1. SUMMARY AND CONCLUSION

The thesis entitled Phytochemical Investigation and Hepatoprotective Activity of Herbal Formulation. The herbal Formulation prepared from *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. leaves extracts deals with pharmacognostical, phytochemical and pharmacological screening of the traditionaly used *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. leaves medicinal plant (in Latur). The literature reveled that only fragmentary information was available on this plant species regarding pharmacognosy, phytochemistry and pharmacological activity by any other researchers. This study was designed first time for the identification, screening of pharmacological activities and isolation of unexplored compound inorder to establish its folklore claims. Plants are becoming potential source for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance and as a prelude to this, it becomes necessary to examine the various pharmacognostical characters of the plant before further investigation. In pharmacognostical studies the macroscopy, microscopy, histochemical studies, physico-chemical constants and inorganic mineral analysis were carried out. Pharmacognostical standards obtained during the observation are valuable tools for the identification of the plant material.

Morphological study had provided a characteristic identity of morphological characters of *Caesalpinia bonduc* L. was observed and the leaves is having pale green colour, odour is characteristic, taste is bitter, size of the leaves is having 2-3.8 by 1.3-2.2 and surface of the leaves is Elliptic- oblong. The macroscopic characters of *Mangifera indica* L. was observed and the Leaves is having dark green colour, odour is characteristic, taste is acride, size of the leaves is 25 cm long and 8 cm wide and surface of the leaves is lanceolate-elliptical, at the boths end pointed. The macroscopic characters of *Ricinus communis* L. was observed and the Leaves was having colour is lightaly green, odour is mild, taste is Astringent as well as salty, size of the leaves was observed as alternative also observed longpetiolare and stipulate, the leaves have palmately lobed and also have 7 lobes or more and surface of the leaves was lanceolated and zigzag margin and at the both end having pointed.

The various distinguishing features of the plant observed through anatomical studies were dried leaves of *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. were prepared course powder separately. Then, the powder were investigated for their microscopic characteristic. The microscopical characteristics of *Caesalpinia bonduc* Linn. which are shown
covering trichomes with having the trichomes attached to epidermis and diacytic stomata, lignified xylem and calcium oxalate crystal shown prism shape. The microscopical characteristics of *Mangifera indica* Linn. which are shown covering trichomes attached to epidermis and paracytic stomata, lignified bordered xylem vessels. The microscopical characteristics of *Ricinus communis* Linn. which are shown covering trichomes and paracytic stomata with covering trichomes, lignified scalariform xylem vessel and non-lignified fibres.

The investigation of phytochemical has been done by chemical tests and using some chemical reagents have been done and it showed the presence of glycoside, flavonoid, tannins, alkaloids and proteins. Various physico-chemical parameters such as ash values, total ash value, acid insoluble ash value, alcoholic extractive values, aqueous extractive value, loss on drying, crude fibre content and foaming index were found to substantiate its standard values. Any significant deviation in the percentage of any parameters reported in this work may indicate adulteration or substitution in the drug. Presence of fluorescence analysis is also a part of diagnostic tool for the presence of chromophore in the particular species.

The pharmacognostical details evolved from the present study would help to fix up the standards for *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. in relation to its identification, authentication and differentiation from other related species and adultrants. This is first report on the pharmacognostical standardisation on the leaves of *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L.

In Phytochemical evaluation various extracts were prepared and studied for qualitative chemical analysis, TLC and HPTLC finger print analysis. The qualitative preliminary phytochemical analysis was performed to detect the nature of the phyto-constituent and their presence in powder and various extracts. The alcoholic and aqueous extracts showed the presence of contain flavanoids, glycosides, proteins, saponins, alkaloids and carbohydrates.

Qualitative chromatographic analysis (TLC) was performed for the identification of different components in the extracts qualitatively. The HPTLC finger print of various extracts were also studied. HPTLC was scanned at 280 nm with the best solvent to detect the maximum number of components and peak abundance qualitatively. HPTLC fingerprint is one of the versatile tool for qualitative and quantitative analysis of active constituents. It is also a diagnostic method to find out the adulterants and to check the purity.
The prepared tablets (ALF-1, ALF-2, AQF-1 and AQF-2) were evaluated for various evaluation parameters such as general appearance, hardness, friability, disintegration & dissolution. The prepared tablets were having the spherical brown colour with smooth surface. All prepared of tablets were having good quality with having better hardness, friability & weight variation. The all form of the tablets formulated with the help of starch paste (10% w/v) as disintegrating agent & binder shown disintegration within 50 min.

The herbal formulations were studied for acute oral toxicity study as per OECD/OCDE guidelines. As per the results, ALF-1, ALF-2, AQF-1 and AQF-2 failed to show any signs of toxicity up to 4500 mg/kg body weight therefore the LD_{50} cutoff values of these herbal formulation were taken to consideration at 5000 mg/kg body weight. The ALF-1, ALF-2, AQF-1 and AQF-2 were screened for the hepatoprotective activity. In the present study, CCl_{4} was used as hepatotoxic agent. It was established that hepatotoxicity by CCl_{4} was due to enzymatic activation to release NAPQI in free state, which in turn disrupt the structure and function of lipid and protein macromolecules in the membrane of the cell organelles.

After the treatment with CCl_{4} there was significant in serum GPT, GOT, ALP and serum Bilirubin levels as compared to control group. The hepatoprotective activities of all extracts were compared with CCl_{4} drug and control group. The said measurements of SGPT, SGOT, ALP and serum Bilirubin levels were recorded in the histopathological observation of rat liver tissue.

The results were obtained from the pharmacological screening have led to conclusion that ALF-2 and AQF-2 shown prominent hepatoprotective activity, from parameters studied i.e. enzymes levels estimated and histopathological studies.

The ALF-2 and AQF-2 formulation were showed decrease in enzyme activity of SGPT, SGOT, ALP and Bilirubin which was shown to be induced of the microsomal enzymes. Thus, hepatoprotective action of this drug were because of its ability to induced microsomal enzymes, thereby accelerating the excretion of CCl_{4} or could be due to inhibition of lipid peroxidation induced by CCl_{4}. The hepatoprotective activity was shown to the combined effect of sterols and flavonoide.

Further studies were focused on structural activity relationship of phytoconstituents isolated from the alcoholic extracts of *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. The isolated compound which are obtained from alcoholic extracts used for the
This scientific study revealed the efficacy of the drug and it would definitely have wide scope in future. Hence, the leaves *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L. can be recommended therapeutically for the investigated medicinal claims. These observations will stimulate further research in the field of phytochemistry and also in the clinical application of phytochemical constituents of *Caesalpinia bonduc* L., *Mangifera indica* L., and *Ricinus communis* L.

**Recommendation:**

I am recommended that Mr. Maheshwar G. Hogade done his work in my college laboratory. When the First time we are meeting and I had described the scheme of thesis topic and I had given general outline of the thesis topic he can work on. During the communication he
asked good questions and appeared intelligent. Then, he visited to traditional practicener and he has collected the material and research papers related to the thesis topic and read all papers and book carefully. He did the independently all work I did not ask him to do this. When second time he meet me all work had done, and I was quite impressed at his great and independence. During that summer, Mr.Maheshwar G.Hogade represented the ability to himself with motivation and enthusiasm. He had spend many long hours and he worked as hard and become my best student. He has collected the all data which are related to the thesis topic. He has done hard work for the collection of plants leaves which were required for the research work. The research work included collection of leaves, determination of foreign material, macroscopical, microscopical, ash value, extractive value, loss on drying, dentification of chemical constituent with the help of chemical test, isolation of compound, determination of acute oral toxicity, preparation of hearbal formulation. Mr. Maheshwar G.hogade did the succesfully work in each one of these areas. He has best skills were excellent. In Sometimes take long time for the testing required long time because some equipment are not working properly and calibration of equipment. He has first person did the long time spend in lab in the morning and the last to leave in the evening.

In research lab there were lot of staff members did the work in same lab that was rather prickly person who had face many problems with students in the past. Mr. Maheshwar G Hogade had to interact with this staff member in order to get his project done. Mr. Maheshwar G Hogade was able to find a problem and interact with staff person and solved the problem. During the summer vacation he has worked continuously and our other staff member also done the work due the Mr. Maheshwar G Hogade.

He vigorously to read the literature himself and created the interesting hypotheses. We met about every other week, and at continually meetings he discussed me about his work and information that was new to me. At the time of finished his work he was shown or discussed me to scientific papers that were related to his work of thesis study that I had not seen previously he had prepared good manuscript of his thesis work. Mr. Maheshwar G Hogade also discussed the important problem with me and I had not given solution then, he has ability to find out the solution. Some time the instrumentation system having problems during his study his experiment. Mr. Maheshwar G Hogade spent a time and solved the troubleshooting the system. Mr. Maheshwar G Hogade is going to become corresponding author of his work manuscript that
he has published paper in journal. He followed rule and regulation during the preparation of manuscript. He had done his work successfully and that work level of motivation. So, finally I want to say that Mr. Maheshwar G Hogade is hard worker and motivational scholar and best student I have worked.

Scope for further works:

In present study I have done phytochemical investigation and hepatoprotective activity of herbal formulation was done and the herbal formulations were shown significant activity. Even I have done thin layer chromatography and high performance thin layer chromatography and
identified the isolated compounds compaired with standared drug. After that have the best scope will do the further study.

- Identification of the isolated compound which are obtained from alcoholic extracts of Mangifera indica L., Ricinus communis L.& Caesalpinia bonduc L.using chromatographic technique (IR, UV AND NMR)

- The isolated compound which will be used for the Preparation of herbal formulation

- Further studies were focused on structural activity relationship of phytoconstituents isolated from the alcoholic extract.

- This scientific study revealed the efficacy of the drug and it would definitely have wide scope in future.

- These are will be chances prepare nanoparticals and will determine activity.

- After preparation of nanopartical from isolated compound and evaluate the hepatoprotective Activity against carbon tetrachloride induced hepatotoxicity.

- Histopathological studies of different dose of Hearbal Formulation.