CHAPTER 4

Conclusion

The essential oil was obtained yield 0.04-0.24 % (w/w) from essential oil extracts by simultaneous distillation extraction (SDE) technique and analyzed by gas chromatography-mass spectrometry (GC-MS) using HP-5MS column. The results showed that the most of the major class of compounds were alcohols and ester. The main compounds were phytol, methyl palmitate, methyl linolenate, methyl linoleate, geraniol, phenylethyl alcohol, *p*-ethylphenol, linalool and *α*-terpineol. Phytol, a key acidic diterpene alcohol is a precursor in biosythesis of vitamins E and K1. It is an antioxidant and a preventive agent against exoxidoinduced breast carcinogenesis.[97] Methyl linolenate (more efficient antioxidant than α-eleostearic acid against oxidative DNA damage), tetradicanoic acid, *n*-hexadecanoic acid, and linoleic acid have been reported to show various toxic effects such as cancer and neurotoxicity etc.[102-104]

Solvent extraction is the most common technique used to isolate natural products. Fresh leaves of Assam tea were used as sample for the selection of a suitable solvent for extraction and for optimization of HPLC conditions. Thirteen solvents were used to examine the effects of extraction solvent on total phenolic content and antioxidant activity. It was found that 80% aqueous acetone provide significantly better result, including extraction yield (18.76%), total phenolic content (786.65 mgGAE/g DW) and antioxidant activity (IC_{50} = 0.24 mg/mL and FRAP = 114.83 mgGAE/g DW), than those of the other solvent system.

The HPLC method was found to be applicable to the analysis of phenolic compounds and caffeine in fresh leaves, steamed leaves, various fermented leaves, steamed water, and various fermented water samples. HPLC-UV conditions for the separation and simultaneous determination of phenolic compounds and caffeine were optimized using a Wakosil-II 5C18 HG column.

The optimum separation of phenolic compounds (GA, GC, EGC, C, EC, EGCG, GCG and ECG) and caffeine was obtained by adjusting detection wavelength and composition mobile phase. The optimum HPLC condition was achieved with the use of the mixture of 1% ethyl acetate in methanol and 0.1% (v/v) phosphoric acid solution in gradient elution program at the flow rate of 0.45 ml/min and the detection wavelength at 270 nm. This method could be used to separate the nine compounds with resolution greater than 1.0 in approximately 90 min under gradient condition at room temperature.

The accuracy expressed in terms of percentage recovery of this method for GA, GC, caffeine, EGC, C, EC, EGCG, GCG, and ECG ranged between 90-96, 84-95, 92, 93-97, 95-97, 97, 83-92, 85-92 and 92-94, respectively of fresh leaves and 81-93, 85-92, 89-98, 90-96, 92-94, 86-92, 87-96, 91-92 and 92-95, respectively of 45 days fermented leaves. The repeatability of peak area of each compound expressed as %R.S.D. was found to be 0.05-1.03%. The reproducibility of the peak area obtained was 0.16-4.45%. The detection limits of GA, GC, caffeine, EGC, C, EC, EGCG, GCG, and ECG were 0.10, 2.45, 0.05, 0.75, 0.35, 0.55, 0.48, 0.75 and 0.70 ppm, respectively.

Prior to HPLC analysis of phenolic compounds and caffeine, fresh leaves, steamed leaves, various fermented leaves of Assam tea samples were subjected to extraction using 80% aqueous acetone as extraction solvent. The highest amount of GA was detected in steamed leaves extract. GC and EGC were detected in high amount in fresh leaves extract, while the steamed leaves had the highest amount of C, EGCG, GCG, and ECG (0.494, 16.976, 4.814 and 15.357 mg/g DW, respectively). The highest amount of caffeine and EC contains were detected in the 120 and 15 days fermented leaves extracts. In the water samples, the 30 days fermented water contains the highest amount of C, EGCG, and GCG at 0.3679, 0.861 and 0.240 mg/g DW, respectively, while the highest amount of GC and EC was detected in the 150 days fermented water. The highest amount of GA, caffeine, ECG and EGC contents were detected in 120, 15, 60 days fermented water and steamed water, respectively.

The total phenolic content of volatile oil, leaves extracts, and water samples were determined spectrometrically according to Foiln-Ciocalteau method. The highest phenolic content with were found in 30 days fermented water, follow by 150 fermented leaves extract and 45 days fermented volatile oil with the value of 644.04 ± 0.01, 457.26 ± 0.02 and 40.60 ± 0.01 mgGAE/g DW, respectively.

The antioxidant activities of volatile oils, leaves extracts and water samples were evaluated using DPPH and FRAP assay. Due to low % radical scavenging, IC50 values volatile oil was not determined. However, among the volatile oil 45 days fermented leaves showed the highest % radical scavenging, while the 15 days fermented leaves showed the highest antioxidant activity in FRAP assay. For sample extracts and water samples, the steamed leaves extract had the highest antioxidant activity in DPPH assay. However, the 30 days fermented water showed the highest antioxidant activity in FRAP assay.

The correlation coefficient (r^2) was the highest (r^2= 0.982) between DPPH (%radical scavenging) and FRAP activity of water samples than that of TPC and FRAP activity of leave extracts (r^2= 0.977) followed by TPC and FRAP activity of solvent extracts (r^2= 0.854), TCP and DPPH of essential oil (r^2= 0.782) and DPPH and FRAP of leave extracts (r^2= 0.735), respectively, which showed significant correlation ($P<0.01$). Similarly a lower correlation was observed between TPC and DPPH of water sample (r^2 = 0.576) followed by TPC and FRAP of solvent extract and essential oil, respectively. These showed significant correlation ($P<0.01$). The correlation between the results of measuring TPC and antioxidant activity of each sample showed that phenol compounds largely contribute to the antioxidant activities of the plant.

Recommendation for Future Work

Other bioactivity of the sample extracted such as antibacterial or anticancer activities should be tested.