1. ABSTRACT

Herbal medicine is still the mainstay of about 75% of the world population, especially in the underdeveloped and developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, in the last few years there has been a major increase in their use in the developed world. In Germany and France, many herbs and herbal extracts are used as prescription drugs. Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace.

The medicinal plants contribute to cater 80% of the raw materials used in the preparation of drugs. 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 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.

India is one of the most important countries in the world in term of floristic diversity. The Ministry of Environments and Forests, Government of India has established 8 biosphere reserves, 85 national parks and 448 sanctuaries for conservation of flora and fauna. India has a network of about 140 botanical gardens for ex-situ (outside natural habitat) conservation and demonstration of plants. 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. 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 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. In Indian medicinal systems the most practitioners formulate and dispense their own recipes; hence, this requires proper documentation and research. 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. 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. In 1997, it was estimated that about 20% of the world’s population lived in extreme poverty and lacked basic medicines. 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 stands at US$ 120 billion and is expected to reach US$ 7 trillion by 2050. Indian share in the world trade, at present, however, is quite low. According to WHO, 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. About 25% of modern medicines are descended from plants first used traditionally.

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 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.

In Japan, total expenditure for all types of pharmaceutical products was approximately $8.3 billion (US) in 1976, whereas only about $12.5 million (US) was spent on Kampo (herbal) 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]

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. 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. The wheat plant is an annual grass. It is
mainly grown as a winter annual in milder climates, with seeding in the fall and harvest from June through August depending on the length of the winter. For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in the levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant. The young germinated plant is a factory of enzyme and growth activity. In the early stages of growth they store large amounts of vitamins and proteins in the young blades. After jointing stage, the nutritional level in the leaves drops rapidly while the fiber content increases rapidly. This stage represents the peak of the cereal plant's vegetative development.

Although over 30,000 varieties of wheat exist, they are of two major types: bread wheat and durum wheat. Agriculturally, Important species of Triticum include – (I) Triticum aestivum, (II) Triticum durum and (III) Triticum dicoccum. Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. The movement for the human consumption of wheatgrass began in the western world in the 1930s and was initiated by Charles F. Schnabel, known as the father of wheatgrass. He said ‘Fifteen pounds of wheatgrass is equivalent to 350 pounds of the choicest vegetables.’ Later Wigmore (1940) 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. 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.

Some research workers have studied chlorophyll, one of the major ingredients present in wheatgrass. Chlorophyll is not so unique in its chemical structure. The chemical similarity between hemoglobin and chlorophyll was first suggested by Verdel in 1855. 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. 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.
The iron-induced liver damage in thalassemia may play a major role in the depletion of lipid-soluble antioxidants like vitamin A and vitamin E. Degradation of chlorophyll following ingestion by humans produces several chlorophyll derivatives, of which pheophytin, pyro-pheophytin, 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). 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.

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. One of the most important parts of prevention is good nutrition. 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. To ensure the quality and safety of herbal products, standardization is of vital importance. The effectiveness of herbal 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.\[17\] It has also been claimed that wheatgrass juice may have antibacterial activity, especially against anaerobic bacteria.\[33\] Chlorophyll solutions provide significant relief of pain, reduction of inflammation, and the control of odor for patients with serious mouth diseases.\[34\] Chlorophyll has also been used successfully to treat chronic and acute sinusitis, vaginal infections, and chronic rectal lesions.\[35\] 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.

An ulcer (from Latin *ulcus*) is an open sore of the skin, eyes or mucous membrane, often caused, but not exclusively, by an initial abrasion and generally maintained by an inflammation, an infection, and/or medical conditions which impede healing. 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. 80% of ulcers are associated with *Helicobacter pylori*. Ulcers can also be caused or worsened by drugs such as Aspirin and other NSAIDs. 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.\[36\] 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. Delivery of drugs to the skin is an effective and targeted therapy for local dermatological disorders. This route of drug delivery has gained popularity because it avoids first pass effects, gastrointestinal irritation, and metabolic degradation associated with oral administration.\(^{[37]}\) Due to the first past effect only 25-45% of the orally administered dose reaches the blood circulation. In order to bypass these disadvantages the gel formulations have been proposed as topical application.\(^{[38]}\) 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.

Potency of herbal product is significantly affected by environmental factors. To ensure the quality and safety of herbal products, standardization is of vital importance.\(^{[32]}\) 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. Hence, we also evaluated the potency of wheatgrass formulation, using UV spectra and HPTLC methods.
In nutshell the objectives of the present project were:

1. To carry out pharmacognostic study of different varieties of Triticum grass (Wheatgrass) using suitable analytical techniques.
2. To analyze wheatgrass for its vitamins, minerals and amino acid contents.
3. To characterize and develop scientific method for standardization of Triticum aestivum (Wheatgrass).
4. To investigate therapeutic benefits of wheatgrass in skin diseases.
5. To evaluate effectiveness of wheatgrass in ulcer.
6. To prepare formulations of wheatgrass for oral and topical application.
7. Standardization of wheatgrass formulations, using flamephotometery and HPTLC methods.

In our investigation, certified samples of three major species of wheat viz. Triticum aestivum, Triticum durum and Triticum dicoccum were acquired from the Wheat Research Center, Gujarat Krushi University, Junagadh, Gujarat. Adequate quantity of unpolished wheat grain of these varieties were soaked overnight in water and were grown in plastic trays filled with soil, on the next day. Trays were watered adequately everyday, for 8 days. On 9th day the wheatgrass was harvested. To characterize and differentiate among the three varieties of wheatgrass, all three varieties of the grass were subjected to microscopic study, which included transverse sections, surface preparations and powder study. These wheat varieties were grown in plastic trays as per the standard procedure. Microscopic studies of transverse sections, surface preparations and powder studies of the three species of wheatgrass were conducted using high-resolution microscope.

In confirmation with the description in literature; the leaves were mainly near glabrous, auriculate, with blades narrowly to broadly linear; broad to narrow; 2-20 mm wide; flat; without cross venation. The leaf blade was linear and parallel-veined with mid rib projecting on the back continuing someway along the sheath. In T. Dicoccum the hairs on the swollen base of leaf were longer than those of other species. The longest leaves were possessed by T. durum. Observations in microscopic studies of different species also confirmed characteristics reported in literature.
transverse section, the wheatgrass leaf showed 1. elaborate epidermis with characteristic stomata and trichomes 2. green assimilating parenchyma, 3. conducting vascular bundles and 4. longitudinal strands of fibrous stereome or supporting tissue. The upper surface of the leaf showed a series of longitudinal ridges or ribs, the lower surface being almost flat. At the summit of each ridge was a single row of elongated thick-walled and pitted cells alternating with hairs. The trichomes or hairs were always unicellular, and vary much in length and stoutness. On the leaves of *T. aestivum*, ample numbers of hair were present, while in *T. dicoccum* and *T. durum* they were sparsely distributed on the surface of the leaf. Stomata were observed at the base of the ridge arranged in single or double lines. Each stoma on the leaf consisted of four cells; the two guard cells being narrow, with specially thickened walls round the stomatal pore and thin-walled widely dilated ends. Pores of the stomata were seen to be in communication with large intracellular cavities in the mesophyll, called lacune. In surface preparation, trichomes or hairs of various lengths were found scattered along the rows at more or less at regular intervals except in *T. durum*. The pharmacognostic characteristics observed in our study were in confirmation with that reported in the literature.

Various chemical tests suggests that wheatgrass contains phenolics, flavonoids and proteins/amino acids in methanol, water and acetone extract, whereas these were absent in petroleum ether, benzene and chloroform extract.

HPTLC and flame photometry methods were used for quantitative estimation of various constituents present in wheatgrass. Samples of methanol and acetone extracts of fresh wheatgrass and methanol extracts of various formulations were used for determination of pigments using TLC and HPTLC.

TLCs of the methanol and acetone extracts of fresh wheatgrass were carried out for estimation of pigments, using n-hexane: acetone: methanol (13.5: 7: 0.25) solvent system. Chromatograms of the extracts were recorded. Individual spots (components) in chromatogram were scanned between 200-700 nm wavelength to obtain their spectra, for the purpose of identification of these components in further investigations.

Methanol extract of fresh wheatgrass showed 13 spots at different Rf values viz. (1) 0.03 (orange), (2) 0.17 (light green), (3) 0.23 (light green), (4) 0.30 (yellow), (5) 0.34 (yellow), (6) 0.44 (green), (7) 0.51 (green), (8) 0.58 (light green), (9) 0.64 (dark
green), (10) 0.69 (yellowish green), (11) 0.71 (light grey), (12) 0.84 (grey), (13) 0.95 (orange). One year old methanol extract showed 10 spots.

TLC of acetone extract of fresh wheatgrass was also carried out and chromatograms (tracks) were recorded. Acetone extract showed 13 spots at different Rf values viz. (1) 0.02 (orange) (2) 0.06 (light green), (3) 0.21 (yellowish green), (4) 0.29 (yellow), (5) 0.33 (green), (6) 0.37 (light green), (7) 0.45 (green), (8) 0.52 (green), (9) 0.57 (green), (10) 0.71 (green), (11) 0.72 (light grey), (12) 0.84 (grey), (13) 0.94 (orange).

TLC of aqueous extract of wheatgrass was carried out in n-propanol: chloroform: acetic acid: ammonia: deionised water (7:4:5:1:1) solvent system and scanned at 254 nm and 210 nm wavelength for determination of amount of vitamins present in wheatgrass. Aqueous extract showed 10 spots at different Rf values viz. (1) 0.0, (2) 0.3230, (3) 0.3412, (4) 0.5832, (5) 0.5843, (6) 0.7012, (7) 0.7153, (8) 0.8652, (9) 0.8951, (10) 0.9127. With the help of standard mixture analysis and calibration curves of vitamins, 7 different vitamins were identified and quantified in aqueous extract of wheatgrass.

TLC of aqueous extract of wheatgrass was carried out in n-butanol: acetic acid: water (5:2.5:2.5) solvent system and scanned at 475 nm wavelength for determination of amount of amino acids present in wheatgrass. Aqueous extract showed 14 spots at different Rf values viz. (1) 0.0471, (2) 0.09, (3) 0.10, (4) 0.1192, (5) 0.1796, (6) 0.1912, (7) 0.1926, (8) 0.2951, (9) 0.3312, (10) 0.3610, (11) 0.405, (12) 0.4535, (13) 0.5124, (14) 0.5686. With the help of standard mixture analysis and calibration curves of amino acids, 10 different amino acids were identified and quantified in aqueous extract of wheatgrass. It was not possible to identify other amino acids as their Rf values and UV spectra were similar to each other.

Wheatgrass is rich in many minerals that are useful for our body. Now-a-days, many formulations of wheatgrass are available in market, but no proper method is available to detect the actual amount of minerals present in the formulation. Present work of determining mineral content, was based on flame photometry which is simple, inexpensive and less time-consuming method. This method is properly validated using standard chemicals and it can be applied to any formulation. In our project, the contents of sodium, potassium, calcium and lithium in methanol and aqueous extracts of wheatgrass, were determined in terms of mmol/L, ppm and mg/100 g of fresh wheatgrass. Results show that wheatgrass contains 80-84 mg calcium per 100 g fresh
wheatgrass in aqueous extract, 60-68 mg calcium per 100 g fresh wheatgrass in methanol extract, 23-25 mg sodium per 100 g fresh wheatgrass in aqueous and methanol extract, whereas 74-75 mg potassium per 100 g fresh wheatgrass in water extract and 39-43 mg potassium per 100 g fresh wheatgrass in methanol extract. As per this result lithium was absent in water and methanol extract. We also tried to detect amount of elements in acetone extract, but in acetone; flame was not stable and the intensity of flame was very high, so it was not possible to conduct analysis of acetone extract.

Anti-inflammatory activity of aqueous, methanol and acetone extracts of *Triticum aestivum* (wheatgrass) was determined by carrageenan-induced paw-edema, using plethysmometer, in albino wistar rats with subcutaneous administration of 1.43 g/kg bodyweight, dose. The maximum anti-inflammatory activity was observed at 5 hours. Compared to control group, the % inhibition with acetone extract, methanol extract, aqueous extract and diclofenac were found to be 50.64, 73.38, 63.81 and 80.87 respectively. The anti-inflammatory activity of methanol extract, was highest among all other extracts but lesser than the standard drug. Application of one-way ANOVA test (Dunnett multiple comparisons) between various groups showed the significance of the result at p<0.01. The activity was attributed to the presence of phytoconstituents in the tested extract. Further studies in this context may come up with safe and effective treatment for inflammation.

Anti-ulcer activity of wheatgrass juice was determined by ethanol induced mucosal damage, in wistar rats. While, administration of ethanol produced severe hemorrhagic gastric lesions, in positive control group, pretreatment with wheatgrass juice and omeprazole 20 mg/kg (reference drug) significantly reduced the number of ulcers. The parameters studied in this model included ulcer index. Average ulcer index found in ethanol induced control rats was $3.6233 \pm 0.2098$, whereas ulcer index in animals treated with omeprazole and wheatgrass juice were $0.75 \pm 0.1176$ and $1.216 \pm 0.1195$ respectively. One way ANOVA test (Dunnett multiple comparisons) between various groups showed significance at $p < 0.01$.

Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Getting fresh wheatgrass is difficult, during travelling. As a pharmaceutical scientist, preparation of a suitable dosage form is the prime area of research in development of new drug formulations. In the present investigation, we decided to prepare suitable formulation that is as effective as juice.
Methanol extract and combined extracts obtained through successive extraction of wheatgrass using petroleum ether, acetone, methanol and water were used to make vatis using suitable excipients. Random samples of these vatis were subjected to various pharmaceutical quality control tests like colour, shape, hardness, thickness, diameter, friability, weight variation, determination of vitamins, amino acids and pigments. Vati-F1 was round shaped with flat surface having dark green colour, whereas F2 was round with convex surface having light green colour with dark green spots on surface. Average weight of vati-F1 and F2 were 912 mg and 1017 mg respectively. Hardness of vati-F1 was less (i.e., 3.3 kg/cm²), compared to vati-F2 (i.e., 5.2 kg/cm²). Size of vati-F1 was also less (i.e., 11.116 mm diameter and 6.05 mm thickness), compared to vati-F2 (i.e., 12.2 mm diameter and 7.53 mm thickness). Friability of vati-F1 was also less (i.e., 0.4112 %), compared to vati-F2 (i.e., 0.885 %). Vati-F1 disintegrated within 11.20 minute, while disintegration time of vati-F2 was 6.6 minutes. Both formulations confirmed to the standards of quality control parameters. Stability of constituents present in these two prepared vatis, F1 and F2 were also checked by determining their contents after six months from date of manufacturing.

Wheatgrass contains chlorophyll. Chlorophyll solutions provide significant relief of pain, reduction of inflammation, and the control of odor for patients with serious mouth diseases, used successfully to treat chronic and acute sinusitis, vaginal infections, and chronic rectal lesions.\textsuperscript{[34,35]} Therefore, in the present study, a suitable topical wheatgrass gel formulation was developed, using carbopol 940. The gel was evaluated for various physicochemical parameters like clarity, pH, viscosity, homogenity and content of amino acids, vitamins and pigments. Stability of constituents, present in wheatgrass gel was also checked by determining its contents, after six months of manufacturing.

TLCs of vati-F1, F2 and gel were carried out in n-hexane: acetone: methanol (13.5: 7: 0.25) solvent system for determination of amount of pigments present in formulations, when these were freshly prepared and then after six months of manufacturing.

Methanol extract of F1 showed 13 spots at different $R_f$ values viz. (1) 0.03 (orange) (2) 0.17 (light green), (3) 0.23 (light green), (4) 0.30 (yellow), (5) 0.34 (yellow), (6) 0.44 (green), (7) 0.51 (green), (8) 0.58 (light green), (9) 0.64 (dark green), (10) 0.69 (yellowish green) (11) 0.71 (light grey), (12) 0.84 (grey), (13) 0.95 (orange).
Methanol extract of wheatgrass vati prepared from whole extract-F2 showed 17 spots at different values viz. (1) 0.03 (orange) (2) 0.06 (light green) (3) 0.17 (light green) (4) 0.21 (green) (5) 0.23 (yellowish green) (6) 0.29 (dark yellow) (7) 0.33 (green) (8) 0.37 (green) (9) 0.44 (light green) (10) 0.52 (green) (11) 0.56 (green) (12) 0.58 (green) (13) 0.64 (dark green) (14) 0.69 (yellowish green) (15) 0.71 (light grey) (16) 0.84 (grey) (17) 0.95 (orange).

In HPTLC, methanol extract of wheatgrass topical gel showed 17 spots at different R_f values viz. (1) 0.02 (orange) (2) 0.07 (light green) (3) 0.17 (light green) (4) 0.21 (green) (5) 0.23 (yellowish green) (6) 0.30 (dark yellow) (7) 0.33 (green) (8) 0.43 (light green) (9) 0.46 (yellowish green) (10) 0.52 (green) (11) 0.56 (green) (12) 0.59 (green) (13) 0.65 (dark green) (14) 0.69 (yellowish green) (15) 0.70 (light grey) (16) 0.84 (grey) (17) 0.94 (orange).

Both formulations, F1 and F2, contained 74-95% of pigments compared to pigments presents in fresh wheatgrass (except component P-53 %).

TLCs of vati-F1, F2 and gel were carried out in n-propanol: chloroform: acetic acid: ammonia: deionised water (7:4:5:1:1) solvent system for determination of amount of vitamins present in formulations, when these were freshly prepared and then after six months, of manufacturing. Results of the study showed that vitamins were sufficiently stable for six months, when formulations were stored in a room temperature, protected from light.

TLCs of vati-F1, F2 and gel were carried out in n-butanol: acetic acid: water (5:2.5:2.5) solvent system for determination of amounts of amino acids present in formulations, both for freshly prepared and after six months of manufacturing. Results showed that amino acids remain stable even after six months, if formulations are stored at room temperature and protected from light.