Summary
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Tender Wheatgrass is consumed either as it is or as its fresh juice extract as a health supplement. It has several useful nutrients as well as compounds that have potential antioxidant activity. However, quantitative studies on wheatgrass are scarce. In view of this, a systematic study on tender wheatgrass has been undertaken which is the focus of this thesis. Accordingly, the work in this thesis encompasses various aspects of wheatgrass such as its total essential elemental content, their bioaccessibility and total antioxidant activity as a function of growth period under different growing conditions. The thesis is divided into five chapters. The subject of the thesis, aim and scope of the work is introduced in the first chapter. In the second chapter, various analytical and biochemical methods used to determine the elemental content and antioxidant activity are described. Chapters 3, 4 and 5 respectively cover the details of results and discussion on total elemental content of wheatgrass as a function of growth period.

Bulk and trace elements in human nutrition and their sources in human diet are described in chapter 1. It enlists the various analytical techniques giving details of the radio-analytical techniques particularly neutron activation analysis, for trace elemental determination. Knowledge of bioavailability of trace elements from food materials is important in the human nutrition. Developments in in vitro methods to determine bioaccessibility of elements are described in this chapter. To understand the efficacy of wheatgrass as an antioxidant, a few aspects like production of reactive oxygen species (ROS), oxidative stress caused by them, its implication in many of the degenerative diseases and endogenous and dietary antioxidant defense against ROS are also briefly described. Further, the chemistry and mechanism involved in the assays performed to determine the total antioxidant activity of wheatgrass are included. This chapter is ended with a brief review of the present status on elemental content and antioxidant activity of wheatgrass followed by scope of the present investigations.

Second chapter gives the experimental details of the present investigations. It describes in details the wheatgrass growth and sample collection for the estimation of total elemental content. Details of instrumental neutron activation analysis (INAA) for
trace element determination and the modified in-vitro experimental methods used to
determine the bioavailability of essential trace elements from wheatgrass samples are
described. Lastly, the materials and methods used for sample preparation for antioxidant
activity assays and the biochemical protocols of the various assays are included.

The third chapter deals with the determination of elemental concentration profiles
in tender wheatgrass over a growth period up to 20th day under four different conditions
namely (i) tap water, (ii) nutrient compounds with tap water, (iii) soil and tap water and
(iv) soil and nutrient solution. Samples of shoots and roots of wheatgrass collected over
this period were analyzed by INAA. A total of 15 elements were determined in these
samples. In addition, a commercially available wheatgrass tablet was analyzed for
comparison. Accuracy of the method was evaluated by analyzing two biological
reference materials, SRM 1573a (Tomato leaves) from NIST, USA and ICHTJ-CTA-vtl-
2 (Tobacco Leaves) from INCT, Poland with each set of samples. It was observed that
the elements such as K, Na, Ca and Mg increased linearly in plant shoots with the growth
period whereas the concentrations of the elements namely Zn, Mn and Fe remained
constant in shoots after 8th day of growth for all four conditions. However, it was observed
that the shoot to root concentration ratio in all the conditions increased linearly for K, Na,
Ca, Mg and Cl and decreased for Zn, Fe, Mn, and Al with growth period. A comparison
of Recommended Daily Allowance (RDA) by Indian Council of Medical Research
(ICMR, 1987) of various essential elements suggests that the amounts are much less than
the RDA values for all the cases. It can be concluded that tender wheatgrass is beneficial
as a medical herb rather than as a food supplement.

The fourth chapter gives the details of the estimation of bioaccessibility of
essential elements from wheatgrass for the growth period of 8-10 days. The
bioaccessibilities are evaluated by two in-vitro digestion methods namely gastric and
gastro-intestinal (pancreatic) digestion. The bioaccessibility values for essential elements
obtained were compared with the different food grains and wheatgrass tablet. It is
observed that the bioaccessibility values of elements obtained by gastric digestion are
varying as follows: % bioaccessibility for fresh wheatgrass is the highest (37 to 57 %)
followed by wheat grains (9 to 38 %) and wheatgrass tablet (17 to 43 %). The values
obtained by gastro-intestinal digestion method were 40 to 80 % for fresh wheatgrass, 15
to 55% for wheat grain and 20 to 70 % for wheatgrass tablet. In addition, during this work, adjustment of pH during gastro-intestinal digestion in the reported method has been modified, standardized and validated. In the reported method NaHCO₃ was used to adjust pH. Since we are using NAA, NaHCO₃ cannot be used. We have used NH₄HCO₃ for pH adjustment during this step. It is found that % bioaccessibilities obtained by this method are not significantly different for elements such as Fe, Zn and K. This work is added as an annex to this chapter.

The fifth chapter describes the evaluation of the antioxidant activity of wheatgrass grown under different conditions over a period of 6, 7, 8, and 10 and 15 days along with a commercially available wheatgrass tablet. The antioxidant activity of wheatgrass was estimated at different levels of protection using aqueous and ethanolic extracts of wheatgrass. The methods employed include FRAP (ferric reducing antioxidant power), ABTS (2, 2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid) and DPPH (1, 1'-diphenyl-2-picrylhydrazyl) assays. Lipid peroxidation and oxygen radical scavenging capacity (ORAC) were determined and utilized to check the potency of a few select extracts. To explain reasons behind the observed differences, total phenolic and flavonoid contents of the extracts were measured. These contents increased with growth under all the conditions. The ethanolic extracts were found to possess more phenolic and flavonoid contents than the aqueous extracts. The highest FRAP values found were observed on 15th day of the growth under condition 4. In aqueous extracts no specific trend was observed with DPPH assay for different conditions as well as growth period. In the case of ethanolic extracts, however, it increased with growth period and the wheatgrass grown in condition 4 was found to be most effective. These extracts were also found to significantly inhibit ascorbate-Fe²⁺ induced lipid peroxidation in rat liver mitochondria. The ORAC values of aqueous and ethanolic extracts of 10th day with condition 4 were found to be higher than those reported for many natural extracts or vegetables.

The systematic investigations gave an insight on the potential of tender wheatgrass as a medicinal herb. Detailed analysis on RDA along with bioavailability of various elements indicated that, tender wheatgrass is not a food supplement but a good medicinal herb. Antioxidant studies showed that, tender wheatgrass grown even with pure water, is an excellent source of antioxidants.