Medicinal plants play a key role in the human health care. About 80% of the world populations rely on the use of traditional medicine which is predominantly based on plant materials. Although herbal medicines are effective in the treatment of various ailments very often these drugs are unscientifically exploited and/or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants can lead to the development of invaluable plant drugs for many diseases. According to one estimate, only 20% of the plant flora has been studied, of these, 15% have been evaluated phytochemically and a reported 6% have been screened for biologic activity. This necessitates continuous exploration and rigorous experimentation of plant products for plant derived drugs in addition to new pharmaceuticals for many of the human ailments. Hence the present research programme has been envisaged. The study explores the medicinal potentialities of three plants of Mulberry belonging to the genus Morus viz. Morus alba, Morus serrata and Morus laevigata which are claimed to possess several medicinal properties in folklore/traditional systems.

- Mulberry is a monoecious or dioecious plant grows up to 10-12m high. Mulberry (Morus) is believed to have originated at the feet of Himalayan ranges. This plant is widely distributed in India, China, Japan, North Africa, Arabia, South Europe etc. Mulberry can be grown both in tropics and in the temperate regions. It is also raised in rain fed and irrigated conditions. In India, mulberry is grown on an extensive scale in various parts, namely Punjab, Himachal Pradesh, Uttar Pradesh, Madhya Pradesh, Bihar, Orissa, Assam, Manipur, Karnataka, Andhra Pradesh and Tamil Nadu. It is reported to grow in many Asian and African countries of the world.

- The medicinal properties attributed to mulberry are extensive. Topically, it is applied for the treatment of wounds. Internally, it is used to relieve insomnia, regulate bleeding during menstruation, treat digestive disturbances, to ease cough and asthmatic breathing, reduce fever and inflammation, as hypo-lipidemic, anti-ageing, anti-filariosis, diuretic, and antiulcer agent. Mulberry is known to possess hypoglycemic, antibacterial, astringent, diaphoretic, antiviral and anticancer effects among a wide range of pharmacological activities reported for this plant. It
is evident that large numbers of biomolecules of medicinal interest are present in mulberry. But extensive work has been carried out by mainly targeting *Morus alba* and thus warranting detailed studies utilizing various other species as phytochemicals vary in terms of species, varieties and within different parts of plants and also influenced by seasons and different agronomic conditions.

- The objectives of the present research programme is to extract leaf materials of three species *viz.* *Morus alba, Morus serrata* and *Morus laevigata* by sequential extraction method and subject the extracts to qualitative phytochemical analysis of the extracts, to isolate bioactive compounds and evaluate the extracts for Pharmacological studies to screen activities *viz.* *in vitro* antioxidant activities, *in vitro* and *in vivo* anticancer, analgesic and anti-inflammatory, wound healing, CNS depressant/stimulant, anthelmintic and antimicrobial activities in addition to perform *in silico* studies on isolated compounds.

- The three different species of the genus *Morus* *viz.* *M. alba* (IC573059), *M. serrata* (EC493838) and *M. laevigata* (IC313789) were collected from Mulberry Germplasm Center, Central silk board, Govt. of India, Hosur, Tamil Nadu, India. Leaves were subjected for shade drying consequently to extraction procedures and the extracts were used for the phytochemical and pharmacological activities. The shade dried powder was successively extracted with petroleum ether, chloroform and methanol in batches of 200g each in the soxhlet extractor. After each extraction the marc was air-dried. The solvent was removed under reduced pressure using rotary vacuum evaporator and the extracts were stored in a desiccator. All these extracts were used for phytochemical investigations and pharmacological screening for various activities.

- The phytochemical analysis revealed the presence of phenols in the petroleum ether extract of *Morus alba, Morus serrata* and *Morus laevigata* along with tannins in *Morus alba* and *Morus serrata*, while their chloroform extracts showed the presence of steroids, terpenoids, alkaloids, phenols, tannins, glycosides. However, saponins were present only in *Morus alba* and *Morus laevigata* in the said extract. Further, the methanol extract of all the species showed the presence of steroids, terpenoids, alkaloids, flavonoids, tannins, glycosides and saponins.
Column chromatography performed utilizing different solvent system and a total of four different compounds were isolated from the different solvent extracts of three mulberry species. The structures of the isolated compounds were determined using IR, $^1$H-NMR and Mass spectral analyses. They were identified as 5-Androstenediol, Cathafuran, Ursolic acid and Moracin M.

The acute toxicity studies were carried out as per stair case method (Ghosh, 1984). Animals weighing 20-25g were used for the study and were divided into groups of 6 animals each. The test extracts was administered intra-peritoneally as a suspension in Tween 80 to the different groups in increasing dose levels of 1000, 2000, 3000, 4000 and 5000mg/kg body weight. The animals were then observed continuously for 3h for general behavioral, neurological, autonomic profiles and then every 30 minutes for next 3h and finally for death after 24h (OECD, 2001). Accordingly the LD50 of all the extracts calculated and the therapeutic dose was selected between $1/10^{th}$ and $1/5^{th}$ of this dose for the evaluation of pharmacological activities. 1% Tween-80 was used as the vehicle to prepare various fractions and administered intra-peritoneally. The doses for all the solvent extracts of three mulberry species viz. Morus alba, Morus serrata and Morus laevigata were found to be safe up to 2000 mg/kg body weight (IP) as animals did not produce any noticeable signs of toxicity and mortality. Therefore, the therapeutic dose was selected between $1/10^{th}$ and $1/5^{th}$ of this dose for the evaluation of pharmacological activities.

In the present investigation, both quantitative viz. total phenolic, flavonoid and total antioxidants and qualititative assays viz. DPPH radical scavenging, nitric oxide radical scavenging, hydroxyl radical scavenging, iron chelating and reducing power were performed to assess and correlate the antioxidant activity of different solvent extracts of Morus alba, Morus serrata and Morus laevigata and the results revealed that, methanolic extracts of Morus have exhibited significant antioxidant activities for scavenging, chelating and reducing power when compared to chloroform and petroleum ether extracts. Further, it was proved by quantitative assays viz. total phenolic, flavonoid and total antioxidants. Among the three species, M. alba was proved to be superior to the other two species. The data therefore suggest that the extracts of Morus are a potential source of natural antioxidants.
Correlation analysis, a bivariate analysis was used in the present work for ascertaining the strength of the relationship between the variables. The degree of such relationship can be established by calculating the correlation coefficient (r), which gives a quantitative measure of the degree of closeness between the variables. On the other hand, regression analysis was used for measuring the probable form of the relationship between the variables. The degree of correlation ranging between 0.7 and 0.9874 suggests a simple, positive and high degree of correlation existing between the variables tested. It is evident from the results that the quantitative estimations performed in the study are positively correlated with the antioxidants studies wherein total antioxidant and total phenolics recorded a high ‘r’ value of 0.9151 and 0.9117 respectively followed by total flavonoid with 0.7989. Further, the average ‘r’ value was found to be highest in DPPH radical scavenging activity (0.9258) followed by other assays under study irrespective of quantitative phytochemical estimations, indicating that it is a best suited and reliable radical scavenging activity. The linear expression obtained from regression analysis is helpful in measuring the variables in term of qualitative and quantitative parameters.

The anticancer effect of the methanol leaf extracts of three mulberry species viz. Morus alba, Morus serrata and Morus laevigata were screened under non-tumorigenic and tumorigenic model systems. The non-tumorigenic model was carried out by cytotoxic assay against human cancer cell lines (MCF7 and 3T3) and MTT assay was used to analyze the cell growth inhibition and cell viability. Among the methanolic extracts of three species of Morus, the results were observed in a dose dependent manner. The methanolic extract of Morus laevigata showed highest growth inhibition in MCF7 (breast cancer cell lines) with an IC\textsubscript{50} value of 546.08µg/ml when compared to Morus serrata (579.16µg/ml) and Morus alba (595.33µg/ml). Similar results are observed with respect to 3T3 (non-cancer immortal cell lines), wherein Morus laevigata, Morus serrata and Morus alba registered IC\textsubscript{50} values of 359.17µg/ml, 386.59µg/ml and 376.66µg/ml respectively.

\textit{In vivo} anticancer activity was performed using EAT cell lines in mice for the methanolic extracts of three species of Morus viz. Morus alba, Morus serrata and Morus laevigata. Male Swiss albino mice were divided into 5 groups (n=6). All
the groups were injected with EAT cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally except the control group. This was taken as day zero, from the first day to every alternative day. The drugs were administered intra-peritoneally for 14 days and after the administration of last dose followed by 18h fasting, 3 mice from each group were sacrificed for the study of antitumor activity. The remaining animals in each of the groups were kept to check the mean survival time (MST) and percent increase in life span of the tumor bearing hosts. Further, anticancer effects of *Morus* species were assayed by observation of change with respect of body weight, ascitic tumor volume, packed cell volume and viable cell count.

- The mean survival time of the EAT cell line bearing mice showed 10.2 days, while it increased to 13.2 and 14.4 days in *Morus alba* methanolic extract treated groups and 12.84 and 13.86 days in *Morus serrata* methanolic extract treated group at 200 and 300mg/kg concentration. *Morus laevigata* however recorded the highest mean survival time of 14.2 and 16.2 at 200 and 300mg/kg concentrations respectively. Further, methanolic extract of *Morus laevigata* at 200 and 300mg/kg concentrations showed remarkable activity in terms of reduced body weight (7.2 and 6.3g), reduced tumor volume (5.4 and 4.8ml), reduced packed cell volume (2.64 and 2.2ml), reduced viable tumor cell count (44.55 and 37.25) in a dose-dependent manner when compared to that of EAT control group. Among the methanolic extract of *Morus alba* at 200 and 300mg/kg concentrations, the anticancer effects observed in terms of reduced body weight, reduced tumor volume, reduced packed cell volume and reduced viable tumor cell count in a dose-dependent manner respectively when compared to that of EAT control group. It was interesting to note that, among the three species under study, *Morus laevigata* was found to be potent for all the parameters evaluated substantiating its anticancer effects.

- The analgesic activity in Mulberry was performed by Tail immersion test. The basal reaction time to radiant heat source was taken by placing the tip of the tail on the radiant heat source. The animals were screened for the sensitivity test by immersing the tail of the mice gently in hot water maintained at 55° C. The time the animal’s tail spent in the water before reacting to the pain is recorded with a stop watch. The animal immersing the tail from hot water within 5 seconds was
selected for the study. The drugs were administered @ 200mg/kg & 400mg/kg intra-peritoneally. After administration of the drugs, the reaction time was measured (in seconds) after 60 minutes. The selected mice were then divided into twenty groups of six mice each.

- The data recorded for analgesic effects after one hour showed that, the control group registered reaction time of 2.43 and standard 9.17 whereas, the different plant extracts of the three species showed variations in the analgesic effects in a dose dependent manner. Animals treated with a dose of 200mg/kg, significant increase in reaction time was shown by all the groups except the petroleum ether extracts of the three plants. Animals treated with 400mg/kg dosage, all the extracts showed significant analgesic activity in terms of reaction time except petroleum ether extract of *Morus serrata* and *Morus laevigata*. It was 5.15±0.13; 8.23±0.15; 10.58±0.16 in petroleum ether, chloroform and methanol extracts of *Morus alba* respectively. The chloroform and methanol extracts of *Morus serrata* recorded 7.52±0.16 and 9.92±0.27 respectively and *Morus laevigata* recorded a reaction time of 6.93±0.13 and 9.37±0.15 for chloroform and methanol extracts respectively (P<0.01). It is evident from the data that, amongst the different solvents used; highest analgesic activity was shown by methanol extract followed by chloroform and petroleum ether. Further, among the three species under study, it is notable that *Morus alba* was found to be superior followed by *Morus serrata* and *Morus laevigata* in terms of pain reliving abilities.

- The anti-inflammatory activity of *Morus* was carried out by Carrageenan-induced paw edema in Mice. The albino rats of either sex were divided into twenty groups of six animals each. The initial paw volume was measured plethysmographically before carrageenan injection. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% carrageenan into the sub-plantar region of the right hind paw of rats. The right paw served as a reference to non-inflamed paw for comparison. The relative increase in paw volume was measured in control, standard and treated group at 60, 120, 180 and 240min after carrageenan injection. The percentage increase in the paw volume over the initial reading was calculated. This increase in the paw volume in the animals treated with the standard drug and the different doses of the crude extracts were compared with increase in paw.
The result reveals that administration of carrageenan to the rats showed a rise in paw volume at different time intervals in control group whereas; it was reduced in the indomethacin treated group. Even though plant extracts treated groups exhibited differential anti-inflammatory activity, significant (P<0.01) reduction in paw volume was recorded at 400mg/kg by *Morus alba* and *Morus serrata* at the third and fourth hour interval in all the extracts. However, in *Morus laevigata*, significant (P<0.01) anti-inflammatory effect was noted in forth hour in chloroform and at third and fourth hour intervals in methanol extract treated group. It is clear from the study that, the anti-inflammatory effect was more pronounced at later phases of time interval viz. third and fourth hour and was proved to be highest in methanolic extracts followed by chloroform and petroleum ether. Further, the order of efficiency in terms of anti-inflammatory effect were *Morus alba > Morus serrata > Morus laevigata*. It can be inferred that the inhibitory effect of the extracts on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis.

Wound healing property was assessed by incision and excision wound models in three leaf extracts of *Morus alba*, *Morus serrata* and *Morus laevigata*. The drugs were prepared for each of the extracts for topical administration. 5g of each extract was mixed with 45g of white petroleum jelly to get 10% (w/w) concentration and were applied topically in the form of ointments. 0.2% (w/w) nitrofurazone was used as a standard reference. All the formulations were prepared freshly before the commencement of each experiment. Eleven groups of animals containing six each were used for each of the excision and incision wound models. The animals of group I was considered as the control and received 5% sodium alginate ointment base and group II animals received 0.2% (w/w) nitrofurazone.

The results of the excision wound revealed that, even though significant wound closure was noted on 8th day in only chloroform (44.31±2.12) and methanol (46.14±1.16) extracts of *Morus alba* (P<0.01) and on 12th day by chloroform and methanol extracts (64.21±1.06, 76.05±1.84) and methanol extract of *Morus*...
serrata (66.17±1.46), all the treated groups of Morus alba have demonstrated significant wound contraction on 16th day (85.12±1.52, 88.18±2.21 and 92.06±1.62) while chloroform and methanol extract of Morus serrata (85.13±1.18, 88.21±1.32) and methanol extract of Morus laevigata (86.50±1.57) have exhibited wound healing effects (P<0.01). As far as mean time required for complete epithelialization, the control group animals took a mean time of 23.11 days whereas in standard group it was found to be 16.36 days. Only methanol extracts of the three species recorded a significant reduction in mean time of 17.52, 18.18 and 18.53 days respectively when compared to untreated groups for complete epithelialization.

- The breaking strength of the skin in incision wounds was increased in the M. alba extract treated groups (methanol, chloroform and petroleum ether) to a significant extent, ie. 1498.88, 926.52 and 628.18 while in M. serrata and M. laevigata extract treated groups it was 1312.55, 686.78 and 1258.33, 876.67 in methanol and chloroform extracts respectively. The results were comparable to the standard drug, nitrofurazone. It is evident from the results that, the Morus alba was potent in terms of wound healing followed by Morus serrata and Morus laevigata. Further, methanol solvent was proved to be the ideal solvent to achieve higher wound healing efficiency.

- The locomotor activity was studied in petroleum ether, chloroform and methanol extracts at two doses of 200 and 400mg/kg and evaluated by using photoactometer. Mice were placed individually in photoactometer and counts were taken for the duration of 5 min. The basal reaction time was noted before and 1 hour after the administration of treatment. The animals were divided in to XX groups of six mice each.

- The data showed after one hour of observation that, the different plant extracts of the three species showed variations in the locomotor effects in a dose dependent manner. The results revealed a significant reduction in locomotor activity by the animals treated with petroleum ether, chloroform and methanol extracts of Morus alba, Morus serrata and Morus laevigata at 400mg/kg concentration. Even though the reduction in locomotor activity was observed at 200mg/kg dosage the data are statistically not significant indicating dosage efficiency. Further, perusal of the results revealed that the extracts of Morus alba were more effective in CNS
depression than *Morus serrata* and *Morus laevigata* in all the three extracts and the depression was more pronounced in petroleum ether extracts of the three species.

- The anthelmintic assay was carried out as per the method described by Ghosh *et al.* Six worms of nearly equal size were placed in petri-plates containing 15ml of solution of either standard drug or test extracts solutions at room temperature. 20% Tween 80 used as control sample was also tested. Thus a total of five groups (20, 40, 60, 80 and 100mg) in each extract along with one negative control (20% Tween 80) and one positive control (Albendazole 20mg/ml) were subjected to the evaluation of anthelmintic property. Observations were made for the time taken to paralyze, and death of individual worms. Paralysis was said to occur when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C. The sequential extracts of *Morus alba*, *Morus serrata* and *Morus laevigata* possess potent anthelmintic property in a dose dependent manner for the parameters studied *viz.* paralysis and death which is quite comparable with standard anthelmintic drug in the organism tested.

- The results of the antibacterial activity revealed that all the extracts showed noticeable effects in dose dependent manner against the organisms studied at concentrations of 20, 40, 60 and 80mg/ml. Considering the overall mean values of the zone of inhibition, *Salmonella typhi* was found to be highly sensitive followed by *Bacillus subtilis*, whereas *Shigella flexneri* appears to be highly resistant followed by *Proteus vulgaris*. It is also very clear from the data that, the methanolic extracts and *Morus alba* irrespective of the species and solvent extracts exhibited better antibacterial activities respectively. The overall mean values of the zone of inhibition with respect to antifungal activity suggest that, *Candida albicans* is highly sensitive to all the extracts than *Aspergillus niger*.

- The MIC of isolated compounds assessed by tube dilution method indicated highest bacteriostatic effect by ursolic acid at 100ug/ml concentration against *Staphylococcus aureus* and *Pseudomonas aeruginosa* while cathafuran B against the latter only whereas, MIC was found to be 200ug/ml for other compounds against the tested organisms. The minimum bactericidal concentration of the
isolated compounds against *Staphylococcus* aureus was found to be 200μg/ml for ursolic acid and 400μg/ml for 5-androstenediol, cathafuran B and moracin M. At 200μg/ml concentration cathafuran B and ursolic acid showed minimum bactericidal effect against *Pseudomonas aeruginosa* while it was 400μg/ml for 5-androstenediol and moracin M.

- *In silico* ADME-Toxicology studies were performed for each drug molecule by submitting the competent drug candidates to the molinspiration, admetSAR and molsoft server which also provided the information about solubility, permeability, and drug-likeness of each drug molecule along with Pharmacokokininetic and pharmacodynamic properties like GPCR ligand, ion channel modulator, protease inhibitor, kinase inhibitor, enzyme inhibitor, nuclear receptor inhibitor, aqueous solubility, TPSA, blood brain barrier, human intestinal absorption, Caco-2 permeability, AMES toxicity, carcinogenicity and acute oral toxicity. Rule of 5 was employed for the screening of the drug molecules and the results revealed that all the drug candidates satisfied the rule of five except cathafuran B (6.8) and ursolic acid (6.7) where violation was found only in terms of ClogP. The results of drug likeliness prediction revealed that the compound moracin M has shown the highest drug likeliness score of 1.24 when compared to 5-androstenediol (0.40), cathafuran B (0.15) and ursolic acid (0.65). Further, results of in silico ADME-toxicity studies revealed that, all the four compounds were found to be non-toxic, non-carcinogenic and very soluble in aqueous solvent.

- *In silico* pharmacology involves the preparation of ligand and target molecules. The protein structural files were fetched out from protein data bank. The structure of the active pocket was predicted using ligplot and the residues forming the active pocket were identified. Protein–ligand interactive visualization and analysis was carried out in Pymol viewer 1.5.4. Automated docking using Lamarckian genetic algorithm, implemented in the program AutoDock4.2, was used to assess the orientation of the ligand molecules bound in the binding site of different target molecules. Among the four compounds tested, 5-androstenediol showed efficient binding in terms of binding energy with glucosamine-6-phosphate synthase (-9.01), cathafuran B with Cox II (-11.71), and GABA receptor (-11.96), ursolic acid with P38 MAP Kinase (-9.97), GSK 3-β (-10.96) and p450 14-alpha-sterol
demethylase (-11.12) and moracin M with β- tubulin (-10.09kJ/mol).

- The present research programme generated interesting results in terms of health benefits of mulberry by documenting biological activity for multifarious pharmacological parameters tested viz. anticancer, anti-inflammatory, wound healing, CNS depressant, anthelmintic and analgesic properties in addition to antioxidant and antimicrobial activities and establishes the fact that, the mulberry species possess important secondary metabolites which has contributed to the manifestation of their bioactivity and validates the folk claims of therapeutic benefits of Mulberry. The study lead to the isolation of four bioactive compounds viz. 5-Androstenediol, Cathafuran B, Ursolic acid and Moracin M. The isolated compounds were analyzed for in silico toxicological and pharmacological studies in connection with the identification of lead molecule. The study also established that these therapeutic benefits are variable with respect to species.

- Even though Morus alba was found to be superior for most of the pharmacological activities studied, it is interesting to note that Morus laevigata was proved to be ideal material in terms of anticancer effects which therefore warrants detailed in vitro and in vivo studies to identify specific bioactive molecule/s and there upon deduce mechanism based validation. The study also supports several of the health benefits of Morus and extends it to two different nearly unexplored species viz. Morus serrata and Morus laevigata. Since the work is carried out with a comparative outlook could therefore form the basis for the identification and development of highly health-supporting mulberry species and varieties. These results, demonstrating superior phytochemical traits of mulberries, may also provide a basis for planning breeding strategies as well as selecting cultivars with high phytochemical profiles and antioxidant capacities as functional foods for consumers. The present study acts as a prelude for efforts in exploitation of health benefits features of mulberry in addition to its role as a forage/feed in sericulture industry. Any experiments targeting a single pharmacological parameter utilizing the appropriate compound will be highly rewarding.

*****