SUMMARY

The present investigation entitled "Studies on Isolation and Pharmacological Screening of Extracts of *Momordica dioica* Roxb and Semi-synthetic Constituents of *Azadirachta indica* A.Juss" has been carried out and presented in the thesis.

*Momordica dioica* Roxb

*Momordica dioica* Roxb belongs to the family Cucurbitaceae. It is a perennial dioecious climber with tuberous roots found throughout India. It is often cultivated around villages for its edible fruit.

The work involves extraction, pharmacological screening of the extracts, isolation and characterization of constituents by using modern analytical techniques like UV, IR, $^1$H NMR, $^{13}$C NMR, MS, HPLC etc. Keeping in view of the various medicinal uses of the plant mentioned in the literature, pharmacological evaluation was carried out to substantiate the folklore claim.

Hexane extract (HE) and ethyl acetate soluble fraction of methanolic extracts (EASFME) were prepared from the fruit pulp of *Momordica dioica*.

Preliminary phytochemical screening reveals the presence of carbohydrates, proteins, amino acids, sterols and flavanoids in both HE and EASFME and in addition presence of alkaloid was observed in EASFME.

HPTLC finger prints for both the extracts were developed.

- Toxicity studies result revealed that MLD for both HE and EASFME in intraperitoneal route was 3.2 g/kg and 1.6 g/kg respectively. By
the same, orally it was found to be was 3.2 g/kg for EASFME and exceeds 3.2 g/kg for HE.

- Analgesic activity - HE was found to be active in both acetic acid induced writhing reflex model and hot plate method, whereas EASFME was active only in hot plate method.

- Anti-inflammatory activity - Both HE and EASFME exhibited a dose dependent anti-inflammatory activity in carrageenan-induced paw oedema method.

- Hepatoprotective activity - HE was found to be more active in reducing the elevated levels of marker enzymes SGOT, SGPT and ALP when compared to EASFME. Bilirubin level was reduced equally by both the extracts. Total protein, albumin, globulin and A/G ratio levels were improved in HE treated groups as compared to standard treated group.

- Glucose lowering effect and Hypolipidemic activity - For OGTT, HE treated group found to be more active, when compared to EASFME in maintaining blood glucose levels at various time intervals 0, 30, 60, 90 and 120 min.

By intraperitoneal administration of extract of *Momordica dioica* in alloxan- induced diabetic model - HE was found to be much more active than EASFME in maintaining blood glucose levels at various time intervals 0, 1, 3 and 5 h.

By oral administration of extract (for 15 days) of *Momordica dioica* in alloxan- induced diabetic model - both HE and EASFME reduces the elevated blood glucose level to that of standard drug Glibenclamide. HE reduced the cholesterol level and EASFME reduces the triglycerides level. Total Protein
quantity level was improved by both the extracts. EASFME was found to be more active in reducing the urea and creatinine levels below the normal value, whereas HE does not have any influence on these two parameters.

- **Antimicrobial Activity** - *Staphylococcus epidermidis, Shigella dysenteriae, Salmonella typhi, Proteus vulgaris and Staphylococcus aureus* exhibits sensitivity towards HE and EASFME. *Bacillus cereus, Bacillus subtilis* and *Klebsiella pneumoniae* also exhibit sensitivity towards HE, whereas *Escherichia coli* and *Pseudomonas aeruginosa* initiate sensitivity towards EASFME.

- **Antifeedant activity** - EASFME was found to be more active than HE, when compared to the standard azadirachtin.

- **Phytochemical analysis** - from the HE Linoleic acid, Palmitic acid and mixture of Oleic acid & Ursolic acids were isolated using column chromatography and identified by spectroscopic techniques like, UV, IR, $^1$H NMR, $^{13}$C NMR, MS etc.

*Azadirachta indica* A.Juss

*Azadirachta indica* A.Juss belongs to the family Meliaceae. It is a large ever green tree, 12-18 m in height and 1.8 - 2.4 m in girth with a straight bole and long spreading branches forming a broad crown, commonly found throughout the greater part of India and often cultivated.

- Fruit skin of *Azadirachta indica* was taken for the present investigations.

- Fruit skin was extracted with CCl$_4$ and concentrated CCl$_4$ extract was studied for its phytochemical and pharmacological activities.
- HPLC profile for CCl₄ extract was studied and compared with isolated constituents.

- HPTLC profile was studied for the CCl₄ extract.

- Based an HPLC profile, Azadiradione was isolated from the fruit pulp of CCl₄ extracts and confirmed by spectroscopic techniques.

- Nimbin was isolated from seed oil of *Azadirachta indica* and confirmed by standard techniques.

- Analgesic activity was screened for CCl₄ extract, Azadiradione and Nimbin (at a dose of 50 and 100 mg/kg body weight) in both acetic acid induced writhing reflex method and hot plate method. Azadiradione was more active in acetic acid induced writhing reflex model, whereas Nimbin exhibited better activity in hot plate method. CCl₄ extract, was found to be less active in both the models.

- Anti-inflammatory activity - Nimbin at a dose of 100 mg/kg body weight was found to be more active in carrageenan-induced paw oedema method, when compared to standard drug and Azadiradione. CCl₄ extract was found to be less active than Nimbin and Azadiradione.

- Antimicrobial activity - CCl₄ extract, Azadiradione and Nimbin (at concentrations of 100, 200, 300, 400 and 500 μg/disc) were exhibits sensitivity to all eight microorganisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysenteriae*) which were screened for antimicrobial activity.

**Semi-synthetic modifications**
Isolated constituents (Azadiradione and Nimbin) were subjected to

- oxidation using NBS
- deacetylation using ED
- reduction using sodium borohydride
- reduction using tetrabutylammonium borohydride and
- epoxidation using H$_2$O$_2$ / NaOH.

All the modified products were confirmed by spectroscopic techniques like, UV, IR, $^1$H NMR, $^{13}$C NMR, MS etc.

**Pharmacological Screening of modified products**

The semi-synthetically modified products were subjected to pharmacological screening like analgesic, anti-inflammatory and antimicrobial activities. It was observed that all the modified products were active in both the models (acetic acid induced writhing reflex method and hot plate method) of analgesic activity and carrageenan induced paw oedema model of antiinflammatory activity when compared to unmodified Azadiradione and Nimbin. None of these products exhibited antimicrobial activity against the screened microorganisms and found to be resistant upto 500 μg/disc dose levels.

C log P value was determined for all the modified products. The results of analgesic and anti-inflammatory activities were correlated with C log P value.