Summary
6. SUMMARY

The ethanol extract of *Mentha spicata* L. was fractionated into hexane, chloroform, ethyl acetate and aqueous fractions following standard protocols. Total antioxidant activity and relative antioxidant activity-relative to quercetin, beta-carotene, ascorbic acid and glutathione were estimated using ABTS radical decolorization assay. Polyphenolics (phenolics and flavonoids) and pigments (chlorophyll and carotenoids) were also quantitated.

The ethyl acetate fraction had shown the highest total antioxidant activity (95% at 20μg/ml) followed by aqueous (84% at 30μg/ml), chloroform (53% at 50μg/ml) and hexane (41% at 50μg/ml) fractions. Compared with quercetin (TAA = 87% at 5μM/ml), the relative antioxidant activity of ethyl acetate fraction of *M. spicata* alone was greater than one at 20μg/ml concentration. Compared with β-carotene (TAA = 77% at 7.5μM/ml), ascorbic acid (TAA = 72% at 15μM/ml) and glutathione (TAA = 61% at 15μM/ml), the relative antioxidant activity with values > 1.0 were found for ethyl acetate fraction at concentration of ≥ 15μg/ml and for aqueous fraction at concentration of ≥ 20μg/ml.

Expressed in mM quercetin equivalents/g of the solvent fraction, the ethyl acetate fraction contained highest amount of quercetin equivalent antioxidant activity (309.54mM/g) followed by aqueous (212.00mM/g), chloroform (47.57mM/g) and hexane (41.28mM/g) fractions. Similarly, the value of β-carotene, ascorbic acid and glutathione equivalents of ethyl acetate
fraction were found highest followed by aqueous, chloroform and hexane fractions.

The total phenolic content was highest in ethyl acetate fraction (54.0mg/g) followed by aqueous (32.0mg/g), chloroform (30.0mg/g) and hexane (14.0mg/g) fractions. The total flavonoids were highest in aqueous fraction (24.20mg/g) followed by ethyl acetate (22.0mg/g), chloroform (16.20mg/g) and hexane (15.0mg/g) fractions of *M. spicata*.

The total chlorophyll content was greater in chloroform fraction (29.07mg/g) followed by hexane (14.40mg/g), ethyl acetate (13.20mg/g) and aqueous (4.73mg/g) fractions. The total carotenoids content was high in chloroform fraction (7.17mg/g) and less in the aqueous fraction (0.93mg/g). The ethyl acetate and hexane fractions, respectively, contained 5.07mg/g and 3.07mg/g carotenoids. Total antioxidant activity positively correlated with total phenolic and flavonoid content of the solvent fractions.

*Mentha spicata* solvent fraction and ethanol extract were evaluated for *in vivo* antigenotoxic potential in mice bone marrow cells using 4-nitroquinoline-1-oxide (4-NQO) as reference mutagen. The mutagen, 4-NQO, enhanced the frequency of micronucleated polychromatic erythrocytes (MnPCEs) by about four times the control value, 15.78 MnPCEs/2500 PCEs. Pretreatment with ethanol extract significantly reduced the mutation frequency induced by the mutagen. The reduction was greatest at the highest dose, 320mg/Kg bwt (by about 69%) and lowest at the lowest dose, 80mg/Kg bwt (about 50%).
Pre-treatment with hexane fraction significantly reduced the 4-NQO induced mutation rate ranging from 42 to 58 percent. Dose differences were significant only at higher doses (320, 160mg/Kg bwt Vs mutagen control). For chloroform fraction, the reduction was in the range 45 - 61 percent. The dose depended differences were not very apparent.

For ethyl acetate fraction, the reduction of MnPCEs was effective at a dose of 160mg/Kg bwt (in the sense the reduced mutation rate was comparable with the value of control group). In the lowest dose (80mg/Kg bwt) the reduction was statistically significant and occurred to the extent of 51 percent of the mutagen group. For aqueous fraction the reduction varied from 46 percent at the lowest dose (80mg/Kg bwt) to 67 percent at the highest dose (320mg/Kg bwt).

Ethanol extract and solvent fractions significantly decreased mutagen induced lipid peroxidation by positively modulating all the measured antioxidants (SOD, CAT and GPx, GST, Vitamin E and C). The magnitude of modulation varied with the dose and solvent fraction. The modulated values at the highest dose were comparable with that of the controls and in some instances the effects were more pronounced than the control values.

The present investigation suggests that the solvent fractions had shown both antigenotoxic and antioxidant properties. The antigenotoxic activity of the solvent fractions was ranked as follows: ethyl acetate fraction > aqueous fraction > chloroform fraction > hexane fraction. This order also corresponds to the order of their antioxidant activities.