1.2 MATERIAL AND METHODS

Nine genotypes of cabbage (namely C1, C2, C3, C5, C7, SEL 1, SEL 7, DARL 851 and DARL 852) were evaluated in a complete, randomized block design with three replications at the experimental field. The experimental site was located in Pithoragarh (Uttarakhand) at an altitude of 5500 ft above sea level. The site was situated in the western Himalayas, which extends from lat 29°29′ N to lat 30°49′ N and long 85°05′ E to long 81°31′ E. The annual rainfall is approximately 1250 mm, out of which 70–75% is received during the rainy season. The temperature of the place ranged from a maximum of 35°C in summer to a lower of –2°C during winter. The seedlings of cabbage genotypes were transplanted in an open field. The mature heads were selected in each replication. Leaves were separated individually from each head and grouped into outermost leaves (L1–L3), outer middle leaves (L4–L10), inner middle leaves (L11–L17) and innermost leaves (L18–L30). The leaves in the four groups were screened for ascorbic acid (mg/100 g), β-carotene (μg/100 g), total chlorophyll (mg/100 g) and antioxidant activity (IC_{50}).

1.2.1 Nutraceutical Evaluation

The chemical analysis of fresh heads included determination of ascorbic acid by the 2,6-di-chlorophenol indophenols method (AOAC 1990). The fresh fruit samples of cultivars were analysed for antioxidant activity using the DPPH (2,2-diphenyl-1-picryl–hydrazyl radical) method (Hatano et al. 1988). β-Carotene was estimated through a spectrophotometer, and the results were expressed as μg/100 g (AOAC 1980). Chlorophyll estimation was carried out by the method given by Witham et al. (1971).

1.2.2 Estimation of Ascorbic Acid

Ascorbic acid was estimated by the volumetric method. Three grams of the fresh sample were extracted with 4% oxalic acid and the volume was made up to 100 mL and centrifuged. Five milliliters of this supernatant was pipetted out, combined with 10 mL of 4% oxalic acid and the titration was done against the dye. Ascorbic acid reduces the 2,6-dichlorophenol dye to a colorless leuco base and gets oxidized to dehydroascorbic acid. It was estimated in mg/100 g.

1.2.3 Estimation of β-Carotene

For the estimation of β-carotene, 5 g of dried sample was taken in 150-mL glass stoppered Erlenmeyer flask and 40 mL water saturated butanol (WSB) was added. The contents of the flasks were mixed vigorously for 1 min and kept overnight (16–18 h) at room temperature in the dark for a complete extraction of β-carotene. The contents were shaken and filtered through the Whatman Filter Paper No. 1 into a 100-mL volumetric flask. The optical density of clear filtrate was measured at 440 nm using an ECIL, double-beam UV-VIS Spectrophotometer 5704SS. Pure WSB was used as a blank. The WSB was prepared by mixing n-butanol with distilled