VI. Summary
1. 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.

2. The objective of the present study is to explore the medicinal potentiality of the plant, viz. *Anogeissus latifolia* Roxb. It is a medium sized deciduous tree belonging to the family Combretaceae and it is commonly known as *Dhava*. It attains height of about 30-40 feet. The bark is effective in anemic conditions, urinary discharges and piles. Stem bark is astringent, haemostatic, constipating, depurative and useful in diarrhea, dysuria, cough, colic, liver complaints, snakebite, skin diseases and vitiated conditions of *kapha* and *vata*. Leaf decoction is reported to be effective in epileptic fits. Gum is used as tonic and generally consumed after delivery. Pawara tribes of Satpura hills, use the gum with a cup of water or milk during early morning for lactation. The plant proved to possess wound healing, anti-fungal, antioxidant and antiulcer activities.

It is evident that the plant is used for various ailments in folklore system and such medicinal claims of the plants are largely remain unexplored warranting systematic pharmacological evaluation. Therefore, in the present research programme the plant is subjected to experimentation for following studies viz. pharmacognosy, phytochemistry, pharmacological screening for hepatoprotective, antihyperglycemic, hypolipidemic, anti-inflammatory, analgesic and anthelmintic activities.
3. Leaves and stem were subjected to pharmacognostic studies. Leaves are alternate or subopposite, elliptic or oblong-elliptic, obtuse or very often shortly cuspidate, glabrous when fully grown, pale dull glaucous-green, midrib prominent, pink; main nerves 6-10 pairs, arching prominent on the lower side, the veins between them reticulate; petioles 6-13 mm long.

Transverse section of the *Anogeissus latifolia* leaf shows a typical dicotyledonous leaf structure having upper and lower epidermis with cuticle, the upper epidermis is multilayered showing the xerophytic character. In the centre upper portion of the midrib just below the epidermis a group of cells which gives strength by having thick end wall and a group of cells just above the lower epidermis gives rise to strengthening, these cells constitute collenchyma. The vascular tissues like xylem and phloem surrounded by bordered sheath. The type of stomata was found to be Anomocytic or Ranunculus stomata due to irregular arrangement of subsidiary cells. Leaf surface data for *Anogeissus latifolia* were 07.70, 12.53, 05.66, 05.30 and 03.58 for stomatal number, stomatal index, palisade ratio, vein islet number and vein termination number respectively.

The microscopy of stem shows secondary growth in which outermost layer form the periderma. Just below the periderma many layered zone viz. secondary cortex and phloem are seen. Vascular bundles are conjoint collateral and open. Parenchymatous pith is very small in the centre.

Moisture content of *Anogeissus latifolia* was found to be 13.4 and 11% for leaf and stem samples respectively. Powder of *Anogeissus latifolia* leaf and stem showed extractive values of 4.76, 0.98, 10.9, 16.91 and 0.79, 0.2, 9.33, 8.78% for petroleum ether, chloroform, ethanol and aqueous extracts respectively. The total, acid insoluble and water-soluble ash values were 11, 2.85, 4.5 and 10.9, 0.65 and 0.9 % for leaf and stem respectively.
4. The powdered bark and leaves of the plant was collected and extracted with different solvents *viz.* petroleum ether, chloroform and methanol. From 2 Kg powdered leaves and bark of *Anogeissus latifolia*, the yield of petroleum ether, chloroform and methanolic extracts were 95.2, 20.0 and 238 g and 15.8, 4.00 and 176 g respectively.

5. All the dried extracts were subjected to qualitative chemical tests. Phytochemical analysis of petroleum ether extract of both leaf and bark extracts of *Anogeissus latifolia* gave positive result for triterpenoids tannins, steroids, and cardiac glycosides. Triterpenoids, steroids and tannins were present in both leaf and bark extracts of chloroform whereas bark of *Anogeissus latifolia* showed additional flavonoids. In case of methanolic extract, both leaf and bark showed the presence of flavonoids, tannins, cardiac glycosides, triterpenoids, carbohydrates, proteins and steroids. However only the methanolic bark revealed the presence of alkaloids.

6. Since the qualitative analysis revealed the presence of several important secondary metabolites in extracts, further isolation of compounds and pharmacological screening has been carried out. The shade dried powdered leaf of the plant was subjected to Soxhlet extraction with different solvents from petroleum ether, chloroform, acetone, ethyl acetate and Methanol. The methanol fraction was chromatographed over silica gel column, eluted with 100% chloroform and with increasing concentrations of ethyl acetate in chloroform. The collected elutent from column chromatography was monitored by TLC, fractions with similar TLC patterns was combined to yield single spot PV4001. The latter was purified further by preparative TLC in chloroform and ethyl acetate which resulted in white coloured compound and crystallized in a desiccator.
The shade dried powdered bark of *Anogeissus latifolia* was extracted with methanol. The concentrated extract was dissolved in water and successively extracted with hexane, ethyl acetate and 1-BuOH. The butanol extract was evaporated under vacuum to yield flavonoidal fraction, which gave positive Shinoda test. The extract was subjected to purification over silica gel column using chloroform:acetone:formic acid as eluent, which yielded the compound PV4002.

The isolated compounds were further subjected to melting point, Mass, IR and Proton NMR spectral analysis for structural elucidation. The spectral analysis of PV4001 was identified as Gallic acid and PV4002 as Myricetin.

7. The various extracts of the plant was subjected to various pharmacological screening. Albino mice were used for acute toxicity studies and Albino Wistar rats were used for the pharmacological screening. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted was approved by the Institutional Animal Ethical Committee (IAEC) of National College of Pharmacy (Registration Number 144/ 1999/ CPCSEA/ NCP/ IAEC/ CLEAR/ P.COL./ 01/06/2007-08), Shivamogga, Karnataka, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The 1/10th of LD₅₀ was selected as the therapeutic dose for the evaluation of pharmacological activities. Maximum tolerated dose was chosen as effective doses for hypolipidemic study. In experimental studies each group comprised of six animals.

8. All the experimental data were expressed as Mean ± S.E. for six rats in each group. Statistical comparisons were performed by one-way ANOVA followed by Tukey’s post-test using Graph Pad Prism software version 4.0, USA.
9. For evaluating hepatoprotective activity nine groups of animals were used for the study. The animals of Group I served as the normal control and received the vehicle for 14 days. Group II – IX received CCl₄ for 10 days. The standard drug Silymarin was administered to Group III and Group IV-IX was treated with various extracts for 14 days. On 14th day of extract/ Silymarin/ vehicle treatment, the blood serum was subjected to analysis of total protein, total bilirubin, Aspartate aminotranferase (AST), Alanine aminotranferase (ALT) and Alkaline phosphatase (ALP) activity. Further, all the animals were sacrificed and livers were excised for histopathological studies.

Animals treated with single oral dose of CCl₄, developed significant liver damage as evident from significant increase in serum activities of SGPT (ALT), SGOT (AST), direct bilirubin concentrations and decrease in total protein compared to normal control rats. Oral administration of reference standard silymarin and various test extracts of Anogeissus latifolia at various doses daily for 13 days on 14th day CCl₄ treated rats exhibited significant reduction in serum activities of SGPT, SGOT, total and direct bilirubin concentration, whereas significant increase in serum total protein concentration compared to CCl₄ treated group. Methanolic extract of both leaf and bark have considerably protected the toxic effects of CCl₄ on liver.

The liver of normal and treated animals were sectioned and stained with haematoxylin and eosin. Degeneration and necrotic damage produced by CCl₄ was observed microscopically. The liver sections of both the normal and treated animals treated rats showed a marked protection in CCl₄ induced liver damage with evident reduction in necrosis and fatty infiltration compared to CCl₄ treated group.

10. The antihyperglycemic model was studied in streptozotocin induced hyperglycemic models of rats. The albino rats were kept fasting for 24 hrs and thereafter diabetes was induced by intraperitoneal injection of
streptozotocin. The rats with blood sugar level above 300mg/dl as well as exhibiting polydipsia, polyurea and polyphagea were selected for the experiment. The experimental animals were divided into nine groups. The drug administration was done orally daily a single dose up to seven days. The blood samples were withdrawn and the blood samples were analyzed for blood glucose levels by one touch profile method using Gluco-Meter. The results obtained revealed that diabetic control has showed increase in blood glucose level from 1\textsuperscript{st} hour onwards in an increasing trend with maximum glucose level recorded on 7\textsuperscript{th} day. Among the bark extracts highest blood glucose lowering activity was noticed in methanol followed by petroleum ether and chloroform. Similar effect was noticed in leaf extracts wherein methanol exhibited potent effect whereas chloroform and petroleum ether exhibited more or less similar effects. From the data, it is also evident that among the two sources of extracts, bark proved to be more effective when compared to extracts from leaf in terms of antihyperglycemic activity.

11. Gum ghatti of \textit{Anogeissus latifolia} was used to study the effect on serum lipid profile. The animals were divided in to six groups. Rats were made hyperlipidemic by the oral administration of cholesterol along with cholic acid in coconut oil for 20 days. The rats were then given drug treatment for 10 days. During these 10 days all the groups also received cholesterol in the same dose as earlier. On 30\textsuperscript{th} day blood samples were withdrawn from rats, after an overnight fasting by retro orbital sinus puncture under anesthesia. Serum total cholesterol, triglyceride and HDL were determined on 10\textsuperscript{th} day after treatment of gum ghatti using commercially available enzymatic kit. The results revealed that feeding of atherogenic diet increased the serum total cholesterol, triglyceride and decreased serum HDL-cholesterol level when compared to normal group. Administration of 250mg/kg of gum ghatti has shown significant decrease in only serum triglyceride level whereas in case
of 500 mg/kg, significant decrease has occurred both in the levels of total cholesterol and triglyceride. In case of 750mg/kg, significant decrease has occurred both in the level of total cholesterol, triglyceride and significant elevation in the HDL level. Thus compared to all the three concentrations 750mg/kg has shown efficient and comprehensive lipid lowering activity.

12. For anti-inflammatory activity, acute inflammatory condition is induced in the animals by Carrageenan injection. The animals were divided into eight groups. Group I received only vehicle while group II served as standard. Group III to VIII were treated with sequential extracts of leaf and bark of *Anogeissus latifolia* at different doses. The initial paw volume was measured plethysmographically before carrageenan injection. Later an inflammatory edema was induced in the left hind paw by injecting carrageenan solution in the plantar tissue of the paw of all the animals. The relative increase in paw volume was measured in control, standard and treated group at 60, 120 and 180 min after carrageenan injection. Administration of carrageenan to the control group of rats showed rise in paw volume and standard drug Indomethacin treated group showed a significant reduction in paw edema volume at different time intervals viz. 1, 2 and 3h. Chloroform leaf extracts exhibited significant reduction whereas, the reduction in paw volume was more prominent for methanol leaf extracts by recording lower mean values and percent inhibition at all the time intervals under study. Similarly bark extracts also exhibited a significant anti-inflammatory activity by reducing the paw volume and percentage inhibition for chloroform and methanol extracts. The latter manifested potent anti-inflammatory effect when compared to chloroform extract. It is evident from the data that the reduction in inflammation is positively correlated with duration wherein highest reduction in paw volume was observed at 3h. Further, results also revealed
that clear cut superiority of bark over leaf for anti-inflammatory activity in terms of both mean values as well as inhibition percentage.

13. Analgesic activity was carried out by tail flick method. The basal reaction time to radiant heat source was taken by placing the tip of the tail on the radiant heat source. The rats were divided into 8 groups. The reaction time was recorded at 0, 30, 60, 90 and 120 minutes. Rats treated with petroleum ether of leaf recorded significant increase in reaction time over the control by exhibiting 5.76±0.21, 5.99±0.05 sec; chloroform leaf extract 6.02±0.09 and 6.36±0.11 sec at 90 and 120 min while leaf methanol extract 4.21±0.15, 5.52±0.09, 6.39±0.11 and 7.32±0.07 sec at 30, 60, 90 and 120 min respectively. Among bark extracts, petroleum ether and chloroform extracts elevated the reaction time significantly at 60, 90 and 120 min time interval whereas methanol bark extract showed analgesic effect at 30, 60, 90 and 120 min indicating potent analgesic effect. Standard drug paracetamol recorded the reaction time of 2.93±0.13, 4.62±0.10, 5.98±0.04, 6.93±0.28 and 7.45±0.17 sec at different time intervals. However, it was noted that the effect of various extracts are more prominent in later phases of treatment and activity was shown in the order of petroleum ether leaf< petroleum ether bark< chloroform leaf< chloroform bark< methanol leaf< methanol bark. Further, the bark extracts of corresponding solvents proved to be effective in terms of analgesic property compared to leaf extracts.

14. The anthelmintic activity was evaluated on Indian adult earthworm *Pherithima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being. Thirty two groups each containing of 6 earthworms were released into 25ml of desired formulation. Each group was treated with one of the following, vehicle, albendazole and petroleum ether, chloroform, methanolic extracts of bark and leaf of *Anogeissus latifolia* in normal saline containing 1% Tween-20.
Observations were made for the time taken to cause paralysis and death time of individual worms. The results reveal that all the extracts of the leaf and bark possess dose dependent anthelmintic activity for the two parameters studied viz. paralysis and time taken to death and the effects comparable to standard drug albendazole on earthworms. For paralysis, among leaf extracts petroleum ether has exhibited maximum reduction in time followed by chloroform and methanol and the values are statistically significant compared to standard. Among bark extracts, chloroform has shown the maximum reduction in time followed by petroleum ether and methanol.

In regard to the time taken to death, both bark and leaf extracts have shown reduction in time required for majority of concentration tested. Among leaf extract, petroleum ether at all the concentrations exhibited reduction in time required than the standard while, chloroform has shown significant reduction at all the concentrations except at 10mg/ml. However, the methanol exhibited significant reduction at two higher concentrations viz, at 40mg/ml and 50mg/ml. Among bark extracts chloroform and petroleum ether recorded time reduction range of 6.20±0.54 to 4.82±0.18 and 7.80±0.17 to 6.30±0.27 respectively proving potent anthelmintic activity at all the concentrations tested followed by methanol which has shown lesser time (8.93 ± 0.26) than standard at 50mg/ml. From the data it is evident that among the two sources of extracts bark proved to be more effective when compared to leaf in terms of anthelmintic activity irrespective of solvents for the parameters studied.

15. The present scientific study on the plant *Anogeissus latifolia* establishes the potential medicinal values and justifies some of its ethano-medicinal claims of many folklore systems. The phytochemical investigation in the plant led to the isolation of two compounds viz. Gallic acid and Myricetin. The systematic pharmacological screening for hepatoprotective, antihyperglycemic, hypolipidemic, anti-inflammatory, analgesic, and
anthelmintic activities and the positive results obtained evidence the presence of important secondary metabolites of pharmaceutical importance in these plants which could therefore form the basis for the development of new drugs. An extensive pharmacological study involving pure compounds is therefore highly encouraging and rewarding.