6.1 PHYTOCHEMICAL SCREENING

6.1.1. Selection, collection and authentication of plant/plant material

The different fresh plant parts viz., leaves of *Gymnema sylvestre*, fruits of *Momordica charantia*, rhizomes of *Curcuma longa*, seeds of *Eugenia jambolana* and fruits of *Emblica officinalis* were collected in the months Jan 2014 to March 2014 from the in and around local areas of Bhopal District of M.P. and identified & authenticated by Dr Zia Ul Hasan, Professor, Head Dept. of Botany, Safia College of science, Bhopal, M.P., dated 22/04/2014. M.P. and were deposited in Laboratory, Voucher specimen No. 470/Bot/Safia /2014 for leaves of *Gymnema sylvestre*, 469/Bot/Safia /2014 for fruits of *Emblica officinalis*, 468/Bot/Safia /2014 for fruits of *Momordica charantia*, 467/Bo/Safia /2014 for seeds of *Eugenia jambolana* and 466/Bot/Safia /2014 for rhizomes of *Curcuma longa*.

6.1.2. Pharmacognostical Evaluation

6.1.2.1. Morphological features

The macroscopy /morphology of different parts of the selected plant such as color, odor, size, shape, taste, surface characters and fractures were carried out. (Dutta AC , 1964)

6.1.2.2. Anatomy and histological studies

The specimens of the proposed study were collected, care was taken to select healthy part and for normal organs. Then required samples of organ were fixed in FAA (formalin-5ml+ Acetic acid 5ml+ 70% Ethyl alcohol-90ml). Free hand transverse sections of fresh stem were taken, cleaned in chloral hydrate solution with gentle warming, stained with phloroglucinol and concentrated hydrochloric acid. They were mounted on slide in glycerine and studied under microscope.
Microphotographs of sections were documented using microscope with camera, Nikon (14 mp). Descriptive terms of the anatomical features are as given in the standard anatomy book (Sardana S, 2007, Jackson BP 2005). The figure and details are given in the results.

6.1.3. Physicochemical Evaluation

The dried parts were subjected to standard procedure for the determination of various physicochemical parameters (The Ayurvedic Pharmacopoeia of India 2001, World Health Organization 1998).

6.1.3.1. Determination of foreign organic matter (FOM)

Accurately weighed 100 g of the drug sample and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6X). Separate and weigh it and the percentage present was calculate.

6.1.3.2. Determination of moisture content (LOD)

Place about 10 g of drug (without preliminary drying) after accurately weighing in a tared evaporating dish and kept in oven at 105\(^\circ\) C for 5 hours and weigh. The percentage loss on drying with reference to the air dried drug was calculated.

6.1.3.3. Determination of ash value

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash

Accurately about 3 gms of air dried powder was weighed in a tared silica crucible and incinerated at a temperature not exceeding 450\(^\circ\)C until free from carbon,
cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

**Acid insoluble ash**

The ash obtained in the above method was boiled for 5 minutes with 25ml of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

**Water soluble ash**

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

**6.1.3.4. Determination of swelling index**

Swelling index is determined for the presence of mucilage in the seeds. Accurately weigh 1 g of the seed and placed in 150 ml measuring cylinder, add 50 ml of distilled water and kept aside for 24 hours with occasional shaking. The volume occupied by the seeds after 24 hours of wetting was measured.

**6.1.3.5. Determination of extractive value**

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.
Cold maceration

Place about 4.0g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerate with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay. Calculate the content of extractable matter in mg per g of air dried material. For ethanol-soluble extractable matter, use the concentration of solvent specified in the test procedure for the plant material concerned; for water-soluble extractable matter, use water as the solvent.

6.1.4. Extraction of Plant Material (Harborne, J.B. 1998)

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered (250gms) were loaded in Soxhlet apparatus and was extracted with ethanol until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined.

6.1.5. Preliminary Phytochemical Screening of Extract

The ethanolic extract obtained after extraction were subjected for phytochemical screening to determine the presence of various phytochemical present in the extracts. The standard procedure were adopted to perform the study. (Kokate C.K. 1997, Divakar MC. 2002)
6.1.5.1. Tests for carbohydrates

**Molisch’s test**

To the Sample 2-3 drops of 1% alcoholic - naphthol solution and 2 ml of conc. sulphuric acid was added along the sides of the test tube. Appearance of purple to violet ring at the junction of two liquids shows the presence of carbohydrates.

**Fehling test**

To the sample add fehling reagent, appearance of brick red precipitate shows presence of carbohydrates.

6.1.5.2. Test for glycosides

**Legal’s test**

To the sample add 1 ml of pyridine and few drops of sodium nitroprusside solutions and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

**Borntrager’s test**

Sample was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

**Baljet’s test**

To the sample add picric acid, orange color shows presence of glycosides.

6.1.5.3. Test for alkaloids

A small portion of the sample was stirred separately with few drops of dilute hydrochloric acid and was tested with various reagents for the presence of alkaloids. The reagents are
Dragendorff’s reagent - Reddish brown precipitates
Wagner’s reagent - Reddish brown precipitates
Mayer’s reagent - Cream color precipitates
Hager’s reagent - Yellow color precipitates

6.1.5.4. Test for proteins and free amino acids

Small quantities of the sample was dissolved in few ml of water and treated with following reagents.

- Million’s reagent: Appearance of red color shows the Presence of protein and free amino acid.
- Ninhydrin reagent: Appearance of purple color shows the Presence of Proteins and free amino acids
- Biuret’s test: Equal volumes of 5% sodium hydroxide solution & 1% copper sulphate solution was added. Appearance of pink or purple color shows the presence of proteins and amino acids.

6.1.5.5. Test for tannins and phenolic compounds

A small quantity of the sample was taken separately in water and test for the presence of phenol compounds and tannins was carried out with the following reagents.

- Dilute Ferric chloride solution (5%) - Blue color or green color
- 10% lead acetate solution - White precipitates
6.1.5.6. Test for flavonoids

**Alkaline reagent test**

To the test solution add few drops of magnesium hydroxide solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates presence of flavonoids.

**Shinoda’s test**

Small quantities of the sample was dissolved in alcohol, to them piece of magnesium followed by conc. hydrochloric acid drop wise added and heated. Appearance of pink, crimson red, green to blue color shows the presence of flavonoids.

6.1.5.7. Tests for fixed oils and fats

**Spot test**

A small quantity of sample was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

**Saponification test**

Few drops of 0.5 N alcoholic potassium hydroxide were added to a small quantity of sample along with a drop of phenolphthlein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

6.1.5.8. Tests for steroids and triterpenoids

**Libermann-burchard test**

Treat the sample with few drops of acetic anhydride, boil and cool. Then add con. sulphuric acid from the side of test tube, brown ring is formed at the junction two
layers and upper layer turns green which shows presence of steroids and formation of deep red colour indicates presence of triterpenoid.

**Salkowski test**

Treat the sample with few drop of conc. sulphuric acid, red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

**6.1.5.9. Test for mucilage and gums**

- Small quantities of sample was added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitates was dried in oil and examined for its swelling property for the presence of gum and mucilage.

- To the sample add ruthenium red solution, pink color shows presence of mucilage.

**6.1.5.10. Test for waxes**

To the test solution add alcoholic alkali solution, waxes get saponified.