Wheatgrass has been traditionally used to treat various diseases/disorders and also as a health tonic. Presently, there are a number of wheatgrass suppliers, in almost all cities of India, supplying fresh wheatgrass daily to their regular customers. To ensure that proper quality of wheatgrass is supplied to the consumer, it is essential to characterize wheatgrass, hence, in this project we had decided to carry out morphological, microscopic and phytochemical studies of wheatgrass. Wheat species differ from one another both morphologically and genetically.

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 and grown in plastic trays as per the standard procedure. 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. To strengthen laboratory testing of wheatgrass quality, we carried out microscopic and phytochemical studies of different species of wheatgrass. Microscopic studies of transverse sections, surface preparations and powder studies of the three species of wheatgrass were conducted using high-resolution microscope. Observations in microscopic studies of different species also confirmed characteristics reported in literature. In 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 number 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 consists 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 are seen to be in communication with large intracellular cavities in the mesophyll, called lacune. In the furrow between two ridges is a band of three to seven rows of motor cells.
Vascular bundles are collateral, with the xylem towards the upper surface of the leaf and the phloem below. 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*.

Phytochemical tests suggested that wheatgrass contains phenolic compounds, flavonoids, proteins and amino acids in methanol, water and acetone extracts, whereas these were absent in petroleum ether, benzene and chloroform extract. Alkaloids and cardiac glycosides were not detected in our chemical tests.

Many scientific reports on nutritional analysis of wheatgrass have been published. These reports and the phytochemical analyses of wheatgrass, in the present project, revealed that wheatgrass is a rich source of chlorophyll, vitamins, amino acids, various minerals like iron, magnesium, calcium, selenium, zinc, chromium, phosphorus, antioxidants like beta carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B12, folic acid, pyridoxine and many other enzymes, which have significant nutritious and medicinal value and insoluble dietary fibers.

HPTLC and flame photometry methods were used for quantitative estimation of various constituents present in wheatgrass. TLCs of methanol and acetone extract of fresh wheatgrass were carried out in n-hexane: acetone: methanol (13.5: 7: 0.25) solvent system for estimation of pigments. Chromatograms of 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 components of wheatgrass 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. Spots at Rf value, 0.51, 0.58 and 0.69, were not detected in one year old extract. It showed that some pigments may have been degraded during one year.

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).

Among these 13 spots, 9 components detected at different R_f values viz. 0.02, 0.21, 0.29, 0.37, 0.45, 0.52, 0.72, 0.84 and 0.94 were common in methanol and acetone extract.

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 R_f 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. We found that wheatgrass contains, pantothenic acid, thiamine, folic acid, pyridoxine, ascorbic acid, riboflavin and D-biotin. It was not possible to identify other vitamins present in wheatgrass. Wheatgrass can be useful in cleaning up the residues of cigarette smoke and other forms of air pollution, in scurvy and in clearing occluded (blocked) arteries, particularly the coronary artery, due to its ascorbic acid content. Because of the presence of folic acid and pyridoxine, wheatgrass can also be useful in anaemia. Wheatgrass may be useful in treatment of other vitamin deficiency disorders like dermatitis, beriberi and parasthesia because of its biotin, pantothenic acid and thiamine contents, respectively.

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 R_f 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. Because of its amino acids contents wheatgrass can be useful in many ways like to improve hearing, nerve and thyroid function, as an anti ageing substance, for brain stimulation, for providing energy and for glutamic acid absorption.
Further, analyses can be made far more rapidly by the flame photometer than by the best gravimetric and volumetric methods when the instrument is properly operated. With flame photometry, results can be produced promptly because of the direct procedure, which is impossible with chemical methods. 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. As per our analysis potassium and calcium is present in more amount in aqueous extract than in methanol extract.

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.

Potassium is the major cation found inside of cells.\[^{316}\] The proper level of potassium is essential for normal cell function. An abnormal decrease of potassium (hypokalemia) can profoundly affect the nervous system and heart, and when extreme, can be fatal. Helping to regulate the body's fluid levels is one of the mineral potassium's greatest functions. It also has a great part in regulating the blood pressure. 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.\[^{30}\]
Failing to meet the standard recommended daily intake levels of potassium can lead to a variety of negative consequences for both physical well being and mental health. Physical symptoms can include muscular cramps and twitching, muscular weakness, even actual muscle damage, poor reflexes, fatigue, fragile bones, irregular heartbeat and other cardiovascular irregularities, kidney failure, lung failure, and cardiac arrest. Mental symptoms can include nervous disorders of various types, anorexia, insomnia, a slowdown of cognitive processes, and depression.\[32\]

Sodium is the major extracellular cation and it plays a role in body fluid distribution. Reduction in the concentration of sodium ions inside the plasma (extracellular) causes, hyponatremia. 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.\[33\]

Calcium strengthen our bones. Most (99%) of calcium is found in bones and teeth \[34-35\] with the remaining 1 % in the soft tissues and watery parts of the body where calcium helps to regulate normal processes of the body.\[35\] A constant supply of calcium is necessary throughout our lifetime, but is especially important during phases of growth, pregnancy, and lactation (breast feeding). Calcium is responsible for construction, formation and maintenance of bone and teeth, muscle contraction. Calcium is a vital component in blood clotting systems in the production of enzymes and hormones that regulate digestion, energy, and fat metabolism and also helps in wound healing. Calcium helps to control blood pressure, nerve transmission, and release of neurotransmitters, to transport ions (electrically charged particles) across the membrane, to reduce the incidence of premature heart disease, especially if adequate intakes of magnesium are also maintained, to reduce the occurrence of osteoporosis, maintaining all cells and connective tissues in the body, may help to prevent periodontal disease (gum disease).\[14,35,36,52\] Calcium Deficiency can lead to loss of calcium from the bone (initially from the jaw and the backbone), which can lead to deformity, can cause extreme nerve sensitivity, muscle spasms, and leg cramps (called tetany) at very low levels in the blood.\[14,52,72,73\]

In today’s fast lifestyle and fast-food world, deficiency of any or many of nutritional factors could easily occur culminating into a disease or disorder. Ensuring regular consumption of recommended intake of vitamins, mineral and other nutrients that our body needs, is vital in maintaining healthy physic and mind.\[30\] This may be difficult in context of today's lifestyle. This concern can be addressed by nutritional
supplements. Findings of our study suggest that wheatgrass and its formulation can be effectively used in most of the above-mentioned disorders caused by deficiency of vitamins, amino acids and minerals like sodium, potassium and calcium. As reported by Ann Wigmore in her famous book on wheatgrass ‘young grasses and other chlorophyll-rich plants are a safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, pancreas and liver problems, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, halitosis, body odor and constipation’. Thus, results of our study fully endorse the claims made by Wigmore.[17]

Topical application of wheatgrass juice has been recommended for treatment of skin diseases.[17] For studying beneficial effect of wheatgrass in skin diseases, anti-inflammatory activity in particular, carrageenan-induced hind paw edema in rats was selected as the experimental model in the present project, which also served as primary screening method for anti-inflammatory activity. Development of edema induced by carrageenan is commonly correlated with early exudative stage of inflammation. Results of our study show that the methanol, aqueous and acetone extracts of wheatgrass possess a significant anti-inflammatory effect against paw edema induced by carrageenan. The maximum anti-inflammatory activity was observed after 5 hours. The % inhibition of paw edema in control group was 0.0 %, while it was 80.87% in diclofenac group, 50.64% in acetone extract, 73.38% in methanol extract and 63.81% in aqueous extract. The anti-inflammatory activity of methanol extract was highest among all other extracts, but lesser than the standard drug. Further studies of methanol extract of wheatgrass, in this context, may come up with a novel, safe and effective anti-inflammatory drug molecule.

Several studies document the use of chlorophyll in treatment of ulcers, resistant to more conventional therapies.[35] Since, wheatgrass is a rich source of chlorophyll, in the present study, we evaluated effectiveness of wheatgrass in ulcer. Ethanol is a commonly used ulcerogenic agent and when given by gavage to rats, it produces severe gastric hemorrhagic lesions. The mechanism of ethanol-induced gastric lesions is varied, including the depletion of gastric mucus content, damaged mucosal blood flow and mucosal cell injury. In addition, ethanol-induced gastric mucosal damage is associated with overproduction of free radicals. Results of our study clearly demonstrate that wheatgrass juice confers protection against gross damaging actions of ethanol on gastric mucosa of rats. The anti-ulcer activity of wheatgrass could be
mediated by strengthening of gastric mucosal barrier, anti-oxidant activity, increase in gastric mucosal blood flow, improved gastric mucus content or cytoprotection. So, wheatgrass or its formulation can be effectively used to treat stomach ulcer disease.

Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. According to Ann Wigmore, considered to be an authority in wheatgrass therapy and who is believed to have treated thousands of patients with wheatgrass juice, the daily dose of wheatgrass for a patient suffering from chronic ailments e.g. thalassemia, should be 100 g fresh wheatgrass. To obtain 100 g fresh wheatgrass daily in all seasons may be difficult. Further, consuming such a large quantity of strong pungent wheatgrass juice may reduce patient compliance, particularly in case of female patients and also among child patients, as in thalassemia. Hence, we perceived that, reduced patient compliance or irregularity and insufficient dosing, are major drawbacks in success of wheatgrass therapy. To alleviate this shortcoming in wheatgrass therapy, we attempted to convert recommended daily dose of 100 g wheatgrass in to consistently available and more acceptable dosage form of vati. In our study we found that 100 g of wheatgrass yielded 1.67 g of dried methanol extract. From 1.67 g of dried extract, 3 vatis (F1), weighing 912 mg each, were prepared. Thus each vati (F1) contained 555 mg of dried methanol extract of wheatgrass. Hence, one vati (F1) taken three times in a day would provide medicinal benefits equivalent to the dose of 100 g of fresh wheatgrass in thalassemia. Thus, the vati prepared in our project, may help improve patient compliance and hence, ensure better outcome of long term treatment in thalassemic patients.

Formulation F2 was prepared from combination of petroleum ether, acetone, methanol and water extract obtained during successive extraction. We found that 100 g of wheatgrass yielded 0.08 g petroleum ether extract, 0.75 g acetone extract, 1.5 g methanol extract and 2.5 g water extract. Total sum of these dried extract was 4.83 g. From 4.83 g of combined extract, six vatis (F2), weighing 1017 mg were prepared. Thus each vati (F2) contained 805 mg of combined extract. Hence, two vati (F2) taken three times in a day would provide medicinal benefits in equivalent to 100 g of fresh wheatgrass.

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.

In the present study we formulated a gel formulation of wheatgrass for treatment of skin diseases, 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 the various formulations were carried out when these were freshly prepared and then 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 extracts of equivalent amounts of these formulations were subjected to quantitative HPTLC. Chromatograms of the formulations were recorded. Individual spots (components) in chromatograms were scanned between 200-700 nm wavelength and compared with spectrum obtained in fresh wheatgrass analysis. Using UV spectrum and Rf values, different components were identified.

Area under curve (AUC) of different components were measured in chromatogram of each formulation and analyzed for comparison with that of fresh wheatgrass. Methanol extract of F1 showed 13 spots. Methanol extract of wheatgrass vati prepared from whole extract-F2 showed 17 spots at different values. Both formulations, F1 and F2, contained 74-95% of pigments compared to pigments presents in fresh wheatgrass (except component P-53 %).

In HPTLC, methanol extract of wheatgrass topical gel showed 17 spots. As per this study, components N (Rf-0.07), O (Rf-0.33), P (Rf-0.56) and UN (Rf-0.21) were not present in vat-F1, indicates that these components were not present in methanol
extract of wheatgrass. Component- R (R_f-0.46) was only present in gel formulation. Component-E (R_f-0.34) was not present in gel formulation.

Wheatgrass gel can be useful in radiation, inflammation, bacterial infection, wounds, burns caused by heat, chemicals, and radiation, because of presence of chlorophyll contents. By comparing UV spectrum, standard R_f values and colors reported in literature, spot detected at 0.95 or 0.94 was β-carotene. β-carotene has been positively linked to increased protection against many forms of cancer, including lung, bladder, rectal, oral and dermal (skin) cancers. Thus, wheatgrass and its vatis can be helpful in cancer.

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. With the help of standard mixture analysis and calibration curves of vitamins, 7 different vitamins were identified and quantified in aqueous extract of these wheatgrass formulations. Results of the study showed that vitamins remain stable even after six months, if formulations are stored at room temperature and protected from light. Formulation-F1 contained 90-100% vitamins, compared to contents present in fresh wheatgrass.

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 amount of amino acids present in formulations, when these were freshly prepared and then after six months of manufacturing. 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 these wheatgrass formulations. Other amino acids were not possible to identify, because their R_f values and UV spectrum were almost similar to each other. Results showed that amino acids remain stable even after six months, if formulations are stored at room temperature and protected from light. The vatis-F1 and F2 can also be coated to prevent harmful effect of light and environmental oxygen thereby further increasing their shelf-life.