18.2.6.5 Metal Chelating Activity

The chelating of ferrous ions by various extracts of *C. australe* was estimated by the method of Dinis et al. (1994). Initially, about 100 µL the extract samples were added to 50 µL of 2 mM $FeCl_2$ solution. Then the reaction was initiated by the addition of 200 µL of 5 mM ferrozine and the test tubes were vortexed well and left standing at room temperature for 10 min. The reaction mixture containing deionized water in place of sample was considered as the negative control. Absorbance of the solution was then measured spectrophotometrically at 562 nm against the blank (deionized water). EDTA was used as the standard metal chelating agent and the results were expressed as mg EDTA equivalents/g extract.

18.2.6.6 Hydroxyl Radical Scavenging Activity

The scavenging activities of solvent extracts of *C. australe* on hydroxyl radicals were measured according to the method of Klein et al. (1991). About 100 µL sample extracts and standards (BHT and rutin) were added with 1 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%) and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH-7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1 mL of ice-cold TCA (17.5% w/v). About 3 mL of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as a control. The intensity of the colour formed was measured spectroscopically at 412 nm against the phosphate buffer blank. The percentage hydroxyl radical scavenging activity is calculated by using Equation 18.2:

$$\%\ \text{Hydroxyl radical scavenging activity} = \left[(A_0 - A_1)/A_0 \right] \times 100 \tag{18.2}$$

where,

A_0 is the absorbance of the control and

A_1 is the absorbance of the extract/standard.

18.2.6.7 Superoxide Radical Scavenging Activity

The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system (Beauchamp and Fridovich 1971). About 3 mL of reaction mixture containing 50 mM sodium phosphate buffer (pH-7.6), 20 µg riboflavin, 12 mM EDTA and 0.1 mg NBT was added to 100 µL sample solution, BHT and rutin. The reaction was started by illuminating the reaction mixture with samples for 90 seconds. The illuminated reaction mixture without sample was used as the negative control. Immediately after illumination, the absorbance was measured at 590 nm against the blank (unilluminated