5. CONCLUSION

Nature has been a source of medicinal plants for thousands of year and an impressive number of modern drugs have been isolated from natural sources. Several species of medicinal plant are used in the treatment of diabetes which affecting large number of people worldwide. Medicinal plants generated commercial demand for pharmacopoeial drugs and their products in India. Efforts have been made in recent years to introduce many of these drug plants to common people. The agronomical practices for growing few medicinal plants have been developed and there is now localized cultivation of these medicinal plants commercially in many part of our country. It is evident that many valuable herbal drugs have been discovered by knowing that a particular plant was used by the ancient folk healers for the treatment of some kind of ailments. Moreover, the medicinal plant wealth are our national heritage and it seems to be the first and foremost line of defence for the treatment of various diseases mostly tribal and rural communities and is a worth scientific study.

The urgent need of hour is the evolution of an action plan for spreading awareness about the value and importance of medicinal plants. In addition to curing various ailments, these plants will add to the national needs export potential. We have to link the indigenous traditional knowledge with modern technology. With the coming of chemical revolution, the medicinal plants, which were once used by the primitive folk healers and traditional medicine men, have found wide acceptance and a place of pride in the modern system of medicine. Their chemical examination revealed that they possess chemical compounds of great biological activity. Indeed, these plants are capable to cure some incurable diseases and also cure the diseases from root. People
suffering from chronic diseases and after loosing all hops from allopathic medicine, turn their eyes towards herbal medicine. The add advantage is that the medicinal plants are easily available, cheaper and without any side effect. Hence, the prime need is to make uses of medicinal plants for solving the health problem and major ailments of the people.

The word herbal is the symbol of safety in contrast to the synthetic ones, which have adverse effect on human health. With the development of civilization, people come to known more and more about the healing properties administration & duration. Herbal medicines are widely used by all section of the community, whether directly as folk remedies or the medicaments of the different indigenous system as well as in modern medicines. Herbal plants have global market worth about us $.62 billions per annum and Indian’s share is only 0.2%, which will grow up to about 15% in near future. According to the WHO, approximately 80% of the world’s population uses herbal drugs as part of their normal health care routine.

Herbal medicines represent the fasted growing segment to heal the various ailments. Possibly, herbal user desire to assume control over their own health care needs. Perhaps the large, impersonal health care system is unpatable to many and they turn to herbal medicines as an alternatives patient may feel alienated by increasingly busy physicians who have less time to spend with them, and they may turn to herbal drugs because they feel they can gain some control, obviously if peoples are going to use herbals as part of their health care routine, they must find out about the herbs and what they do. The natural drugs somehow contain the vital force that is going to improve their health.

This research work comprises of ‘Development of Standardisation Parameters of Guizotia abyssinica (L.f.) Cass. with Special Reference to its Pharmacological Approaches’. The parameter employed for this purpose include development of standardization parameters as per WHO Guidelines, successive extraction of plant material, preliminary phytochemical screening, certain in vitro biological and pharmacological activities along with formulation and evaluation of suitable dosage form.
Madhya Pradesh sustains a very rich traditional medicinal plant wealth and inherits unique plant and animal communities. *Guizotia abyssinica* (L.f.) Cass. belonging to family Asteraceae (Compositae), commonly known Niger, black seed (E) Ramtil, Kalatil (H). It is an erect, stout, branched annual herb, grown for its edible oil in many parts of Madhya Pradesh, India. During the course of present investigation an well documented questioner was prepared and the ethomedicinal claims and folk-lore uses of the plants were documented as described by various selected informants of the study area. It was concluded from present work that the herb is locally known as ramtil and various part such as root, leaves, stem, seed and seed oil were used in the treatment of various diseases viz., microbial infection, burns, cough, contraceptives, arthritis and syphilis by the various informants selected in different doses and duration along with specific method of preparation.

Further the status of the species was also recorded which conclude that the species is very rare and going to endangered in almost all the study sites, except budelkhand region where the species is still cultivated by the farmers therefore, there is urgent need of conservation of the species. Hence, it was concluded from the present wok that the selected species of *Guizotia* is very useful in the treatment of certain kind of disease and various ex and in-situ conservation strategies along with cultivation of species may be adopted to prevent the species from extinction. Hence, it was realised that the selected species have diversified medicinal uses as ascertain by its folk lore.

Various standardisation parameters which include macroscopy, anatomical studies, quantitative microscopy, physicochemical evaluation and fluorescence analysis of powdered drug were carried out.

The macroscopic studies of stem, leaves and seed were studied and the results are presented in chapter 4. The anatomical study of the stem revealed the presence of multicellular trichomes, oil glands, conjoint and collateral vascular bundle arranged in rings, stone cells and pith, while powder microscopy revealed the presence of xylem vessels, tracheids, starch grain, fibres and oil glands. The anatomical study of leaves revealed the presence of multicellular trichomes, stomata, chloroplast, conjoint and
collateral vascular bundle, while powder microscopy revealed the presence of xylem vessels, parenchyma, calcium oxalate crystals etc. Anatomy of seeds (Powder as well as T.S.) reveals the presence of fibres, epidermis, endodermis, parenchymatous cells hexagonal in shape having alternate arrangement, endosperm containing, starch grains, oil globules, lattice form of calcium oxalate crystals and testa. Hence, these anatomical studies can be used as a diagnostic tool for the correct identification of the species of *Guizotia* and aims at setting the anatomical standards to establish quality control parameter for the raw material. Therefore, these features are useful in detecting the adulterants if any in this plant and will lead to efficacy and purity of the selected plant. Hence, these findings will be helpful in the correct identification, identity and purity of the selected medicinal plant.

The quantitative microscopy of leaves revealed various leaf constants such as Stomatal number: Upper epidermis (2-5), Lower epidermis (4-9); Stomatal index: Upper epidermis (6.25-8.57), Lower epidermis (29.41-31.66); Pallisade ratio: Base:middle:apex (4.06:4.18:4.37) and Vein-islet number (18). These parameters are very much useful in developing the standards for the leaves.

The physicochemical evaluation of stem, leaves and seed of *Guizotia abyssinica* (L.f.) Cass. was carried out. In this study ash values (total ash, acid insoluble ash and water soluble ash), moisture content, swelling index and foreign organic matters were determined. The TA was found to be 13.8632, 10.2080 & 2.8632 % w/w; WSA was found to be 5.7690, 4.3294 & 1.2944% w/w; AIA was found to be 3.7542, 2.2526 & 0.5752 % w/w; MC was found to be 2.1684, 3.9372 & 2.7562 % w/w; SI was found to be 6.3340, 4.1684 & 1.8082 % w/w; FOM was found to be 1.2020, 0.8010 & 0.2022 % w/w; water soluble extractive value was found to be 18.7638, 27.7802 & 20.4694 % w/w; alcohol soluble extractive value was found to be 6.8902, 27.7802 & 9.1078 % w/w and pet. ether soluble extractive value was found to be 4.302, 8.3476 & 5.2854 % w/w of GAS, GAL & GASe.

The Fluorescence analysis of powdered drug of stem, leaves and seed powders of *Guizotia abyssinica* (L.f.) Cass. in various solvents were examined under ordinary light and UV light (short and long). The powder was also treated with various chemical
reagents viz., 5% FeCl₃, 1M H₂SO₄, dil. HNO₃, 5%NaOH, 5%NaOH + Water, 5% Iodine, conc. HNO₃, Ethanol and dil. HCl and the changes in colour were recorded and were presented in chapter 4.

Exhaustive successive extraction of the powdered plant material were carried by shade dried coarsely powder of stem, leaves and seed of *Guizotia abyssinica* (L.f.) Cass. with petroleum ether, chloroform, ethanol and water in a soxhlet apparatus. The percentage yield, color, nature and pH of the extract were recorded. The percentage of PEEGAS was found to be 2.33±0.19% w/w, CEGAS was 1.83±0.21% w/w, EEGAS was 2.66±0.39% w/w, AEGAS was 16.66±0.31 w/w, PEEGAL was 3.13±0.38 w/w, CEGAL was 1.43±0.24 w/w, EEGAL was 6.86±0.42 w/w, AEGAL was 29.33±0.24 w/w, PEEGASw was 15.13±0.42 w/w, CEGASw was 1.0±0.09 w/w, EEGASw was 0.83±0.09 w/w and AEGASw was 29.60±0.49 w/w respectively. Other parameters viz., color, nature and pH of extract were also recorded and were presented in table.

Preliminary phytochemical screening of pet. Ether, chloroform, ethanol and aqueous extract of stem, leaves and seed of *Guizotia abyssinica* (L.f.) Cass. were carried out which revealed the presence of various active phyto-constituents. EEGAS showed presence of alkaloids, protein, amino acid, fixed oil, fats, steroids, triterpenoids and waxes; AEGAS showed presence of carbohydrates, alkaloids, protein, amino acid, tannins, phenolic compounds, flavonoids, fixed oil, fats, steroids, triterpenoids and waxes; CEGAL showed presence of fixed oil and fats; EEGAL showed presence of carbohydrates, alkaloids, protein, amino acid, tannins, phenolic compounds, fixed oil, fats and waxes; AEGAL showed presence of carbohydrates, alkaloids, protein, amino acid, steroids and triterpenoids; PEEGASw showed presence of fixed oil, steroids and triterpenoids; CEGASw showed presence of fixed oil, steroids and triterpenoids; EEGASw showed presence of alkaloids, protein, amino acid, tannins, phenolic compounds, flavonoids, fixed oil, steroids, triterpenoids, fats and waxes and AEGASw showed presence of carbohydrates, alkaloids, protein, amino acid, flavonoids, fixed oil, steroids, triterpenoids, fats and waxes.

The dried seeds of *Guizotia abyssinica* (L.f.) Cass. was subjected to solvent extraction method and traditional method for extraction of fixed oil. The percentage
yields of fixed oil by solvent extraction method was found to be 27.96% v/w and by traditional method 30.38% v/w, the color of oil was yellow, taste was nutty and nature was Clear, excellent and slow-drying. The phytochemical screening revealed the presence of fixed oil, protein, amino acid and flavonoids. The fixed oil obtained was analysed for refractive index, optical rotation, specific gravity, saponification value, unsaponification matter, iodine value, acetyl value, acid value, ester value and R.M. value and the values obtained were 1.4684, +2.16°, 0.9202, 190.8, 0.5, 122.5, 22.7, 4, 186.7 and 1.2 respectively.

For the first time HPTLC profile for the ethanolic and aqueous extracts of *Guizotia abyssinica* (L.f.) Cass. leaves, seeds and stem were developed. A broad screening programme, covering the most important phytochemical group of compounds was developed on the basis of HPTLC. Chromatographic screening studies gave 6 to 10 spots of different extracts at selected mobile phases. Thus, it could be concluded that HPTLC can be used as a pharmacognostical tool in the pharmaceutical industry to identify medicinally important plant. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic. Present study also supported the view that the leaves and seeds of *Guizotia abyssinica* (L.f.) Cass. could be a potential source of natural antimicrobial and anti-inflammatory drugs. Further in near future the separation and characterization of the bioactive compound from the plants is also to be evaluated. The results obtained from qualitative evaluation of HPTLC fingerprint images will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of sample of *Guizotia abyssinica* (L.f.) Cass. can provide standard fingerprints with selected solvent system and can be used as a reference for the proper identification/authentication and quality control of the drug. The HPTLC fingerprint pattern developed with aqueous and ethanolic extract will be helpful in differentiating the species. This feature can be used to differentiate the authentic samples from that of market and adulterated drug.

The aqueous and ethanolic extracts of leaves and seeds of plant of *Guizotia abyssinica* (L.f.) Cass. were screened for acute toxicity study by OECD guideline no. 423 for determination of LD$_{50}$ and ED$_{50}$ No mortality in either extract was observed at
the dose of 5000 mg/kg, therefore, the LD$_{50}$ was 5000 mg/kg and ED$_{50}$ was 500 mg/kg. Hence, two doses of 100 and 200 mg were selected for further investigation.

The aqueous and ethanolic extracts of *Guizotia abyssinica* (L.f.) Cass. Syn. *G. oleifera* D.C., *Polymnia abyssinica* L.f., Suppl., *Verbesina sativa* Roxb., *Jaegeria abyssinica* Spr., leaves and seed were screened for antimicrobial activities against some pathogens viz., *Escherichia coli*, *Pseudomonas aeruginosa* *Staphylococcus aureus* and *Enterobacter faecalis*. Extracts were found to produce significant inhibition against all the pathogens. Ethanolic extract were observed to be more active than aqueous extract as compared to the standard drug viz., Cefitaxime & Amoxycillin. The extract of various concentrations i.e., 10, 20, 40 and 60 µg/ml were evaluated for Anti-microbial activity and were compared to standard drug.

The attempt was made to study *in vitro* anthelmintic activity of aqueous and ethanolic extract of leaves and seeds. The anthelmintic activities of the extract were determined and it was found that AAGASe possesses higher activity than other extract and found to be significant when compared with the standard drug.

The aqueous and ethanolic extracts of leaves and seeds of plant of *Guizotia abyssinica* (L.f.) Cass. were screened for acute toxicity study by OECD guideline no. 423 for determination of LD$_{50}$. The results showed that the aqueous and ethanolic extracts i.e., AEGAL, EEGAL, AEGASe and EEGASe were belonging to category-5(unclassified). Hence, LD$_{50}$ was 5000 mg/kg, therefore, ED$_{50}$ was 500 mg/kg.

The aqueous and ethanolic extract of *Guizotia abyssinica* leaves and seed were evaluated for anti-inflammatory activity in animal models and the results were summarized in chapter 4. The result obtained indicates that the extract found to have significant (P < 0.01) anti inflammatory activity in rats. The AEGAL and EEGAL at the test doses 100 and 200 mg/kg b.w. reduced the oedema induced by carrageenan by 42.68%, 47.56%, 35.97% and 39.63% respectively at 6 h, whereas the AEGASe and EEGASe at the test doses 100 and 200 mg/kg b.w. reduced the oedema induced by carrageenan by 47.56%, 53.04%, 37.19% and 45.73% as compared to standard drug which showed 63.41% of inhibition as compared to the control group.
As per the results of pharmacological screening on anthelmintic, anti-microbial and anti-inflammatory activities two extracts viz., AEGAL & AEGASe were selected for formulation of herbal tablets. The $\lambda_{max}$ (maximum wavelength) were determined for both the extract and was found to be 216 nm and 252 nm respectively. Standard curve were plotted and regression coefficient were determined. FTIR was carried out to study the extract excipients compatibility and data revealed that no any in compatibility was there between extract and excipients. FT-IR spectra did not show the presence of any additional peaks for new functional groups. The major peaks of the extract remained unchanged in the mixtures. These results suggest absence of any chemical interaction between the extract and the excipients used in tablet formulations. Peak values and functional groups for both the extracts were presented in table which revealed the presence of various functional groups in the extracts.

The various composition of the prepared herbal tablet formulations containing AEGAL and AEGASe are given in chapter 4. The micromeritic properties were determined for all the physical mixtures. The results of angle of repose, Carr’s Index and Hausner ratio indicated that the powder mixtures possess good flow properties and good packing ability. The physical property of tablet was determined and the results of the uniformity of weight, hardness, drug content and friability of the tablets were given in chapter 4. All the samples of the test product complied with the official requirements of uniformity of weight. The drug content was found to be close to 100% in all formulations. The low friability indicates that the herbal tablets are compact and hard. On the basis of results obtained after evaluation of herbal tablets two formulations i.e., F7 of AEGAL and F7 of AEGASe were optimized and selected for the stability studies for three month duration at $25^0C/60\% RH$, $30^0C/60\% RH$ and $40^0C/75\% RH$. The results are reproducible, even on tablets that had been stored for 3 months at $25^0C$, $30^0C$ & $40^0C$ at relative humidity.

From the results of stability studies it was revealed that the F7 of AEGASe having promising results at $30^0C/60\% RH$, followed by F7 of AEGAL at same condition than that of other two conditions selected for the investigation.
Hence, it was concluded from the present work that the herbal tablet containing AEGASe has promising effect than that of herbal tablet containing AEGAL and serve as a potential drug in the treatment of microbial and inflammatory conditions.

Further research and investigation in *Guizotia abyssinica* (L.f.) Cass., in the isolation and characterization of novel compounds from the extracts will lead for the development of formulation of various other dosage forms viz., novel drug delivery system i.e., ethosomes, phytosomes, niosomes etc, which may be used in the treatment of many diseases and associated disorders and may results in the development of some safe effective and herbal preparations.