2. INTRODUCTION

Ayurveda is the oldest healing system of medicine. Major formulations used in Ayurveda are based on herbs. The prime objective of Ayurveda, the ancient Indian system of medicine, is the prevention and cure of disease process. According to WHO report, over 80% of the world population relies on traditional medicine largely plant based for their primary healthcare needs 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. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace.

According to The National Medicinal Plants Board, Ministry of Health and Family Welfare, Govt. of India, department of Ayush, India has 15 Agroclimatic zones and 17000-18000 species of flowering plants of which 6000-7000 are estimated to have medicinal usage in folk and documented systems of medicine, like Ayurveda, Siddha, Unani and Homoeopathy. About 960 species of medicinal plants are estimated to be in trade of which 178 species have annual consumption levels in excess of 100 metric tons.

The effectiveness of these drugs mainly depends upon the proper use and sustained availability of genuine raw materials. The domestic market of Indian Systems of Medicine and Homoeopathy is about Rs. 4000 crores (2000), which is expanding day by day. The Ayurved drug market alone is to the order of Rs. 3500 crores. Besides this, there is also a growing demand for natural products including items of medicinal value/pharmaceuticals, food supplements and cosmetics in both domestic and international markets. Presently, India’s export, from medicinal and herbal plants, is Rs. 3000 crores. India, with its diversified biodiversity has a tremendous potential and advantage in this emerging area. There are several stakeholders in the medicinal plants sector, right from herb collectors and growers to manufacturers and consumers. More than 700,000 practitioners of Ayurveda, Siddha, Unani, Yoga, Naturopathy and Homeopathy are registered in the Indian Systems of Medicine and also a sizeable number of practitioners are not registered.

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.\textsuperscript{[40]} With a view to strengthen the medicinal plants sector all over the country as well as to conserve the wild stock, the NMPB was set-up by the Government of India on 24 November 2000.\textsuperscript{[41]} 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. Land based developmental activities provide means of livelihood, health and opportunity for employment. India has global position in the field of traditional medicines. About 90\% herbal raw drugs used in the manufacture of vegetable drugs are obtained from the wild source which is limited. There are about 45,000 plants species in India which are in abundant in the regions of eastern Himalaya, western Ghats and Andman and Nicobar Islands. The rich heritage of flora is due to diversified and varied agro-climatic conditions. The official documented plants with medicinal potential are 3,000 but traditional practitioner use about 8,000 vegetable drugs. India is the largest producer of medicinal herbs and approximately called the botanical garden of the world.\textsuperscript{[2]} In Indian medicinal systems the most practitioners formulate and dispense their own recipes; hence, this requires proper documentation and research. There are currently about 2,50,000 registered medical practitioners of the Ayurvedic system; the total for all traditional systems being nearly 2,91,000 as comparable to 7,00,000 of the modern medicines. In rural India, 70\% of the population is dependent on the traditional system of medicine. In western world also the practitioner of herbal medicines is steadily growing and approximately 40\% of the population is taking herbs to treat diseases.\textsuperscript{[3]} Public, academic and governmental interest in traditional medicines is growing exponentially due to increased incidence of the adverse drug reactions and economic burden of the modern synthetic drugs.\textsuperscript{[4]} In 1997, it was estimated that about 20\% of the world’s population lived in extreme poverty and lacked basic medicines.\textsuperscript{[5]} The herbal drugs, which are cheap with less side effects, will be helpful to cure diseases of all the people including countries of the third world.

Medicinal plants are not only a major resource base for the traditional medicine & herbal industry but also provide livelihood and health security to a large segment of Indian population. The domestic trade of the AYUSH industry is of the order of Rs. 80 to 90 billion (1US$ = Rs.50). The Indian medicinal plants and their products also account of exports in the range of Rs. 10 billion. There is global resurgence in traditional and alternative health care systems resulting in world herbal trade which

In the UK retail sale of complementary medicines were estimated to be 72 million pound in 1996, an increase of 36% in real terms since 1991. According to the detailed analysis of herbal medicines market in Germany and France, total sales of herbal medicines in those countries in 1997 were US$ 1.8 billion and US$ 1.1 billion respectively.[7] Estimates of expenditure on herbal medicines vary, but data generally show that the global market for herbal products has grown rapidly in the past decade. In the USA, annual retail sales of herbal medicines were estimated to be US$ 1.6 billion in 1994, [8] and almost US$ 4 billion in 1998.[9] Retail sales of herbal products in the European Union (EU) were estimated to be US$ 7000 million in 1996.[10] A detailed analysis of the European herbal medicines market reported that Germany and France make up more than 70% of the market share.[11] In 1997, total sales of herbal products (using wholesale prices) were US$1.8 billion in Germany and US$1.1 billion in France. In the UK, retail sales of herbal products are reported to have increased by 43% in the period from 1994 to 1998, with retail sales of licensed herbal medicinal products reported to be £50 million in 1998.[12] These figures demonstrate that herbal medicinal products are being used increasingly by the general public on a self-selection basis to either replace or complement conventional medicines.

The system of traditional medicine in Japan, known as Kampo, is an adaptation of Chinese traditional medicine. Kampo formulations are essentially multicomponent mixtures of natural products, primarily plant extracts. In 1976 more than 69 kinds of Kampo formulae were introduced into the National Insurance Scheme in Japan, and this number has doubled since that time. The total expenditure for all types of pharmaceutical products in Japan was approximately $8.3 billion (US) in 1976, whereas only about $12.5 million (US) was spent on Kampo medicines. Thus in that year, Kampo medicines in the Japanese health care system amounted to only about 0.15% of total pharmaceutical expenditures. In 1983, total pharmaceutical expenditures in Japan were valued at about $14.6 billion (US) and those for Kampo medicines increased to about $150 million (US). Hence, in 7 years, expenditures for
Kampo medicines in the Japanese health care system increased to about 1% of total pharmaceutical expenditures.[13]

The EXIM bank of India, in its report (1997) has reported the value of medicinal plants related trade in India of the order of 5.5 billion US dollars and is growing rapidly. According to WHO, the International market of herbal products is estimated to be US $ 62 billion which is poised to grow to US $ 5 trillion by the year 2050. India’s share in the global export market of medicinal plants related trade is just 0.5%. In India, the herbal drug market is about $ one billion and the export of plant-based crude drugs is around $ 80 million.[42] Herbal medicines also find market as nutraceuticals (health foods) whose current market is estimated at about $ 80–250 billion in USA.[43]

<table>
<thead>
<tr>
<th>Nation</th>
<th>Annual Retail Sales in millions (US $)</th>
<th>%</th>
<th>Per capita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>$ 3,500</td>
<td>50.0</td>
<td>$ 42.9</td>
</tr>
<tr>
<td>France</td>
<td>1,800</td>
<td>25.7</td>
<td>31.2</td>
</tr>
<tr>
<td>Italy</td>
<td>700</td>
<td>10.0</td>
<td>12.2</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>400</td>
<td>5.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Spain</td>
<td>300</td>
<td>4.3</td>
<td>7.6</td>
</tr>
<tr>
<td>Netherlands</td>
<td>100</td>
<td>1.4</td>
<td>6.4</td>
</tr>
<tr>
<td>All others</td>
<td>130</td>
<td>2.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Total</td>
<td>$7,000</td>
<td>100.0</td>
<td>$19.1 (mean)</td>
</tr>
</tbody>
</table>

India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. This can be achieved by judicious product identification based on diseases found in the developed world for which no medicine or only palliative therapy is available; such herbal medicines will find speedy access into those countries. Backward integration from market demands will pay rich dividends.
Strategically, India should enter through those plant-based medicines, which are already well accepted in Europe, USA and Japan. Simultaneously, it should identify those herbs (medicinal plants), which are time-tested and dispensed all over in India. One such herbal drug – Wheatgrass, has been traditionally used, since ancient times, to treat various diseases and disorders, in India.

Wheat, \((Triticum\) species\) a cereal grass of the \(Gramineae\) \((Poaceae)\) family, is the world's largest edible grain cereal-grass crop. Wheat has been a food crop for mankind since the beginning of agriculture. The wheat plant is an annual grass. 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.\(^{14}\)

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 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.\(^{15}\)

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 – (I) \(Triticum aestivum\) (Common wheat, bread wheat, local varieties - Lok1, GW273) - \(Triticum aestivum\) comprises nearly 95 percent of the wheat grown. Its principal use is for flour. It is the most important variety for agriculture. (II) \(Triticum durum\) (Durum wheat, local variety Raj 1555) - \(Triticum durum\) is used mainly for the manufacture of semolina, which is made into macaroni, spaghetti and related products. It is next in importance to \(Triticum aestivum\). (III) \(Triticum dicoccum\) (Emmer wheat, local variety DDK) - \(Triticum dicoccum\) is one of the most ancient of cultivated cereals. Emmer was formerly grown in the United States for feed on a limited acreage but now has substantially disappeared from cultivation.
Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are a number of wheatgrass suppliers, in almost all cities of India, supplying fresh wheatgrass, on daily basis to their regular customers by home-delivery system for various ailments and as health tonic. Dr. Ann Wigmore, U. S. A. founder director of the Hippocrates Health Institute, Boston, U.S.A. was one of the proponents of the ‘Wheatgrass Therapy’. Dr. Wigmore utilized the chlorophyll present in wheatgrass as body cleanser, rebuilder and neutralizer of toxins. 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.\(^{[17]}\)

Some research workers have studied chlorophyll, one of the major ingredients present in wheatgrass. Chlorophyll is not so unique in its chemical structure. It is built around a porphyrin ring, which occurs in a variety of natural organic molecules. The most interesting group of molecules which contain porphyrin rings are those involved in cellular respiration, or the transportation and consumption of oxygen. These include hemoglobin, myoglobin, and the cytochromes. The chemical similarity between hemoglobin and chlorophyll was first suggested by Verdel in 1855.\(^{[18]}\) One of the major differences between chlorophyll and hemin is that chlorophyll contains magnesium while the hemin molecule contains iron as its central atom. Owing to the close molecular resemblance between chlorophyll and hemoglobin, it was hypothesized that chlorophyll is nature's blood-building element for all herbivorous animals and humans.

Some studies have indicated that feeding chlorophyll-rich foods to rats stimulates the regeneration of red blood cells.\(^{[19]}\) Researchers were able to demonstrate that this effect was not due to the iron or copper in the green foods.\(^{[44]}\) Hughes and Latner (1936) fed several doses and forms of chlorophyll to anemic rabbits and found that very small doses of purified chlorophyll or large doses of a crude chlorophyll extract produced a very favorable effect on hemoglobin regeneration.\(^{[45]}\) They suggested that the chlorophyll is acting as a physiological stimulant of the bone marrow and is not really concerned with the actual chemistry of regeneration of the porphyrin.

The deficiency of magnesium in serum or erythrocytes has also been reported in human β-thalassemia. These deficiencies may play a significant role in various cellular abnormalities characteristic of this disorder.\(^{[20]}\) The iron-induced liver damage in thalassemia may play a major role in the depletion of lipid-soluble antioxidants like
Degradation of chlorophyll following ingestion by humans produces several chlorophyll derivatives, of which pheophytin, pyropheophytin, and pheophorbide have been under study for their potential medical benefits. Pheophorbide-a showed antioxidant activity against lipid auto-oxidation. The extent of activity was comparable to that of $\alpha$-tocopherol, a powerful and well-known antioxidant.

Traditionally, wheatgrass has been used as an adjunct in treatment of cancers. Wigmore (1985) suspected that wheatgrass is also useful as anticancer preparation by virtue of its several components like chlorophyll, P4D1 compound, abscisic acid and laetrile (vitamin B17). According to Te et al., (1997) chlorophyllin, which is obtained by hydrolysis of chlorophyll to remove phytol alcohol, is an efficient antimutagenic agent and has been used as a dietary supplement or to diminish the intensity of the discomforting side effects of cancer preventive therapy. It is possible that chlorophyllin may have beneficial effects when used in combination with cancer preventive therapy. More recently, the cancer chemopreventive properties of chlorophylls have come to be recognized. Chlorophyll has been reported to exhibit anti-mutagenic activity in short-term genotoxicity assays. Chlorophyll-rich plant extracts, as well as water solutions of a chlorophyll derivative (chlorophyllin), dramatically inhibit the carcinogenic effects of common dietary and environmental chemicals. Using the standard Ames test, it has been shown that an extract of wheatgrass, when applied to known chemical mutagens (which cause cells to become cancerous), decreased their cancer-causing ability by up to 99 percent. Later studies by the same investigators showed that several green vegetables provide anti-mutagenic protection from a number of cancer causing chemicals. This activity was found to be proportional to the amount of chlorophyll in the vegetables.

The human body needs a balance of nutrients such as vitamins, minerals and amino acids to carry out body functions. Nutritional diseases are diseases in humans that are directly or indirectly caused by a lack of essential nutrients in the diet. Nutritional diseases are commonly associated with chronic malnutrition. 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, 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. Hence, to evaluate usefulness of wheatgrass in nutritional deficiency disorders, we planned detailed analyses of wheatgrass for its vitamins, minerals and amino acid contents.

Owing to long-standing and time-proven use of herbal drugs, along with higher safety margin, WHO has taken necessary, steps to ensure quality control with modern techniques and application of suitable standards for this purpose. The pharmacopoeias of different countries include monographs indicating quality parameters and standards for various herbal drugs and also for some of their products. For the purpose of quality control of herbal drugs, W.H.O. has prepared accordingly the guidelines. The objectives put forth are provisions for recommended general methods and also the general limits for contaminants for herbal drugs. The medicinal plants contribute to cater 80% of the raw materials used in the preparation of drugs. The effectiveness of these drugs mainly depends upon the proper use and sustained availability of genuine raw materials. Hence, in order to entitle wheatgrass, the status of a standard herbal medicine for acceptance in global market, we standardized wheatgrass using HPTLC in this study.

It has been recommended that the topical application of wheatgrass juice is useful for treatment of skin infections. It has also been claimed that wheatgrass juice may have antibacterial activity. Chlorophyll limits the growth of many types of germs not by directly killing them, but by providing an environment, which interferes with their growth especially against anaerobic bacteria, those that do not require oxygen. Chlorophyll solutions provide significant relief of pain, reduction of inflammation, and the control of odor for patients with serious mouth diseases. Chlorophyll has also been used successfully to treat chronic and acute sinusitis, vaginal infections, and chronic rectal lesions. Thus, in classic literature, wheatgrass has been claimed to possess anti inflammatory, analgesic and antibacterial properties. Since, all these three are major contributors in skin diseases; we attempted to investigate therapeutic benefits of wheatgrass in skin diseases, in this project.

Stomach ulcers affect about 4 million Americans every year. More than 40,000 Americans have surgery because of persistent symptoms or problems from ulcers every year. About 6,000 Americans die of stomach ulcer-related complications every year. Peptic ulcer, also known as PUD or peptic ulcer disease is an ulcer of an area of the gastrointestinal tract that is usually acidic and thus extremely painful. 80% of
ulcers are associated with *Helicobacter pylori*. Ulcers can also be caused or worsened by drugs such as Aspirin and other NSAIDs. Contrary to general belief, more peptic ulcers arise in the duodenum (first part of the small intestine, just after the stomach) than in the stomach. About 4% of stomach ulcers are caused by a malignant tumor, so multiple biopsies are needed to make sure. Duodenal ulcers are generally benign.

Chlorophyll has been shown to be extremely effective in speeding the healing of peptic ulcers and wounds, which develop internally in the gastro-intestinal tract. Several studies document the use of chlorophyll in the treatment of ulcers resistant to more conventional therapies. The results are quite impressive. In the Offenkrantz study, 20 of the 27 patients with chronic ulcers were relieved of pain and other symptoms in 24 to 72 hours.\[35\] *Since, wheatgrass is a rich source of chlorophyll and chlorophyll has been claimed to have beneficial effect in treatment of ulcer, we also decided to evaluate effectiveness of wheatgrass in ulcer.*

Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are a number of wheatgrass suppliers, in almost all cities of India, supply fresh wheatgrass, on daily basis to their regular customers by home-delivery system for various ailments and as health tonic. Fresh wheatgrass has been proposed to be used as a juice, which is prepared in a mixer/blender with addition of little water followed by filtration through a cloth.\[36\] To get fresh wheatgrass is difficult during travelling. Wheatgrass powders are available in market by many supplier, but this are not standardised and contains large amount of insoluble fibres, so increases the bulk of the powder and increases dose or dosing frequency. So it is inconvenient to the patients. In a chronic disease like thalassemia, the drug treatment is of long duration, may even be for years. In such a circumstances the factor of patient compliance becomes very important. Outcome of the therapy will largely depend upon regular supply (round the year and in all seasons) and acceptability of the drug by patient. As a pharmaceutical scientist, preparation of a suitable dosage form is prime area of research in the development of new drug formulations. In the present investigation we decided to prepare suitable formulation that is as effective as juice. *For treatment of ulcer, we prepared vatis of wheatgrass extract, while for treatment of skin disease; a gel formulation was developed in this project.*

The major hindrance in the amalgamation of herbal medicine into modern medical practices is the lack of scientific and clinical data and better understanding of efficacy
and safety of herbal products. Potency of herbal product is significantly affected by environmental factors. To ensure the quality and safety of herbal products, standardization is of vital importance.\textsuperscript{[32]} Also, for the purpose of quality control of herbal drugs, W.H.O. has prepared guidelines. The objectives put forth are provisions for recommended general methods and also the general limits for contaminants for herbal drugs. It is well known that the stability of components present in wheatgrass, like chlorophyll, beta-carotene, vitamin A, vitamin E, vitamin C etc. are adversely affected upon exposure to changes in air, light, humidity and temperature. Today, chromatographic methods HPLC, GC, HPTLC are used for identifying active constituents of medicinal herbs. These scientific procedures have brought revolution in the field of herbal medicine, particularly in case of single plant based formulations. The concept of standardization is rapidly catching up with herbal products based on active constituents. \textit{Hence, we also evaluated the potency of wheatgrass formulation, using UV spectra and HPTLC methods.}