Summary & Conclusions
7. SUMMARY AND CONCLUSIONS

- Pharmacognostical and phytochemical analysis of *M. oleifera* confirms authenticity of the plant.

- The extractive values in different fractions of *M. oleifera*: chloroform, ethyl acetate, n-butanol and residual was found to be 1.44%, 1.27 %, 2.44 %, 14.8% respectively.

- Hexane- ethyl acetate: methanol (7.7: 2.0: 0.3), toluene: ethyl acetate- diethylamine (8: 1: 1), chloroform- methanol (9.5:1), Hexane : methanol (5: 5), dichloromethylene - ethyl acetate : hexane (6: 2: 2), dichloromethane: ethyl acetate : hexane (6: 3: 1) were found to be more appropriate solvent systems for ethanolic extract, chloroform, ethyl acetate, n-butanol and residual fraction respectively.

- HPTLC fingerprint profile of ethanolic extract, chloroform fraction, ethyl acetate, n-butanol fraction and residual fraction shows 10, 9, 10, 10 and 8 peaks at different Rf values respectively at 525nm after derivatisation.

- Quantitative estimation of marker compounds by HPTLC analysis showed following percentage weight by weight quantity of ascorbic acid, quercetin, benzylisothiocyanate and glycerol-1-(9-octadecanoate): 0.52 %, 2.57%, 2.77%, 0.47% in ethanolic extract, 0 02 %, 2.23%, 0.00%, 0.21% in chloroform fraction; 0.05%, 0.00%, 2.53%, 0.16% in ethyl acetate fraction; 0.45%, 2.38%, 2.72%, 0.29% in n-butanol fraction, 0.00%, 0 00%, 1.79% and 0.00% in residual fraction respectively. The concentration of β-sitosterol was found to be 2.59% in ethanolic extract, 0.84% in chloroform fraction, 0.38% in ethyl acetate fraction, 3.42% in n-butanol fraction and 0.15% in residual fraction

- The isolation of β-sitosterol from n-butanol fraction was performed by preparative TLC and purified by column chromatography. The yield obtained was 0.34% of the weight of the powder of the dried seeds.

- Characterization and structure elucidation of isolated compound by melting point, UV, IR, Mass, $^1$NMR and $^2$NMR spectral analysis confirms that the compound is β-sitosterol.

- Ethanolic extract of *M. oleifera* showed dose dependent bronchodilatory activity in histamine and acetylcholine induced bronchospasm in guinea pigs.

- The preliminary pharmacological studies in present investigation showed that ethanolic extract and all its fractions possesses bronchodilatory, mast cells stabilizing, immunosuppressive and anti-anaphylactic activities.
• Ethanolic extract and n-butanol fraction were found to be more effective compared to chloroform and ethyl acetate fraction. The active components are possibly present in ethanolic extract and n-butanol fraction. These results correlate well with phytochemical analysis.

• Ethanolic extract and n-butanol fraction showed bronchodilatory activity in chemical- toluene diisocyanate (TDI) induced-immune mediated inflammatory responses in rats and ovalbumin induced airway inflammation in guinea pigs. Both the extracts showed protection by improvement in airway hypereactivity symptoms, oxidative stress parameters (MDA, SOD, CAT and GSH), lung function parameters (tidal volume, respiration rate). It also produced inhibition of infiltration of inflammatory cells (eosinophils and neutrophils), level of TNF-α, IL-4, IL-6, IL-5, mediators like histamine. However, n-butanol fraction produced more promising effects as compared to ethanolic extract.

• β-sitosterol has shown bronchodilatory activity by increasing PCD time against spasmogens (histamine and acetylcholine). It also showed protection against ovalbumin induced chronic airway inflammation by improvement in lung function parameters (tidal volume, respiration rate, minute volume, inspiration time, expiration time and peak expiratory rate). It also produced inhibition of infiltration of inflammatory cells, level of TNF-α, IL-4, IL-6, IL-5 and histamine.

• β-sitosterol appears to be the active principle responsible for the antiasthmatic activity. However, the involvement of other marker compounds, ascorbic acid, quercetin, benzylisothiocyanate and glycerol 1-9-octadecanoate can not be ruled out.

• In conclusion our data suggest that seed extracts of *M. oleifera* produced beneficial effects in antigen induced bronchospasm, mast cell degranulation, immune reactions and anaphylactic reactions. A good co-relation was found between the concentration of marker compounds and pharmacological activities. β-sitosterol appears to be the active principle responsible for the potential pharmacological activities mentioned above. The antiasthmatic activity of n-butanol fraction and its active principle β-sitosterol might be due to the inhibition of infiltration of inflammatory cells, release/synthesis of TNF-α, IL-4, IL-5 and IL-6, mediators like histamine.