal Plants Board, Ministry of Health andFamily Welfare, Govt. of India has 15 agro climatic zones, with 47000different plant species and 15000 medicinal plants The Indian Systems ofMedicine have identified 1500 medicinal plants, of which 500 species aremostly used therapeutically. The medicinal plants contribute to cater 80% ofthe raw materials used in the preparation of drugs. The effectiveness of thesedrugs mainly depends upon the proper use and sustained availability ofgenuine raw materials. Besides this, there is also a growing demand for natural products includingitems of medicinal value/pharmaceuticals, food supplements and cosmetics inboth domestic and international markets. Presently, India' s export, frommedicinal and herbal plants, is Rs. 3000 crores. India, with its diversifiedbiodiversity has a tremendous potential and advantage in this emerging area.(Desai et al,2005)

India is one of the most important countries in the world in term of floristic diversity. About 54% of the country‟s land is under cultivation for food, ornamental and medicinal plant crops and approximately 19% area has varying degree of forest vegetation cover. India is the largest producer of medicinal herbs and approximately called the botanical garden of the world.(Ahmadullah,1999).

The Human diet is enriched with young parts of plants (so called ―green foods‖), which can improve nutrient balance intake in natural way. Wheatgrass (*Triticumaestivum*) refers to young grass of the common wheat plant, which belongs to Poaceae family. This is the most commonly found herb in India, although its nativity is currently unknown. This plant is believed to have many nutritional values; it has been shown to have anti-inflammatory, antioxidant, anti-carcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial. Wheatgrass juice helps in building red blood cells and stimulates healthy tissue cell growth. 100 g of wheatgrass powder is equal to 23 kg of fresh vegetables. Ideally, wheatgrass should be taken about an hour prior to meal. This allows the body to fully metabolize it without competing with other foods, and it may also curb hunger. It is recommended that lot of water (at least a liter) should be consumed with the juice to reap its maximum nutritional benefits. Taking wheatgrass as a supplement in the mid-morning or mid-afternoon is a great time for this "green" energy boost.(SatyavatiRana at al.,2011).

HISTORY

Wheat grass 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.

Ann Wigmore

Ann Wigmore (called mother of wheatgrass) re-discovered the health benefits of wheatgrass in the 1970s, but it was Dr. Charles Schnabel who was first to discover them 1925. Dr. Schnabel was not alone. Scientists, medical doctors and other health practitioners produced a significant volume of research on wheatgrass during the 25 years between 1925 and 1950. Ann Wigmore's bibliographies in her books list many of them, especially Dr. Schnabel. Their research was the scientific basis for many of Ann Wigmore ideas. The research by Schnabel and other scientists used dehydrated wholefood wheatgrass powder that had been grown for 200 days over the winter in special glacial soil in Northeast Kansas(The Ann Wigmorebiography (1909-1994))

[Fig 1: Ann Wigmore]

Ann tested her indoor grasses on her animal friends. Wheat became her favorite grass because the animals chewed more of it and it was sweet tasting, easy-to-find and inexpensive. To further her studies, she even adopted a sick and cancerous monkey. Ann nursed the monkey back to good health with creative techniques and live food recipes including sprouted seeds, fermented nut and seed "yogurt," and rejuvelac, a

cultured sprouted wheat drink. These, along with wheatgrass, would later become the cornerstone of her Living Foods Diet.

Ann began delivering fresh wheatgrass juice to bedridden ill and elderly people in her Boston neighborhood. Then in 1958, she turned an old mansion on Commonwealth Avenue in Boston into The Hippocrates Health Institute. It was founded on the principle of Hippocrates, the Greek father of modern medicine, who along with his Hippocratic oath, is often paraphrased as saying: "The body heals itself. The physician is only nature's assistant." Dr. Ann, as she was fondly called after she became a doctor of divinity, believed that the body can act as its own physician given the proper tools -- living foods. "Living foods for living bodies, dead foods for dead bodies," said Ann (AnnWigmore, 1909-1994).

Dr. Ann has a long list of stories and testimonials from guests who improved their health with the help of wheatgrass. Wheatgrass and its sister, triticum barley, have by testimony helped guests with ailments such as high blood pressure, diabetes, obesity, gastritis, stomach ulcers, pancreas and liver troubles, asthma, glaucoma, eczema, skin problems, constipation, hemorrhoids, diverticulitis, colitis, fatigue, menstrual problems, arthritis, athlete's foot, anemia, bad breath/body odor, and burns. In addition, wheatgrass has served as a wonderful first aid for red eyes, wax in ears, congested nasal passages, bleeding gums, tooth pain, sore throats, and inflamed mucous membranes (Wigmore 1985).

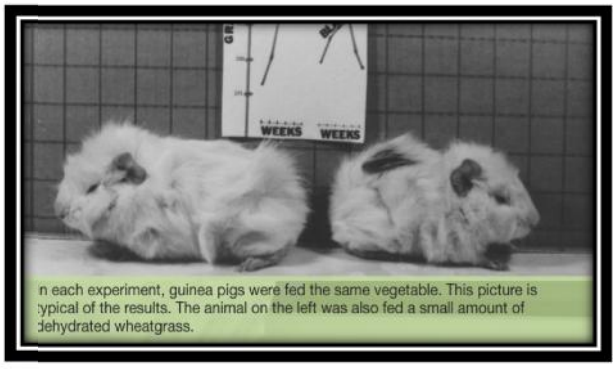
Charles Schnabel

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 hens 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.

[Fig 2: Charles Schnabel]

By 1940, 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.

[fig 3: Old and new laboratory of Charles Schnabel]



[fig 4: Wheat grass experiments]

Steve Meyerowitz

"Sprouts are unique in that they are the only form of agriculture available in all four seasons that can be locally grown---and that means anywhere in the world, from Africa to Alaska! Their harvest cycle, from seed to salad, is only one week. Not only that, one pound of alfalfa seed, for example, yields 10-14 pounds of fresh "mini-salad" greens"

[Fig 5: Steve Meyerowitz]

Wheat, (Triticum species) a cereal grass of the , is the world's largest edible grain cereal-grass crop. Scientific reports on nutritional analysis of wheatgrass have been published frequently in various journals. These reports and the chemical analyses undertaken reveal that wheatgrass is rich in chlorophyll, minerals like magnesium, selenium, zinc, Calcium, Potassium, Phosphorus, Sodium, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value.

Wheatgrass juice is a fast and sure way to cleanse our bodies of environmental pollutants. Its high levels of enzymes and amino acids work like a "natural detergent" to detoxify the liver, eliminate toxic heavy metals from the blood stream, rid the body of waste matter and help to strengthen the immune system.

Wheatgrass juice has been proven over many years to benefit people in numerousways: cleansing the lymph system, building the blood, restoring balance in the body,removing toxic metals from the cells, nourishing the liver and kidneys and restoringvitality as claimed by Dr. Ann Wigmore, U. S. A. founder director of the HippocratesHealth Institute, Boston, U.S.A. She claimed that wheatgrass is a safe and

effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes,gastritis, ulcers, anemia, asthma and eczema.

Dr. Christopher recommended this schedule: when you get up in the morning, take a drink of 1 quart warm water, 2 tablespoons unsulphured molasses, and ½ lemon to clear any leftover digestive liquids from the stomach. In a half hour, take your two ounces of wheatgrass juice. This can be taken straight, diluted half and half with distilled water.

Our bodies are complex systems in which there is a delicate chemical balance that keeps everything functioning as it should. Disruptions to the system are going to have consequences with some being more severe than others. Some of these consequences can take the form of disease or irreversible damage. Prevention is always better than trying to cure illness or repair damage. One of the most important parts of prevention is good nutrition. Making sure that you regularly consume the standard recommended daily intake levels of the vitamins, mineral and other nutrients your body needs is the first vital step in keeping a healthy physic and mind.

In this century medical scientists have found chlorophyll to be effective in the general fields of detoxification, deodorization, in ulcer and the healing of wounds. Researchers observed a side benefit when chlorophyll was used to treat peptic ulcers. Chlorophyll tended to "promote regularity" in the patients studied.According to several investigators, chlorophyll did not act simply to stimulate bowel activity, as does a laxative. Rather, it promoted bowel regularity, stimulating bowel action only when that action was sluggish. The same effect was noted in a 1980 study of the use of chlorophyllin (a water-soluble chlorophyll derivative) to reduce body and fecal odours in a geriatric nursing care facility. Today, patients to deodorize the surfaces and contents of colostomies routinely use chlorophyll tablets. Chlorophyll is also administered to incontinent patients to reduce odours in health care facilities.(Murray S,2010)

But because of today's lifestyle and diet, it is very hard therefore to intake the proper daily amount of potassium necessary for a normal life. For this concern, nutritional supplements are the solution.Helping to regulate the body's fluid levels is one of the mineral potassium's greatest functions. Not only that but it also has a great part in regulating the blood pressure. It also helps to keep the heart thumping steadily,

regularly and is also essential to the nervous system. Wheatgrass is entirely nontoxic. It can be used internally or topically without fear of side-effects.

Potassium works to promote the proper functioning of the tissue that makes up the nervous system. It also serves to enhance muscle control plus the growth and health of cells particularly through its importance in waste product removal. This mineral is also vital to the kidneys in their waste removal tasks. Potassium also plays an important role to mental function as well as to physical processes. It helps to promote efficient cognitive functioning by playing a significant role in getting oxygen to the brain. Potassium (K) is the major cation found inside of cells.The proper level ofpotassium is essential for normal cell function. (Charlene JN,2010)

Sodium (Na) is the major extracellular cation and it plays a role in body fluiddistribution. Sodium is essential to the body for fluid balance, muscle contractions and nerve reactions. Sodium is important in maintaining human body fluid volume and maintaining electric potential in the animal tissue.(Determination of sodium and potassium by flame photometry,http://tera.chem. ut.ee/~koit/arstpr/nak_en.pdf)

Calcium strengthens your bones, particularly before you are 35 years old. Most (99%) of calcium is found in bones and teeth with the remaining 1 % in the soft tissues and watery parts of the body where calcium helps to regulate normal processes of the body. Calcium is responsible for construction, formation and maintenance of bone and teeth,muscle contraction. Calcium is a vital component in blood clotting systems in theproduction of enzymes and hormones that regulate digestion, energy, and fat metabolismand also helps in wound healing.(Sizer F at al,1997)

Every day our bodies accumulate internal waste and harmful toxins from eating overcooked, processed and chemically grown food, breathing polluted air and drinking impure water. If we don’t rid the body of these toxins they can cause long-term damage and disease.wheatgrass use for this purpose majorly.

Wheatgrass juice is also a complete protein source. Proteins are responsible for an array of diverse functions throughout the body ranging from cell renewal and creation of hormones, to building and repairing muscles, blood and organs. Proteins are made up of amino acids, which are essential to proper digestion and assimilation of foods,

immunity against disease, rapid healing of cuts and wounds, proper liver function and regulating our level of mental awareness.

We recommend that you start with 1 ounce of wheatgrass juice followed with a glass of water, for the first few days. Once you are comfortable with thisthan you can increase the amount to 2 ounces per day. Wheatgrass has a strong cleansing effect and may make you nauseous if you start with too much. If you have a hard time swallowing that shot of wheatgrass every day try mixing it with water.

Wheatgrass is a vegetable, harvested prior to the plant forming the flower head. Wheatgrass packs a nutritional punch, including (per 3.5 grams) 860 mg protein, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and an abundance of micronutrients, such as B complex vitamins and amino acids. Phytochemical constituents of wheatgrass include alkaloids, carbohydrates, saponins, gum and mucilages. Its water soluble extractive value is found to be greater than its alcohol soluble extractive value. This is because of the chlorophyll content of wheatgrass, which is about 70% water soluble. Wheat grass juice is high in vitamin K, which is a blood-clotting agent. People taking blood-thinning medications or people with wheat-related allergies shouldn't drink wheat grass juice without consulting a health care professional. Wheat allergies are generally a response to the gluten (a protein) found in the wheat berry . Wheatgrass is available in form of extract, tablets (ready to use) and mixed juice (Wheatgrass wonders).

CHAPTER 2

REVIEW OF LITERATURE

LITRETURE REVIEW

2.1 General Information about wheatgrass

Studies from the Department of Food Science and Nutrition, ClemsonUniversity, South California have identified that chlorophyll present in wheatgrass had the potential toneutralize infections, heal wounds, overcomeinflammations and get rid of parasitic infections. Through investigations, it hasbeen found that wheat grass is an excellent source of vitamin C, E, beta caroteneand vitamin B. It is also said to contain 90 different minerals, 19 amino acids andmore iron than spinach and more protein than meat, fish, egg, beans or dairyproducts.(R.Tusharbindu,2005) Zinc and selenium in highly bio-available form is available. The mostvital ingredient of wheat grass is chlorophyll and hence called by a pet name"concentrated solar energy". 100 g of wheatgrass powder is equal to 23 kg of fresh vegetables. Ideally, wheatgrass should be taken about an hour prior to meal. This allows the body to fully metabolize it without competing with other foods, and it may also curb hunger. It is recommended that lot of water (at least a litre) should be consumed with the juice to reap its maximum nutritional benefits. Taking wheatgrass as a supplement in the mid-morning or mid-afternoon is a great time for this "green" energy boost. Wheat grass juice contains significant concentrationof folic acid, which may lead to reduction of high blood pressure. The role ofwheat grass inreducing blood pressure has been established. Wheat grass juicesupplementation to cancer patients has also proved to have a positive impact onthem.

2.2 Taxonomical Details :

Kingdom Plantae – Plants
Subkingdom Tracheobionta – Vascular plants
SuperdivisionSpermatophyta – Seed plants
Division Magnoliophyta – Flowering plants
Class Liliopsida – Monocotyledons
Subclass - Commelinidae
Order -Cyperales
Family - Poaceae Grass family
Genus -*Triticum*L
Species -*Triticumaestivum*

2.3 Different species of wheat:

The principal cultivated grasses are the cereal grains-wheat, rice, corn, barley, oats, rye and millet.(Baker H.,1978).

Wheat species differ from one another both morphologically and genetically. *Triticum*species can be placed in three groups, according to whether their body cells contain 14, 28 or 42 chromosomes. The basic haploid number being 7, these groups are described as Diploid, Tetraploid and Hexaploid respectively. Diploid species include *T. boeoticum*(Wild Eincorn - most ancient variety of wheat) and *T. monococcum*(Eincorn). *T. boeoticum*was growing in Southwestern Asia before the advent of agriculture. *T. monococcum*is now grown to a limited extent in the mountainous region of Yugoslavia, Asia Minor and North Africa. Diploid species can be readily crossed to yield Tetraploid group. Tetraploid species include *T. dicoccum* and*T. durum* (Durum wheat or Macaroni wheat). *T. dicoccum*is one of the most ancient of cultivated cereals. It was formerly grown in the United States for feed on a limited acreage but now has substantially disappeared from cultivation. It is grown to a limited extent in the nilgiri hills and the neighboring areas and is preferred for the preparation of suji or rawa.

T. durum, next important species to *Triticumaestivum*, is used mainly for the manufacture of semolina which is made into macaroni, spaghetti and related products. Although high in gluten, *T. durum* is not good for baking. Instead, it is often ground into semolina, the basis for excellent pasta, such as spaghetti and macaroni. It is grown to a considerable extent in parts of Gujarat and central peninsular India. It is preferred for preparation of vermicelli or sewian. When crossed with Diploid species, Tetraploid species yield Hexaploid group. Hexaploid species include *T. aestivum*(common wheat, bread wheat, local varieties - Sharbati, Lalkanak). *T. aestivum*is the most evolved and widely cultivated of all wheat species. It is high in protein (10-17 %) and yields flour rich in gluten, making it particularly suitable for yeast breads. (AydosO.S ,*et al* ,2011)

The Middle East is probably the area of origin, and wheat apparently spread throughout Europe not later than the Stone Age. Historians believe it has been growing since Paleolithic times and cultivated for at least 6,000 years. Wheat now

provides one-fifth of total developing country food supply, up from 15 % in the early 1970s.(Kahn EJ,1985)

In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. 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. Although over 30,000 varieties of wheat exist, they are of two major types: bread wheat and durum wheat. In U. S. Dept. of Agriculture-Technical Bulletin 1287 has classified wheat into 10 species of *Triticum*. Six of these are cultivated and four are non-cultivated, or rarely so. Agriculturally, important species of *Triticum*include.

2.4 Health benefits of Wheatgrass :

- Excellent source of easily absorbable vitamins, minerals, enzymes and complete proteins
- Helps to prevent and fight against infections.
- Improves the body's ability to heal wounds.
- Helps remove toxic heavy metals from the body.
- Helps with skin problems such as eczema or psoriasis.
- Helps improve blood sugar disorders.
- Helps reduce and eliminate body odours.
- The high magnesium content builds enzymes that restore sex hormones.
- Helps prevent tooth decay.
- Helps to cleanse the liver.
- Arrests the growth of unfriendly bacteria.
- Natural energizer.
- Great for constipation and proper bowel function.
- Non-allergenic.

Wheatgrass in Cancer prevention:

Although many dietary compounds have been suggested to contribute to the prevention of cancer, there is a strong likelihood that wheatgrass extract, which contains chlorophyll, an antioxidant, may affect cancer prevention**.** Additionally, selenium and lactrile present in wheatgrass have anti-cancer properties. Selenium builds a strong immune system, and can decrease the risk of cancer. (Scott c: Brain cancer &wheatgrass). Wheatgrass contains at least 13 vitamins (several of which are antioxidants) including B12, abscisic acid, superoxide dismutase (SOD), cytochrome oxidase, mucopolysaccharide . SOD converts two superoxide anions into a hydrogen peroxide molecule, which has an extra oxygen molecule to kill cancer cells.(Ernst E, 2006).

Hepatoprotective role of wheatgrass

Triticumaestivum leaf extract affects liver enzyme activities as well as lipid peroxidation (Arya P,2011). Jain et al reported the hepatoprotective role of fresh wheatgrass juice has in CCl4 treated rats. It showed a significant hepatoprotective effect with a dose of 100mg/kg/day in terms of SGOT, SGPT, ALP and Bilirubin in serum. (Jain G,2007)

Wheatgrass as cardio protective and anti- hyperlipidemic agent

Wheatgrass has been claimed to reduce the blood pressure as it enhances the capillaries, supporting the growth of lactobacilli (Locniskar M. 1988). An animal study by Kothari et al. found that wheatgrass reduced total cholesterol, LDL, bad cholesterol, and triglyceride levels in rats treated with wheatgrass juice.

Wheatgrass in thalassemia

The pH factor of human blood is 7.4 and the pH factor of wheatgrass juice is also 7.4, which is why it is quickly absorbed into blood. Wheatgrass is an effective alternative to blood transfusion. Wheatgrass has the potential to increase the hemoglobin (Hb) levels, increase the interval between blood transfusions, and decrease the amount of

total blood transfused in thalassemia Major and intermediate Patients. (Singh K.2010).

Wheatgrass and Diabetes

Abundance of natural fiber in wheatgrass optimizes blood sugar levels. wheatgrass (spray dried powder of juice) confirmed the presence of chlorophyll, which is believed to be the pharmacologically active component in wheatgrass, acting as an anti-diabetic agent. (Shirude A.A.,2011).

Wheatgrass and Rheumatoid Arthritis:

Rheumatoid arthritis affects mainly younger individuals, and is three times more common in females than in males. study showed that when 8.5g of fermented wheatgrass extract taken twice per day with water, in case of 15 Severe Rheumatoid Arthritis patients , showed decreased Ritchie index, and according to a health assessment questionnaire, morning stiffness showed significant improvement. Doses of steroids were reduced in half of patients. This may be due to presence of wheatgrass which contains vitamins A, B1, B2, B3, B5, B6 and B12, vitamin C, E and K, Calcium, Iodine, Selenium, Zinc, and many other minerals, including, superoxide dismutase, muco-polysaccarides, and chlorophyll. Its anti-inflammatory properties exert a positive effect on bone and joint problems, reducing pain and swelling.(Nenonen T.,1998)

Wheatgrass and inflammatory conditions

Chlorophyllin has bacteriostatic properties that aids in wound healing (Young MA.,2006). It has been used to treat various kinds of skin lesions, burns, and ulcers, where it acts as a wound-healing agent, stimulating granulation tissue and epithelialization (Chernomorsky SA et al.,1988). It was reported that rate of healing with chlorophyll is so rapid that its inclusion in armamentarium of burn treatment is suggested because it completely supersedes sulphonamide compounds as primary dressing for clean and potentially infected wounds. (Grunewald J.,2009).

Significance of chlorophyll in health and disease –

The most important light-absorbing pigments, in the thyllakoid membrane of a plant leaf, are the chlorophylls. These are green pigments with polycyclic, planar structures resembling the protoporphyrin of hemoglobin, except that Mg^{+2}, not Fe^{+2}, occupies the central position. The four inward oriented nitrogen atoms of chlorophyll are coordinated with the Mg^{+2}. The heterocyclic five-ring system that surrounds the Mg+2 has an extended polyene structure, with alternating single and double bonds. Chloroplasts of higher plants always contain two types of chlorophyll. One is invariably chlorophyll a, and the second in many species is chlorophyll b. Although both are green, their absorption spectra are slightly different, allowing the two pigments to complement each other's range of light absorption in visible region. Most higher plants contain about twice as much chlorophyll a as chlorophyll b.

Chlorophyll itself is not a single molecule but a family of related molecules, designated chlorophyll a, b, c, and d. Cholorophylla is a bluish-green solid and cholorophyll b is a dark green solid, both giving a green solution in organic solutions. In natural chlorophyll there is a ratio of 3 to 1 (of a to b) of the two components.

All the above-mentioned pigments allow plants to absorb energy from visible light. In addition to this, chlorophyll has been reported to be useful in several clinical conditions. Possibly, the presence of magnesium offers wide range of therapeutically useful effects.

In this century medical scientists have found chlorophyll to be effective in the general fields of detoxification, deodorization, in ulcer and the healing of wounds. Chlorophyll tended to "promote regularity" in the patients studied. The same effect was noted in a 1980 study of the use of chlorophyllin (a water-soluble chlorophyll derivative) to reduce body and fecalodors in a geriatric nursing care facility. Chlorophyll use also reduced the amount of intestinal gas experienced by the patients. And, as chlorophyll has no toxic side effects, the "gratifyingly good results" obtained made it preferable to the use of "drastic laxatives". Today, patients to deodorize the surfaces and contents of colostomies routinely use chlorophyll tablets. Chlorophyll is also administered to incontinent patients to reduce odors in health care facilities.Topical chlorophyll ointments and solutions for healing and deodorizing

wounds are still available, as are chlorophyll-containing toothpastes and chewing gums.(Young R et al., 1980)

Chlorophyll has found diverse applications in both medicine and industry.[66] In response to recent concerns over the safety of chemical additives used as food colorants, the food industry has witnessed a renewed interest in using plant pigments as natural colorants. Chlorophyll, produced mainly from grasses, is one of the top four pigments used in the food industry for the coloring of food and drink.Chlorophyllin is the man-made sodium, iron, or copper salt of chlorophyll in which the central magnesium ion of chlorophyll is replaced by the metal ion. In Japan, chlorophyllin is used as a food additive for coloration.[68] Several in vivo experiments and animal experiments show chlorophyll to be safe. Not just low toxicity, NO toxicity-whether ingested, injected or rubbed onto a surface. In medicine, chlorophyll has attracted great interest as a fundamental component of a healthy diet and as a potential therapeutic agent.(Offenkrantz W.,1950)

Just one ounce of fresh wheatgrass juice can contain the vitamins, minerals, enzymes and amino acids found in 2.5 pounds of raw vegetables.The most potent form of wheatgrass is the fresh juice as nutrients are lost in pasteurizing or preserving. Like many raw and highly nutritious foods, fresh wheatgrass juice is highly active chemically and is thus unstable. Fresh is best.

2.5 Macroscopy:

Wheatgrass is young grass shoots of wheat berry. In appearance, wheatgrass is like any other grass.

Culms are simple, hollow or pithy, glabrous, 1.2 m tall. Leaves flat, narrow, 20-38 cm long, 1.3 cm broad. Spikes long, slender, dorsally, compressed, somewhat flattened; rachis tough, not separating from spikelet’s; 2-5 flowered, relatively far apart from stem, slightly overlapping, nearly erect, pressed closed to rachis; glumes keeled in upper half, firm, glabrous, shorter than lemmas; lemmas awned or awnless, less than 1.3 cm long; palea as long as lemma, remaining entire at maturity, caryopsis free threshing, soft

or hard, red or white.(Shirude A.A.,2011)

2.6 Microscopy

Transverse section of leaflet shows an Upper epidermis covered with cuticle. Only coveringtrichomes emerge from epidermal layer. Dumble shaped types of stomata are seen in the upper epidermis.Mesophyll is made up of uniform parenchyma cells, loosely arranged. Lower epidermis is very similar to upper epidermis. Midrib represents a flat ventral surface and convex dorsal surface. The epidermal layers are continuous over the midrib, collateral and conjoint vascular bundle is prominent occupying the central portion of the midrib. Vascular bundle is surrounded by sclerenchymatous tissues. Diagnostic characters seen in powder drug is Dumble shaped stomata along with the epidermal cells, lignified fragments of fibers, unicellular trichomes along with the epidermal cells and oval shaped Starch grains.

The principal cultivated grasses are the cereal grains-wheat, rice, corn, barley, oats, rye and millet.Various researchers have known that the cereal plant, at this young green stage, contains many times the level of vitamins, minerals and proteins found in the seed kernel, or grain product of the mature cereal plant. The nutrient content of these grasses varies with their stage of growth and growing conditions, rather than with the species of cereal grass. 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, the nutritional level in the leaves drops rapidly while the fiber content increases rapidly. The jointing stage is that point at which the internodal tissue in the grass leaf begins to elongate, forming a stem. This stage represents the peak of the cereal plant's vegetative development;factors involved in photosynthesis and plant metabolism would be expected to increase up to this stage. Sucrose, the simple carbohydrate found in table sugar, is the primary molecule from which all organic (carbon containing) molecules are formed in the plant. At the appropriate times and rates, sucrose is converted into amino acids (which make up all proteins), complex carbohydrates, lipids (fats), and

nucleic acids (DNA and RNA). The degree of conversion of sugars to specific complex nutrients is dependent on the activity levels of specific enzymes in the plant. Enzyme activity levels are dependent on the plant's growth stage. Chlorophyll, protein, and most of the vitamins found in cereal grasses reach their peak concentrations in the period just prior to the jointing stage of the green plant. Although this period lasts for only a few days, cereal grasses which are consumed, as food supplements should be harvested precisely during this stage of the wheat or barley plant's development. After the jointing stage, the stem forms branches and continues to elongate. The chlorophyll, protein, and vitamin contents of the plant decline sharply as the level of cellulose increases. Cellulose, the indigestible plant fiber, provides structural stability for the growing stem. (Shankul K., et al., 2010)

2.7 Exogenous antioxidants

2.7.1 Beta-carotene:

Excited-state derivatives such as singlet oxygen and the excited triplet (diradical) states of other molecules may be quenched by interactions with conjugated diene systems such as those found in carotenestocopherols or the melanins. Vitamin A, itself, can pose a hazard to human health and even the risk of birth defects when taken in excess. Its precursor, carotene, can be taken in virtually any quantity without harmful effect. At the same time it provides the body with a store of the raw material from which it produces vitamin A naturally according to needs. carotene has been positively linked to increased protection against many forms of cancer, including lung, bladder, rectal, oral and dermal (skin) cancers. (Huber W,1980)

2.7.2 Vitamin C:

Vitamin C acts synergistically with vitamin E and assists not only in the prevention of the formation of arterial cholesterol plaque but also, in sufficient quantities, has been shown to actually assist in the chelation ("dissolving") of existing cholesterol plaque thereby help in clearing occluded (blocked) arteries, particularly the coronary artery. Vitamin C is also specifically known to assist in the prevention of many forms of cancer, including pancreatic, rectal, cervical, esophageal and oral cancer. It is also a

powerful free radical scavenger and thereby helps in cleaning up the residues of cigarette smoke and other forms of air pollution.(Forrest et al,1974)

2.7.3 Vitamin E:

As well as the primary defenses (scavenger enzymes and metal-ion sequestration), secondary defenses are also present. Both vitamin C and α-tocopherol seem to minimize the consequences of lipid peroxidation in lipoproteins and in membranes, should this process begin.tocopherol is the most effective lipid-soluble chain-breaking antioxidant in vivo in humans. The content of -tocopherol in circulating low-density lipoproteins helps to determine their resistance to lipid peroxidation and thus may affect the development of atherosclerosis, a disease in which lipid peroxidation is involved. Low plasma levels of α-tocopherol and vitamin C correlate with an increased incidence of myocardial infarction and of some forms of cancer. (Wefers H, et al, 1988)

Wheatgrass is rich in chlorophyll, magnesium, Iron, selenium, zinc, chromium, and antioxidant vitamins like vitamins A, E, C, B12, folic acid, pyridoxine, host of other minerals and amino acids, that have significant nutritious and medicinal value. Since deformation of RBC, caused by oxidation of excess α-chains, is the main causative factor for hemolysis and therefore, increased frequency of repeated blood transfusions in thalassemia; antioxidants and magnesium present in wheatgrass may reduce the need of such repeated transfusions.
(Gey KF, et al.,1987)

Table no 1: Nutritional analysis of wheatgrass(by pines international, Inc. 2004)

NUTRITION	**AMOUNT**	**NUTRITION**	**AMOUNT**
Calories	13	Protein	860 mg
Carbohydrates	1.6 g	Dietary fibres	1 g
Chlorophyll	18.5 mg		
VITAMIN			
Biotin	4µg	Vitamin B5 (Pantothenic Acid)	36µg
Cholin	5 mg	Vitamin B6 (Pyridoxine)	39µg

Lutein	1 mg	Vitamin B8 (Folic Acid)	21 µg
Vitamin A (Betacarotene)	1668 IU	Vitamin B12 (Cobalamin)	0.05 µg
Lycopene	1 mg	Vitamin C	7.5 mg
Vitamin B1 (Thiamine)	11µg		
Vitamin B2 (Riboflavin)	260µg	Vitamin K	35µg
Vitamin B3 (Niacin)	252µg	Zeaxanthin	279V
MINERALS			
Calcium	15 mg	Phosphorus	14 mg
Cobalt	1.7 µg	Potassium	137 mg
Copper	17 µg	Selenium	3.5 µg
Iodine	8 µg	Sodium	1 mg
Iron	870 µg	Sulfur	10.5 mg
Magnesium	3.9 gm	Zinc	62 µg
Manganese	240 µg		
AMINO ACID			
Alanine	69 mg	Lysine	38 mg
Arginine	66 mg	Methionine	18 mg
Aspartic Acid	50 mg	Phenylalanine	36 mg

Protein	**1.959**	**Calories**	**21.0cal**
Carbohydrates	2.09	Moisture	959
Ash	0.0489	Magnesium	24mg
Selenium	<1 ppm	Sodium	10.3mg
Zink	0.33g	Phosphorus,	75.2gm
Calcium,	24.2 mg	Iron,	61mg
Vitamin B1	08 mg	Vitamin B2,	0.13mg
Vitamin B3,	0.11mg	Vitamin B5,	6.0mg
Vitamin C,	3.65 mg	Vitamin B12,	<1µg
Folic Acid,	29µg	Vitamin E,	15.2 IU
Dietary Fiber (total)	0.1gm	Biotin,	10µg
Chlorophyll	42.2 mg	Lecithin,food	0.03g
L-Arginine	135 mg	Choline,	94.4 mg
Aspartic Acid,	260 mg		

All above constituents are present in 100 g juice. Data based on scientific laboratory analysis by (Irvine Analytical Laboratories Inc., Irvine, CA, USA).

[Table no 2: Nutritional Analysis of Wheatgrass]

Name of vegetable	**Protein**	**Fiber**	**Ca**	**Vit. A**	**Iron**	**Se**	**Mg**	**K**
	g	g	Mg	IU	Mg	µg	Mg	Mg
Dehydrated wheatgrass	25	17	515	66080	57.1	99.7	197.5	1,425
Beets(raw)	1.7	0.8	17	22	0.7	-	23.5	339
Bib lettuce(raw)	1.3	0.5	35	964	2.1	-	9	264
Broccoli(raw)	3.6	1.5	103	2,500	1.1	-	24	380
Cabbage(raw)	0.9	0.8	34	90	0.3	1.5	13	163
Cauliflower	2.7	1	25	60	1.1	0.7	24	295
Celery(raw)	0.9	0.6	39	266	0.3	-	21.6	39
Collards	3.6	0.9	401	6,500	1	-	57	401
Corn(cooked)	3.2	0.7	163	396	3	-	20	163
Cucumber(raw)	0.9	0.6	25	245	1.1	0.1	11.2	158
Egg plant(raw)	1.2	0.9	12	10	0.7	-	16	214
Green pepper (raw)	1.3	1.4	9	425	0.8	0.6	18	213
Cale(raw)	4.2	1.3	179	8,900	0.5	-	37	318
Mushroom (raw)	2.7	0.8	6	5	0.8	12	10.8	406
Okra(raw)	2.4	1	249	520	0.6	-	41	-
Onions(raw)	1.5	0.6	27	41	0.5	1.5	11.8	155
Peas(raw)	6.3	2	26	632	1.9	-	34.5	311
Potato(raw)	2.2	0.8	7	5	0.6	-	-	409
Radish(raw)	1	0.7	28	5	1	4.2	14	290
Spinach(raw)	3.5	0.6	97	8,109	3.2	-	80	471
Sweet potato	1.6	1.2	31	3,400	0.7	-	-	233

(baked)								
Tomato(raw)	1.1	0.5	10	905	0.6	0.5	14.1	245
Turnips(raw)	1	0.8	38	5	0.5	0.6	18.8	261

NOTE: all above contents are taken 100 gm as per procedure.

Source: *Nutrition Almanac* and published scientific papers on cereal grass by Dr. George Kohler.((Kohler 1953, Hamilton et al., 1988, Laboratory Analyses 1989).

[Table no 3: Comparison of contents of wheatgrass with other vegetables]

CHAPTER 3

AIM AND OBJECTIVES

Aim and objective

The stability of wheatgrass juice is very poor hence consuming it after few hours would not provide the same benefits as when consumed fresh. But, it is tedious for people to collect fresh grass daily and consume it, since the daily requirement of wheatgrass is 2-3ounceonly. Therefore our aim of the study was to formulate a safe, stable and effective formulation in form of oral nutritional herbal drink which would provide same benefits as the fresh wheatgrass.

The main objectives of the study were:

- Pharmacognostic evaluation of grass with the aim of establishing the diagnostic standards.
- Physicochemical evaluation and phytochemical analysis of grass using various chemical tests and quantitative estimations.
- To Study growth cycle of wheat grass and extraction method from fresh wheat grass.
- Selection of appropriate solubilisation technique, optimization of various process variables and formulation of non-effervescent granules of wheat grass extract.
- Evaluations of various nutrients present in the formulation and its comparison with marketed sample and laboratory grass.

CHAPTER 4

MATERIAL AND METHODS

Materials and Methods

4.1. Pharmacognostical investigation of wheatgrass :

4.1.1. Growing.Drying and authentication of the Grass:

Superior quality of seeds of wheat were obtained. (1/4 - 1/3 Cups dry Grain for a 5 inch square Tray, 1-2 cups dry grain for an 10 inch square tray, 2-4 cups dry grain for for a 10 inch x 20 inch tray). These seeds were soaked overnight for 8-12 hours with warm water. After 12 hours, the soaked seeds were filtered and the remaining water was drained off and the seeds were rinsed thoroughly with cool water. Grass would grow much better, if sprout it prior to planting. After another 12 hours, when proper sprouting had occurred, the seeds were transferred into moistened loam soil. The tray was then properly covered with aluminium foil or paper for proper germination for a period of 12-15 hours under dark conditions. 24 hours later, growth was measured with root hairs. The grass growth is monitored daily for duration of 8 days as the grass grows 2-3cm per day.

The fresh leaves of *Triticumaestivum*was procured in lab,Institute of pharmacy,Nirma University Ahmedabad, Gujarat, India in the month of January 2013. The fresh leaves were identified by comparing their morphological description as described in various standard texts (The Wealth of India, 1998). Further, the voucher specimen was authenticated by Dr. B.L. Punjani, Ethanobotanist, P.G. Centre in Botany, Smt. S.M. Panchal Science College, Talod, Gujarat, India. The dried leaf was stored in airtight plastic container at room temperature until needed.

4.1.2. Macroscopic evaluation:

The leaves of *Triticumaestivum* Linn.was subjected to macroscopic observations, which comprised of the organoleptic characters of the crude drugs viz. colour, odour, taste, shape.

4.1.3. Microscopic evaluation:

4.1.3.iStudy of transverse section of leaf:

Free hand transverse sections of the fresh grass of *Triticumaestivum* Linn. were cut. The section was cleared with chloral hydrate solution and then stained with phloroglucinol hydrochloric acid (1:1) and mounted with glycerin. A separate section was prepared and stained with dil. iodine solution for the identification of starch grains.

Transverse section of leaflet shows an Upper epidermis covered with cuticle. Only covering trichomes emerge from epidermal layer.Mesophyll is made up of uniform parenchyma cells, loosely arranged. Lower epidermis is very similar to upper epidermis. Midrib represents a flat ventral surface and convex dorsal surface. The epidermal layers are continuous over the midrib, collateral and conjoint vascular bundle is prominent occupying the central portion of the midrib. Vascular bundle is surrounded by sclerenchymatous tissues.

4.1.3.ii. Powder study:

Small quantity of powder of grass of TriticumAestivumLinn.was taken and decolorize by using chloral hydrate. Then the decolourised powder was stained with Phloroglucinol hydrochloric acid (1:1).All observations of the microscopic evaluation were made and recorded with the help of special CCD (charged couple device, Lawrence and Mayo) camera attached with microscope.

Diagnostic characters seen in powder drug is vascular bundle, lignified fragments of fibers, unicellular trichomes along with the epidermal cells, parenchyma cell, calcium oxalates.

4.2. Physico-Chemical Evaluation: (The Ayurvedic Pharmacopoeia of India, 2001)

4.2.1. Determination of total ash:

2gm of accurately weighed leaf powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450˚C until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ash less filter paper, incinerated, along with filter paper, evaporated to dryness and ignited at a temperature

not exceeding 450^0 C. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

4.2.2. Determination of acid insoluble ash:

The ash obtained from above procedure was boiled for 5 min. with 25ml of dilute hydrochloric acid and the insoluble matter was collected in a Gooch crucible, or on an ash less filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

4.2.3. Determination of water soluble ash:

The ash obtained from above procedure was boiled for 5 min. with 25ml of distilled water and the insoluble matter was collected in a Gooch crucible, or on an ash less filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The weight of insoluble matter was substracted from total ash giving water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug.

4.2.4. Determination of alcohol soluble extractive:

5gm of the air-dried leaf powder was macerated with 100ml of alcohol of the specified strength in a closed flask for 24 hours, shaking at an interval of six hours. It was then allowed to stand for 18 hours. The macerate was filtered rapidly taking precaution against any loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚C to a constant weight and finally weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

4.2.5. Determination of water soluble extractive:

5gm of the air-dried leaf powder was macerated with 100ml of chloroform water of the specified strength in a closed flask for 24 hours, shaking at an interval of six hours. It was then allowed to stand for 18 hours. The macerate was filtered rapidly to prevent any loss of water. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105˚C to a constant weight and finally

weighed. The percentage of water-soluble extractive was calculated to the air-dried drug.

4.2.6. Determination of moisture content (Loss on drying):

About 10 gm of leaf powder was placed in a tared evaporation dish. It was then dried at 105^0C for 5 hours and weighed. Drying was continued and the leaf was weighed at 1 hr interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30min. and cooling for 30min. in a desiccators, did not show more than 0.01gm difference.

4.3. Preliminary Phytochemical Screening:

4.3.1. Qualtitative estimation:

The extract prepared from TriticumAeustivumgrass andwere analyzed qualitatively for the detection of the major groups of chemical constituents and individual components using standard and specific detecting reagents as described by Evans (2002) and Harbone (1998).

Tests for Alkaloids:

About 500 mg of the dried extract were stirred with about 5 ml of dilute hydrochloric acid and filtered. The filtrate was tested with the following reagents:

(i) Mayer's reagent: Few drops of Mayer's reagent (potassium mercuric iodide solution) were added separately to each filtrate and observed for the formation of white or cream colored precipitates.

(ii) Dragendroff's reagent: Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added separately to each filtrate and observed for the formation of orange yellow precipitates.

(iii) Hager's reagent: Few drops of Hager's reagent (Saturated aqueous solution of picric acid) were added separately to each filtrate and observed for the formation of yellow precipitates.

(iv) Wagner's reagent: Few drops of Wagner's reagent (Solution of iodine in potassium iodide) were added separately to each filtrate and observed for the formation of reddish brown precipitates.

Tests for Carbohydrates:

(i) Molisch's test: A small amount of each extract was dissolved in ethanol and two drops of a 20% w/v solution of a-napthol in ethanol were added to it. Now, about 1 ml of concentrated H_2SO_4 was slowly added along the sides of the test tube. Appearance of red-violet ring at the junction of the two layers indicated the presence of carbohydrates.

(ii) Fehling's test:A small amount of the each extract was dissolved in about 2 ml of distilled water and filtered. An equal amount of Fehling's solution was added to the filtrate and the contents were boiled. Appearance of brick red precipitates confirmed the presence of reducing sugars.

(iii) Benedict test: A small amount of the each extract was dissolved in about 2 ml of distilled water and filtered. About 1 ml of Benedict solution was added to the filtrate. The contents were boiled and observed for the appearance of brick-red precipitates which confirmed the presence of reducing sugars.

Tests for Sterols:

(i) Liebermann-Burchard's test:A small amount of each extract was dissolved separately in chloroform and few drops of acetic anhydride were added. Now, concentrated sulphuric acid was added drop-wisely along the sides of the test tube and observed for the appearance of blue to blood red color as the indication of sterols.

(ii) Salkowski test: A small amount of each extract was dissolved in chloroform. Concentrated Sulphuric acid was added drop wise along the sides of test tube and observed for presence of red or yellow colour at lower layer.

Tests for Saponins:

(i) Foam Test: About 1 ml of each extract (in the respective solvents) was separately diluted to 20 ml with distilled water and further shaken in a graduated cylinder for 15 minutes. Formation of about 1 cm thick layer of foam confirmed the presence of saponins.

Tests for Phenolic compounds and Tannins

(i) Ferric chloride test: Small amount of each extracts were separately shaken with water and warmed. Now about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color.

(ii) Lead acetate test:A few milligrams of each extract were separately stirred with about 2 ml distilled water and filtered. To the filtrate, few drops of 10% w/v lead acetate solution was added and observed for the formation of white precipitates.

Tests for Amino acids and Proteins:

(i) Millon's test:A small amount of each extract was separately dissolved in about 5 ml of distilled water and filtered. To 2 ml of the filtrate, 5-6 drops of Million's reagent (solution of mercury nitrate and nitrous acid) were added and observed for formation of red precipitates as an indication of the presence of proteins.

Tests for flavonoids

(i) Shinoda test:A few milligrams of each extract were separately shaken with ethanol in different test tubes. Now, small pieces of metallic magnesium or zinc were added followed by addition of 2 drops of concentrated HCl and observed for the formation of pink color.

(ii) Aqueous NaOH test: To test solution add 10 % NaOH, yellow color is obtained.

(iii) Mineral acid test: To test solution add conc. H_2SO_4, yellow-orange color is obtained.

(iv) Lead acetate solution test: To the test solution add 10 % of lead acetate solution, yellowish precipitates are obtained.

4.4 Extract preparation from fresh wheat grass

After proper height that is 20cm to 25cm was obtained by the grown grass, it was cut. This fresh mature wheat grass was taken and washed with fresh water in order to remove any earthy matter. This washed grass was then placed into water and methanol in a ratio of 7:3 respectively in order to obtain the proper extract. This mixture was then sonicated for duration of 1-2 hours at room temperature. The resulting solution was filtered with wattman filter paper with a prepared cotton bed placed above it. During filtration, the extract was extensively and carefully squeezed in order to obtain almost all the filterate as well as to prevent any damage to the filter aid. The filterate was then placed in the rotary vacuum bath for solvent reduction for duration of 3-4 hours. The resulting concentrated solution was then centrifuged for 7 minutes at 5000rpm, room temperature. After centrifugation, the supernatant was decanted carefully and the semi-solid mass obtained below thesupernatant was placed in a china dish and dried on water bath at a temperature below 40˚C for a duration it was completely dried. After drying, the product was scrapped from the china dish and slightly hard lumps of the extract were obtained. These hard lumps were properly grinded in a glass mortar pestle followed by sieving using a 100 mesh sieve until a powdered extract is obtained.

4.5 Formulation designing and optimization from extract

4.5.1 Formulation designing and optimization for non-effervescent granules

The powdered extract was taken in a glass mortar pestle and mixed with sufficient amount of isopropyl alcohol 70% (IPA) until a paste of the extract was formed. To this paste, a solubility enhancer was required, for which maltodextrin and βcyclodextrin were used. Finally, the optimised quantity of β-cyclodextrin, a solubility enhancer was added. The resulting dry lump of extract was then passed through 100 mesh sieve. This fine powder was then transferred to a glass mortar pestle and optimised amount of mannitol was added to it and thoroughly mixed followed by sieving through 100 mesh sieve. The final 5gm granules batch obtained can then be consumed with fruit juice, honey, milk or smoothies as per the individual's choice of taste.

Batch	**Extract (gm)**	**Solubility enhancer (gm)**	**Mannitol (gm)**	**IPA 70 % (ml)**
Maltodextrin				
A	0.5	0.5	3.8	0.2
B	0.5	0.5	3.1	0.4
C	1.0	1.0	2.8	0.2
D	1.0	1.5	2.2	0.3
β- cyclodextrin				
A'	0.5	0.5	3.8	0.2
B'	1.0	0.5	3.1	0.4
C'	1.0	1.0	2.8	0.2
D'	1.0	1.5	2.2	0.3

[Table no 4: Formulation designing and optimization for non-effervescent granules]

4.5.2 Formulation designing and optimization for effervescent granules

Sodium carbonate, sodium bicarbonate and citric acid (mixture A) was individually sieved using 40 mesh sieve. They were then combined in a china dish and appropriate amount of IPA (70%) was added until lump formation occurred. Simultaneously, extract was also sieved using 100 mesh sieve and little amount of IPA (70%) was added to form a paste. To this paste, β-cyclodextrin was added and mixed properly

(Mixture B). Mixture A and mixture B were then combined and uniformly mixed with little amount of IPA (70%), passed through 10 mesh sieve and the 5g granules batch was dried at 40˚C for 5-10 minutes.

Batch	**Extract (gm)**	**Sodium carbonate (gm)**	**Sodium bicarbonate (gm)**	**Citric acid (gm)**	**Solubility enhancer (β-cyclodextrin) (gm)**	**IPA (70%) (ml)**
A	0.5	0.6	0.8	0.4	2	0.7
B	1	0.6	1	0.4	1.3	0.7
C	1.5	0.6	1	0.4	0.3	0.7
D	1	0.5	1.5	0.5	1	0.5
E	0.5	0.6	1	0.4	1.8	0.7

[table no 5: Formulation designing and optimization for effervescent granules]

4.6 Approaches for enhancement of solubility in non-effervescent granules

4.6.1 Particle size reduction

The extract was sieved 2-3 times using 100 mesh sieve in order to obtain fine powder in order to obtain particle size reduction hence improve solubility.

4.6.2 Complexation

Extract and β-cyclodextrin were separately sieved through 100 mesh sieve. Both constituents were mixed and again sieved together using the same sieve.

4.6.3 Evaporate to dryness

Extract was combined with solvent in a china dish followed with addition of β-cyclodextrin and heated below 40˚C until complete solvent evaporation. The dried extract was then sieved using 100 mesh sieve.

4.6.4Particle size reduction and complexation

The above approaches for solubility enhancement by particle size reduction and compelxation were combined because this combination was found to give better solubility hence used.

4.7 Quantitative estimations:

4.7.1 Estimation of Phenolic Substances: (Singleton and Rosi, 1965)

1g each of air-dried powder of Triticumaestivumgrass,Extract, Marketed sample, and Formulation isolate were extracted with 100ml methanol by maceration process for 24 hours, separately and filtered. The final volume of the filtrate was adjusted up to100 ml using methanol. 5ml of each extract was diluted with an equal volume of methanol with and was used for the estimation of phenols. To 1 ml of the methanolic extract 10 ml of distilled water and 1.5 ml of diluted (1:2) folinciocalteu reagent were added and the mixture was kept aside for 5 min. after adding 4 ml of 20%w/v Na_2CO_3 solution the final volume was adjusted to 25 ml using distilled water. The absorbance was measured at 765 nm at an interval of 30 min up to 2 hr using distilled water as a blank. Results were expressed in g/100 g of dry matter with respect to Gallic acid serves as a standard.

4.7.2 Estimation of Flavonoids:(Baharamet al., 1996)

1g of air-dried leaf powder of Triticumaestivumand Glycine max protein isolate were extracted with 100ml methanol by maceration process for 24 hours and filtered. The final volume of the filtrate was adjusted to100ml using methanol. Each of 1 ml of these extracts was diluted up to 10ml with methanol and was used for theestimation of flavonoids. To 3ml of the each extract, 3ml of methanolic $AlCl_3$ was added. After 10 min, the absorbance was read at 430 nm. Results were expressed in g/100 g of dry matter with respect to Rutin serves as a standard.

4.7.3 Estimation of Vitamin B_1: (Ozgur M.U., et al., 2002).

Preparation of the stock solution

The vitamin was dissolved in 0.1N hydrochloric acid and then diluted with the same solvent in order to obtain 200ppm concentrations. Working solutions had a concentration.of 20 ppm.

Preparation of the standard solutions

Standard solutions were prepared in 10 ml volumetric flasks containing 4-20 ppm concentration of vitamin B1 and diluted to volume by 0.1N hydrochloric acid. The calibration graphs were prepared by plotting absorbance against concentration.

Preparation of the sample

50 mg of sample was accurately weighed into a 50 ml volumetric flask, dissolved in 0.1N hydrochloric acid and diluted to volume. 1.2 ml of this solution was diluted to 10 ml with the same solvent and the absorbance was recorded.

4.7.4 Estimation of Vitamin C: (I.P. 2007).

Preparation of Standard solution:

0.1 g of ascorbic acid was weighed and dissolved in a mixture of 100 ml of freshly boiled and cooled water and 25 ml of 1 Msulphuric acid. Immediately it was titrated with 0.05 M iodine, using starch solution as indicator until a persistent blue-violet colour was obtained.

1 ml of 0.05 M iodine is equivalent to 0.008806 g of $C_6H_8O_6$.

Preparation of sample:

0.1 g of sample was weighed and dissolved in a mixture of 100 ml of freshly boiled and cooled water and 25 ml of 1 Msulphuric acid. Immediately it was titrated with 0.05 M iodine, using starch solution as indicator until a persistent blue-violet colour was obtained. The concentration of Vitamin C was then calculated.

4.7.5 Estimation of Vitamin E: (Dahot M.U., et al., 1990).

Preparations of standard solution and UV-spectra

Accurately weighed amount of 100mg a-tocopherol acetate was transferred to 100 ml volumetric flask and diluted with ethanol/isopropanol up to the mark. UV-spectra of standard solutions were recorded to determine the λ_{max}.

Preparations of standard dilutions and calibration graph.

Serial dilutions of standard solutions were prepared by pipetting out 1.0 to 5.0 ml solutions into 10 ml volumetric flask and brought the volume upto mark with ethanol and/isopropanol. The absorbance of each dilution was recorded on spectrophotometer at 285 nm and calibration graphs were prepared by plotting absorbance against concentration.

Preparation of Sample:

Accurately weighed amount of 100mg a-tocopherol acetate was transferred to 100 ml volumetric flask and diluted with ethanol/isopropanol up to the mark. The dilutions were made by pipetting out certain volume of sample into 10 ml volumetric flask and brought the volume upto mark with ethanol and/isopropanol. The absorbance of sample was recorded on spectrophotometer at 285 nm.

4.7.6 Estimation of Amino acid (By Ninhydrin method):

Reagents Required:

- **Ninhydrin:** dissolve 0.8g stannous chloride ($SnCl_2.2H_2O$) in 500mL of 0.2M citrate buffer (pH 5.0). add this solution to 20g of ninhydrin in 500mL of methyl cellosolve (2 methoxyethanol).
- **0.2M Citrate Buffer pH 5.0**

  Solution **A:** 0.2 M Citric acid. Solution **B:** 0.2 M Sodium citrate Mix 20.5 ml of solution A with 29.5 ml of solution B and check pH.
- **Diluent Solvent:** Mix equal volumes of water and *n*-propanol, and use
- **Standard*:*** Dissolve 50mg leucine in 50mL of distilled water in a volumetric flask. Take 10mL of this stock standard and dilute to 100mL in another flask for working standard solution.

Procedure:

- Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of standard amino acid solution to the respectivelabelled test tubes.
- Add distilled water in all the test tubes to make up the volume to 1ml.
- Add 1ml of distilled water to the test tube labelled Blank.
- Now add 1ml of ninhydrin reagent to all the test tubes including the test tubes labelled'blank' and 'unknown'.
- Mix the contents of the tubes by vortexing /shaking the tubes.
- Then cover all the test tubes with paper/marble.
- Place all the test tubes in boiling water bath for 15 minutes.
- Cool the test tubes in cold water and add 5ml of diluents solvent to each test tube andmix well.

- Now record the absorbance at 570 nm of each solution using a colorimeter.
- Then plot the standard curve by taking concentration along X-axis and absorbance at 570 nm along Y-axis.

Volume of standard amino acid(ml)	Volume of distilled water(ml)`	Concentration of amino acid(µg)	Volume of Ninhydrin reagent	Incubate in boiling water bath for 15 min	Volume of solvent(ml)	Incubate at room temperature for 10 min
0.0	1.0	00	1		5	
0.2	0.8	20	1		5	
0.4	0.6	40	1		5	
0.6	0.4	60	1		5	
0.8	0.2	80	1		5	
1.0	0.0	100	1		5	
1.0UK	0.0	?	1		5	

[Table no 6: Estimation of Amino acid]

4.7.7Estimation of chlorophyll

- Place the filter containing the 0.1% sample in a centrifuge tube.
- Add about 10 mL of aqueous acetone solution; cap tightly and place in the dark box.
- Repeat Step until the desired number of samples have been processed.
- Remove the cap from the centrifuge tube, insert the microtip, and sonify for 20 seconds at the5 setting.
- Rinse the microtip into the centrifuge tube with approximately 1 mL of aqueous acetonesolution.
- Bring the extract to a volume of 13.0 mL with the acetone solution, cap, mix and return tothe dark box.
- Repeat the steps outlined in Step until all of the samples have been sonified.

- Place the dark box in the 4˚C cold room and allow the extract to steep overnight.
- Clarify the extract by centrifuging the extract for 20 minutes at approximately 500 g. (Mix theextract thoroughly before centrifuging.)
- Carefully transfer the clear extract to a 5.0 cm cell and using the multi wavelength mode on thespectrophotometer, measure the absorbance at: 750, 663, 645, and 630 nm (if uncorrectedchlorophyll is desired) or at 750, 665, 663, 645, and 630 nm if both corrected and uncorrectedchlorophyll are desired)
- For corrected samples: Immediately after measuring the absorbance, add 0.1 mL of 0.1 N HCl tothe spectrophotometer cell, mix, wait 90 seconds and measure the absorbance.

Calculations (Formula)

- Determine the absorbance at 750, 663, 645, and 630 nm directly from the printout.
- Subtract the absorbance at 750 nm from the 630, 645, and 663 nm values (turbidity correction).
- Calculate the uncorrected chlorophyll a concentration by inserting the corrected absorbance valuesin the following equation.

$$\text{Uncorrected chlorophyll a } (\mu g/l) = \frac{[11.64(Abs663) - 2.16\,(Abs645) + 0.10(Abs630)]}{E(F)} \quad V(L)$$

WhereF = Dilution Factor (i.e., if the 663 Abs is >0.99 with the 1 cm cell, dilute,re-analyze and insert the dilution factor in the equation)

E = volume of acetone used for the extraction (mL)

V = volume of water filtered (L)

L = cell path length (cm)

- For corrected samples, determine the absorbance at 665 nm and 750 nm after acidification.

- Subtract the absorbance at 750 nm from the absorbance at 665 nm (turbidity correction).
- Calculate the corrected chlorophyll *a*concentration by inserting the turbiditycorrected absorbance readings in the following equations.

Corrected chlorophyll a (μg/l) = $\frac{26.73(663_b - 665_a)\ E(F)}{V(L)}$

WhereF = Dilution Factor (if the extract requires dilution)E = The volume of acetone used for the extraction (mL)

V = The volume of water filtered (L)

L = The cell path length (cm)

665a = The turbidity corrected Abs at 665 nm after acidification

663b = The turbidity corrected Abs at 663 nm before acidification

4.8 TLC Profile

25 g of fresh wheatgrass was crushed thoroughly, using mortar and pestle. The crushed wheatgrass was completely exhausted by adding small quantities of methanol acetone followed by filtration every time in a successive manner.Methanol and Acetone extract of fresh wheatgrass and extract powder showed spots at different Rf values.

Solvent system-n-hexane : acetone : methanol

13.5 : 7 : 0.25

4.9 Evaluation parameter

4.9.1 Angle of repose

The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface. The blend was carefully pored through the funnel until the apex of the conical pile just touches the tip of the funnel. The radius of the base of the conical pile was measured. The angle of repose was calculated using the following formula:

$$\mathbf{Tan\ \theta = h/r}$$

Where, θ = Angle of repose,

h = Height of the cone,

r = Radius of the cone base.

Values for angleof repose ≤ 30° usually indicate a free flowing material and angles ≥ 40° suggest a poorly flowing material, 25- 30 show excellent flow properties, 31-35 show good flow properties, 36-40 show fair flow properties and41-45 showing passable flow properties.

4.9.2 Bulk Density

15 g powder blend introduced into a dry 100 ml cylinder, without compacting. The powder was carefully leveled without compacting and the unsettled apparent volume, Vo, was read. The bulk density was calculated using the following formula.

$$\mathbf{\rho b = M / Vo}$$

Where, ρb = Apparent bulk density,M = Weight of sample, V = Apparent volume of powder

4.9.3 Tapped Density

After carrying out the procedure as given in the measurement of bulk density the cylinder

containing the sample was tapped 500 times initially followed by an additional taps of 750 times until difference between succeeding measurement is less than 2% and then tapped volume, Vfwas measured, to the nearest graduated unit. The tapped density was calculated, in gm per ml, using the following formula.

ρtap = M / Vf

Where, ρtap = Tapped density
M = Weight of sample,
Vf = Tapped volume of powder

4.9.4 Carr's Index (%)

The Compressibility index (Carr's index) is a measure of the propensity of a powder to be
compressed. It is determined from the bulk and tapped densities. In theory, the less compressible a material the more flowable it is. As such, it is measures of the relative importance of interparticulate interactions. In a free flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater inter-particle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Carr'sIndex which is calculated using the following formulas:

Compressibility index = [(ρtap - ρb) / ρtap] / ×100

Where,ρb= Bulk Density
ρtap = Tapped Density

Compressibility index values

Compressibility index	**Properties**
≤ 10	Excellent
11 – 15	Good

16 – 20	Fair
21 - 25	passable
26 – 31	Poor
32 – 37	Very poor
>38	Very very poor

[table no 7: Compressibility index values]

4.9.5 Hausner's Ratio

Hausner's ratio is an indirect index of ease of powder flow. It is calculated by the following formula.

Hausner's Ratio=Tapped density(ρt) / Bulk density(ρb)

Where ρt tapped density and ρb is bulk density. Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones, between 1.25 to 1.5 showing moderate flow properties and more than 1.5 poor flow.

CHAPTER 5

RESULTS

CHAPTER 6

DISCUSSION

CHAPTER 7

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

- Nature has been a source of medicinal agents for thousands of years and impressive members of drug have been isolated from natural sources.
- Shoots of *Triticumaestivum*Linn., also known as wheat grass, belonging to family Gramineae, possesses high chlorophyll content, essential vitamins, vital enzymes, amino acids and dietary fibers.It also contains different minerals, and more iron content than spinach and more protein content than meat, fish, egg, beans or dairy products.
- The authenticity of grass of *Triticumaestivum* was established by various pharmacognostical and phytochemical analysis as per earlier reports.
- Wheat grass was grown, extraction done, formulated and optimised, evaluated for various parameters as per the standards procedures.
- Predominance of vitamin E, vitamin C, vitamin B1, amino acids, chlorophyll, phenolics and flavonoids could be responsible for treatment of various disease conditions.
- In a foreseeable future, the research is necessary to establish the value of these extracts in the treatment of anti-inflammatory, anti-cancer, anti-oxidant and anti-ulcer activity.
- Long term tolerance studies are also needed before being recommended for human use.

CHAPTER 8

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BIBLIOGRAPHY

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