17.2.2 Collection and Identification of Plant Materials

The fresh material of *B. retusa* were collected from Kotagiri hills in the Nilgiris District, Tamil Nadu, India during the month of October, 2014. The taxonomic identity of the plant was confirmed by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. (No. BSI/SRC/5/23/2015/Tech/531). The stem parts were washed under running tap water to remove the surface pollutants and were air-dried under shade. The dried samples were powdered and used for further studies.

17.2.3 Preparation of Plant Extracts

The powdered stems of *B. retusa* (100 g) were packed in small thimbles and extracted successively with organic solvents (400 mL) such as petroleum ether, chloroform, ethyl acetate and methanol in increasing order of polarity using a Soxhlet apparatus. The different solvent extracts were concentrated by a rotary vacuum evaporator (Yamato RE300, Japan) and then air-dried. The dried extract obtained with each solvent was weighed and the percentage yield was calculated using Equation 17.1.

$$\text{Extract yield percentage} = [A/B] \times 100 \tag{17.1}$$

Where

A = Amount of crude extract,
B = Amount of sample.

The extracts obtained were used for the assessment of various analyses (1 mg/mL of respective organic solvents).

17.2.4 Quantification of Total Phenolic, Flavonoid, Vitamin C and E Contents

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003) and the results were expressed as gallic acid equivalents (GAE). Total flavonoids in the extracts were estimated as rutin equivalents according to the method of Zhishen et al. (1999). The vitamin C content in stem extracts was estimated based on the method of Sadasivam and Manikam (2008) and the results were expressed as milligrams of ascorbic acid equivalents per gram extract. The vitamin E content was determined by Prieto et al. (1999), method and tocopherol were used as standards.

17.2.5 *In Vitro* Antioxidant Assays

17.2.5.1 DPPH$^{\bullet}$ Scavenging Assay

The DPPH radical was used to measure the free radical scavenging activity of plant extracts by the method of Blois (1958). Different concentrations of stem extracts were taken, and 3 mL of a 0.1 mM methanolic solution of DPPH was added to the