total phenolics. For the determination of total non-tannin phenolics (Makkar 2003), 500 µL of each plant sample was incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µL of distilled water taken in a 2 mL eppendorf tube for 4 h at 4°C. After incubation, the eppendorf tubes were centrifuged at 4000 rpm for 10 min at 4°C. The supernatant contains only the non-tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant was collected and the non-tannin phenolics were determined by the same method described for the quantification of total phenolics. The analyses were also performed in triplicate, and the results were expressed in gallic acid equivalents. From these two results, the tannin content of the plant samples were calculated as follows:

$$\text{Tannins} = \text{Total phenolics} / \text{Non-tannin phenolics}$$

18.2.5.3 Quantification of Total Flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by Zhishen et al. (1999). About 500 µL of all the plant extracts were taken in different test tubes and 2 mL of distilled water was added to each test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150 µL of 5% $NaNO_2$ was added to all the test tubes, followed by incubation at room temperature for 6 min. After incubation, 150 µL of 10% $AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 min at room temperature. Then 2 mL of 4% NaOH was added to all the test tubes which were then made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 min at room temperature. The pink colour developed due to the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicate and the results were expressed in rutin equivalents (RE).

18.2.6 *In Vitro* Antioxidant Assays

18.2.6.1 DPPH$^{\bullet}$ Scavenging Activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Braca et al. (1958). Sample extracts at various concentrations were taken and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.004% methanolic solution of DPPH was added to the aliquots of samples and standards (BHT and rutin) and shaken vigorously. Negative control was prepared by adding 100 µL of methanol in 3 mL of methanolic DPPH solution. The tubes were allowed to stand for 30 min at 27°C. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH$^{\bullet}$ concentration.