17.2.5.6 Nitric Oxide and Superoxide Anion Radical Scavenging Assays

The biological radicals such as nitric oxide (Sreejayan and Rao 1997) and superoxide anion radical (Beauchamp and Fridovich 1971) scavenging activity was estimated in the stem extracts according to the standard procedures.

17.2.5.7 Lipid Peroxidation Inhibition Assay

A modified thiobarbituric acid-reactive substance (TBARS) assay was used to measure the lipid peroxide formed using goat liver homogenates (Ohkawa et al. 1979). Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of TBA yielding a pinkish-red chromogen. Liver homogenate (500 µL of 10%, v/v inphosphate-buffered saline pH 7.4) and 500 µL of sample were added to a test tube and made up to 1.0 mL with distilled water. Then, 50 µL of FeSO4 (0.075 M) and 20 µL of L-ascorbic acid (0.1 M) were added and incubated for 1 h at 37°C to induce lipid peroxidation. Thereafter, 0.2 mL of EDTA (0.1 mol/L) and 1.5 mL of TBA reagent (1.5 g TBA, 60 g TCA and 5.2 mL 70% $HClO_4$ in 800 mL of distilled water) were added in each sample and heated for 15 min at 100°C. After cooling, samples were centrifuged for 10 min at 3000 × g and absorbance of supernatant was measured at 532 nm. Inhibition (%) of lipid peroxidation was calculated using Equation 17.2:

$$\%\text{Inhibition} = \left[(\text{Control OD} - \text{Sample OD})/\text{Control OD} \right] \times 100 \tag{17.2}$$

17.2.6 Statistical Analyses

All the experiments were done in triplicate and the results were expressed as mean ± SD. The data were statistically analysed using one-way ANOVA by Duncan's test for all the studies. Mean values were considered statistically significant when $p < 0.05$.

17.3 RESULTS AND DISCUSSION

17.3.1 Extractability of Stems

Soxhlet extraction is a standard method for the extraction of bioactive compounds from plant sources. In the present study, petroleum ether, chloroform, ethylacetate and methanol have been used to extract the lipophilic compounds (oils and fatty acids), pigments (chlorophyll) and polyphenolics (phenolics and flavonoids). The extract yield percentage of *B. retusa* was shown in Table 17.1. The results showed that methanol extract (19.2 g/100 g sample) had a higher extract yield than other solvent extracts. Methanol extraction of medicinal plants generally yielded more components than other solvent extractions. It is worth mentioning that methanol solvent extraction may allow more hydrogen bonding with phenolic compounds (Murugan and Parimelazhagan 2014).