FORMULATION AND EVALUATION OF POLYHERBAL HAEMATINIC CAPSULES

A Dissertation submitted to

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In partial fulfilment of the requirements for the award of the degree of

MASTER OF PHARMACY IN

PHARMACOGNOSY

Submitted by

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DEDICATED TO MY BELOVED PARENTS

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INTRODUCTION

1. Introduction

"THE LORD HAS CREATED MEDICINES OUT OF THE EARTH AND HE WHO IS WISE WILL NOT ABHOR THEM"

-ECCLESIATES, CHAPTER-38, VERSE-4.

Over 120 medicinal plants are found in the bible including aloe, rue, madder, frankincense, myrrh and marigold. This shows the belief people had in herbs.[1]

"NOWHERE ON EARTH IS THERE ANY CREATURE WHICH IS IMMORTAL. YET ALTHOUGH DEATH IS UNAVOIDABLE A MAN MAY AVOID MANY DISEASES. HEALTH BRINGS HAPPINESS."

-SARNGADHARA SAMHITA, SECTION-1.5.

One can avoid disease, when the food what he consumes is itself exhibits rich medicinal value. This is possible when he/she includes our traditional herbs in their diet.[2]

World population is becoming 5 billion and with this rate of growth it is likely to touch 7 billion by 2020. Global estimates indicate that over three forth of the population cannot afford the products of the western pharmaceuticxal industry and have to rely upon the use f traditional medicines, which are mainly derived from plants. As a part f strategy to reduce financial burden on the developing countries which spend 40-50 of their total health budget on drugs , WHO currently encourages recommends and promotes the inclusion of herbal drug in National health care programmes as such drugs are easily available at aprice within the reach of a commaon man and as such are time tested and thus considered to be much safer than modern synthetic drugs.

Plants have curative measure for all ailments but man has to discover it out. Herbs act in almost magical and astonishing ways. However , the ultimate objective of their use is that they should interact directly with our body chemistry. Their active constituents must be absorbed in the blood stream, they circulate, to influence our whole system.[3]

The following statistics gives an alarming picture about anaemia:

- 56% of Indian women suffer fromanaemia[4]
- 4 out of 5 children in the age of 6-35 months suffer fromanaemia[5]
- 20% of maternal deaths are due to anaemia and anaemia indirectly contributes to another 40% of maternal deaths and maternal mortality is staggeringly high at 454 per every 100000 livebirths.[6]
- Anaemia spares none; it affects both adults and children of bothsex.

ANAEMIA[7]

Decrease below normal of haemoglobin concentration or RBC count or hematocrit (PCV).

Reduction of total circulating red cell mass below normal limits.

Decrease in oxygen carrying which leads to hypoxia .

Anemia is characterized by decreased oxygen carrying capacity of blood

WHO criteria for anemia

Adult males: Haemoglobin less than 13g /dl

Adult female: Haemoglobin less than 12g/dl

Grading of anemia

Mild: Haemoglobin 9.1 -10.5g/dl

Moderate: Haemoglobin 6.0-9.0g/dl

Severe: Haemoglobin<6.0g/dl

CLASSIFICATION

I.MORPHOLOGICAL CLASSIFICATION:

It is based on

a) Red cell size(Normocytic,microcytic,macrocytic)
b) Degree of haemoglobinization:(normochromic,hypocromic)

Table 1.1 Types of anemia

Type of anemia	**Microcytic,hypochromic**	**Normocytic, normochromic**	**Macrocytic**
Size of RBC	Smaller than normal	Normal	Larger than normal
Central pallor in RBC	More than 1/3	Normal	Normal
Mean corpuscular volume(MCV)	Reduced(<80fL)	Normal(82-98fL)	Increased(>100fL)
MCHC	Reduced(<30g/dl)	Normal(31-36g/dl)	Normal(31-36g/dl)
Examples	Iron deficiency anemia, Thalassemia	During blood loss, anemia of chronic disease	Deficiency of vitamin B12 and folic acid
Morphology of RBC			

II.ETIOLOGICAL CLASSIFICATION

i) Decrease in RBC production.

ii) Increase in RBC destruction.

iii) Blood loss.

1.IMPAIRED RED CELL PRODUCTION

1.1.a)Disturbed Proliferation and Maturation of Erythroblasts

►Defective DNA synthesis

- Megaloblastic Anemia due to deficiency or impaired utilization of vitamin B12 or Folic acid.

- Anemia of renal failure due to deficiency of erythropoietin
- Anemia of chronic disease due to iron sequestration and relative erythropoietin deficiency
- Anemia of endocrine disorders

b) Defective Haemoglobin Synthesis

►Defective heme synthesis

- Iron deficiency , Sideroblastic anemia

►Defective Globin Synthesis

- Thalassemias

1.2) Marrow replacement

a) Primary hepmatopoietic neoplasm

- Acute Leukemia
- Mylelodysplastic Syndrome

1.3)Marrow Infiltraton

a) Metastatic Neoplasm

1.4) Disturbed proliferation and differentiation of stem cells.

a) .Aplastic anemia, pure red cell aplasia.

2.INCREASED RED CELL DESTRUCTION(Hemolytic anemias)

2.1) Intrinsic (Intra corpuscular) Abnormalities

a) Hereditary

►membrane abnormalities

- Spherocytosis
- Elliptocytosis

►Enzyme deficiencies

- Glucose-6-Phosphate dehydrogenase
- Pyruvate Kinase

► Disorders of hemoglobin Synthesis

►Defective Globin Synthesis

- Thalassemias

- ►Structurally abnormal globin Synthesis
  - Sickle Cell Anemia

b)Acquired

- ►Membrane defects
  - Paroxysmal Noutunal hemoglobinuria

2.2)Extrinsic(Extra corpuscular Abnormalities)

a)Antibody – Mediated

- ► Isohemagglutinins- transfusion reaction
  - Rh disease of New born

- ► Auto antibodies
  - Idiopathic
  - Drug associated
  - Systemic Lupus erythematous

b)Mechanical trauma to RBCs

- ► micro angiopathic hemolytic anemia
  - Disseminated intra vascular coagulation.

c)Infections

- Malaria.

3)BLOOD LOSS

- ►Acute: Trauma
- ► Chronic: Leisons of GIT, Gynecological disturbances.

RED CELL INDICES

- ✓ Red cell indices are useful in Morphological characterization and diagnosis of anemias.
- ✓ They can be calculated automatically or using specialized instruments.
- ✓ The four significant Red Cell Indices are:
  - ★ MCV-Mean Corpuscular Volume.
  - ★ MCH- Mean Corpuscular Haemoglobin.
  - ★ MCHC- Mean Corpuscular Haemoglobin Concentration.

- RDW- Red Cell Distribution width.

I. MCV-Mean Corpuscular Volume

It is defined as the average number of Red Blood Cells.

It is expressed in femolitres(fL)

This proves very useful in diagnosis

Normal range: 82-98 fL

$$MCV = \frac{PCV \times 1000}{\text{RBC Count (millions)}} = 0.45X1000/5 = 90fL.$$

II. MCH- Mean Corpuscular Haemoglobin

It is defined as the amount of Haemoglobin per Red Blood Cell

It is expressed in Picograms

It has limited value in diagnosis

Normal range: 27-32pg

$$MCH = \frac{\text{Haemoglobin (in g/L)}}{\text{RBC (in millions/}\mu\text{L)}} = \frac{15X10}{5} = 30pg$$

III. MCHC- Mean Corpuscular Haemoglobin Concentration

It is defined as the average number of Haemoglobin in RBC taking volume into consideration.

It si expressed in g/dL.

Normal range: 31-35g/dL.

$$MCHC = \frac{\text{Haemoglobin(in g/dL)}}{\text{Packed Cell Volume}} = \frac{15}{0.45} = 33g/dL$$

IV. RDW- Red Cell Distribution width.

It is a quantitative measure of anisocytosis.

Normal range = 11.5% - 14.5%

Normal RDW indicates RBC uniform in size .

Increased RDW tells that RBC are heterogenous in size and shape

Early Iron deficiency Anemia: RDW increased with low MCV

Thalassemia : RDW normal with low MCV

$$RDW = \frac{\text{Standard deviation}}{\text{MCV}} \times 100$$

Increased RDW >15% is the early sign of Iron deficiency anemia.

SIGNS AND SYMPTOMS OF ANAEMIA[8]

Skin

Skin becomes pale, buccal and pharyngeal mucous membrane shows prominent paleness in conjunctivae, lips, ear lobes, palm and nail bed. Skin becomes thin and dry losing its elasticity, early greyness, thinning and loss of hair can occur. Nails become brittle and easily breakable.

Cardiovascular system

Anaemia can cause tachycardia and increased velocity of blood flow and cardiac murmurs are observed.

RESPIRATION

Force and rate of respiration is increased sometimes it may lead to breathlessness and dyspnoea. Oxygen haemoglobin dissociation curve is shifted to right.

DIGESTION

The common symptoms of anaemia are anorexia, nausea, vomiting, abdominal discomfort and constipation.

METABOLISM

Basal metabolic rate is increased in severe anaemia.

KIDNEY

Albuminuria is common and renal function is disturbed.

REPRODUCTIVE SYSTEM

In females, the menstrual cycle is disturbed. Menorrhagia, oligomenorrhagia or amenorrhea is accompanied withanaemia.

NEUROMUSCULAR SYSTEM

Headache, lack of concentration, restlessness, irritability, drowsiness, dizziness or vertigo especially when standing, increased sensitivity to cold and fainting sensation are common symptoms. Muscles become weak and there is lack of energy andfatigue.

Agents used to treat anaemia[9]

Iron

Iron is stored in intestinal mucosal cells as ferritin. Iron deficiency resultsfrom a negative iron balance due to depletion of iron stores, acute chronic blood loss and insufficient intake during periods of accelerated growth in children, heavy menstruation and/or inadequate intake of iron in diet. Supplementation with ferrous sulphate is required to correct the deficiency.

Adverse effect

GI disturbance caused by local irritation are the most common adverse effects.

Folic acid

Folic acid is used in treating deficiency states that arise from inadequate levels of vitamin. Folate deficiency may be caused by increased demand (during pregnancy and lactation), poor absorption caused by pathology of small intestine, alcoholism or treatment with drugs that are dihydro folate reductase inhibitors. Folic acid is normally well absorbed in jejunum. Oral folic acid administration isnontoxic.

Adverse effects

Rare hypersensitivity reactions to parenteral injections have been reported.

Cyanocobalamine

Vitamin B12 deficiency results from low dietary levels, poor absorption of the vitamin due to failure of gastric parietal cells to produce intrinsic factor or loss of receptor activity for the intestinal uptake of the vitamin. Vit.B12 supplementation is required in the large oral doses, sublingually or once a month by the parenteral route.

Adverse effects

Rarely nausea, vomiting and rhinitis have been reported with the intra nasal formulation.

Erythropoietin and Darbepoetin

Erythropoietin is a glycoprotein normally secreted by the kidneys that regulates red blood cell proliferation and differentiation in bone marrow. Human erythropoietin is produced by rDNA technology, is effective in the treatment of anaemia caused by renal disease, anaemia associated with HIV and in some cancer patients. Darbepoetin is a long acting version of erythropoietin that has two carbohydrate chains which improves its biological activity and half-life is increased about three times that of erythropoietin and it shows decreasedclearance.

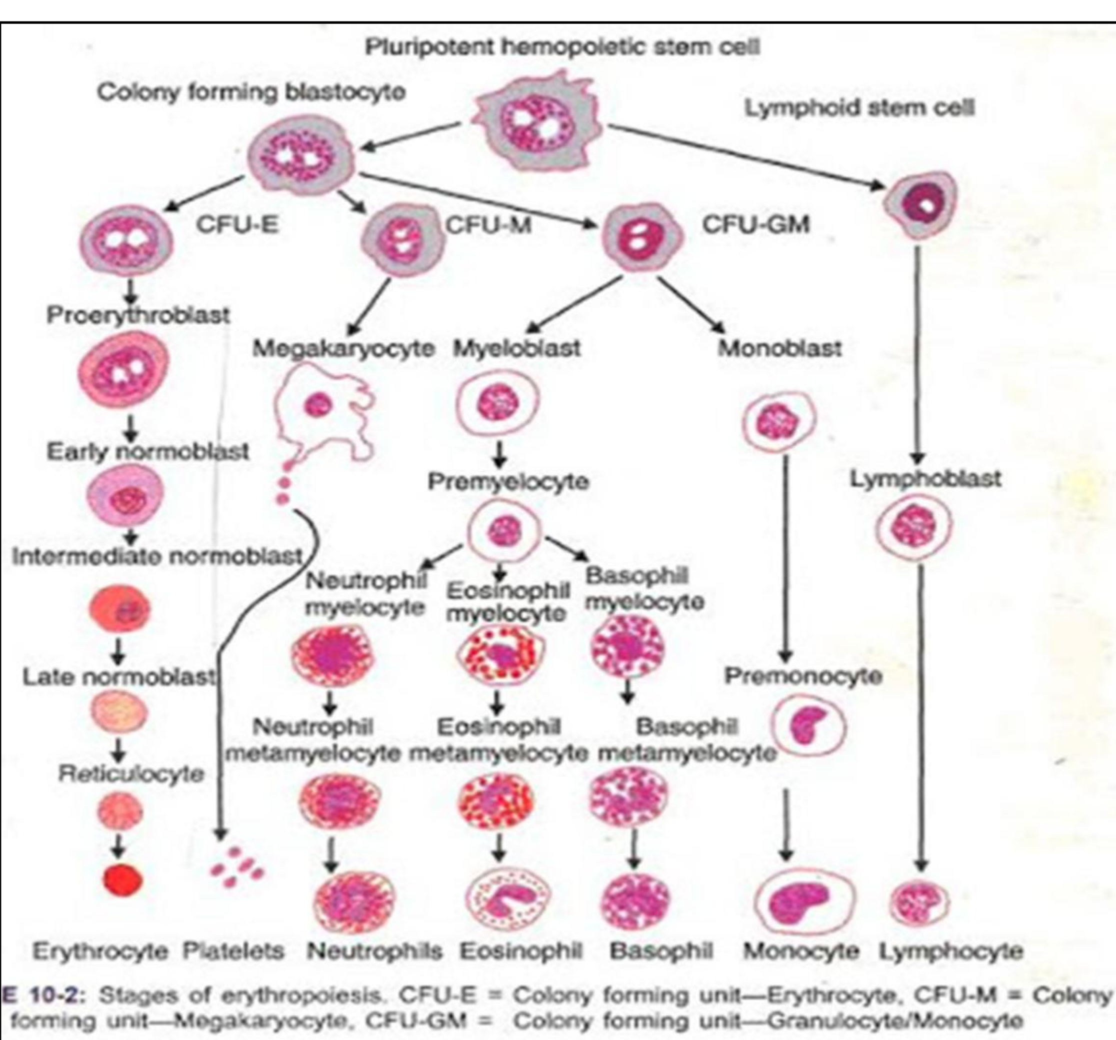
Adverse effects

Cause elevated blood pressure and arthralgia in some cases. When erythropoietin is used to target haemoglobin concentration more than 12g/dl serious cardiovascular events such as (thrombosis and severe hypertension), increased risk of death, shortened time of tumour progression and decreased survival has been observed.

HAEMOPOIESIS[10]

Haemopoiesis or hematopoiesis is the process of the origin, development and maturation of all the blood cells.

Fig.1.1 HAEMOPOISIS



E 10-2: Stages of erythropoiesis. CFU-E = Colony forming unit—Erythrocyte, CFU-M = Colony forming unit—Megakaryocyte, CFU-GM = Colony forming unit—Granulocyte/Monocyte

BLOOD

Blood is the connective tissue in fluid form . it is considered as the fluid of life because it carries oxygen from the lungs to all parsts of the body and carbon di oxide from all parts of the body to the lungs. It is also known as fluid of gowth because it carries nutritive substance from the digestive system and the hormones from the endocrine gland to all the tissues . The blood is also called as the fluid of health because it protects the body against the disease and get rid of the waste products and unwanted substances by transporting them to the excretory organs like kidneys.

BLOOD CELLS

THREE TPYES OF CELLS PRESENT IN BLOOD

- Red blood cells or Erythrocytes
- White blood cells or Leukocytes
- Platelets or Thrombocytes

PROPERTIES OF BLOOD

COLOUR

Blood is red in colour . Arterial blood is scarlet red because it contains more oxygen and venous blood is purple red because of more carbon dioxide.

VOLUME

The average volume of blood in a normal adult is 5 litres. In new born baby it is 450ml. It increases during growth and reaches 5 litres at the time of puberty. In female it is slightly less and is about 4.5 litres. It is about 8 % of the body weight in a normal young healthy adult weighing about 70Kg.

pH

Blood is slightly alkaline and its Ph in normal conditions is 7.4.

SPECIFIC GRAVITY

- The specific gravity of Total Blood: 1.052 to 1. 061
- The specific gravity of Blood cells: 1.092 to 1.101

- The specific gravity of Plasma : 1.022 to 1.026

VISCOSITY

Blood is five times more viscous than water. It is mainly due to red blood cells and plasma proteins.

FUNCTIONS OF BLOOD

- **NUTRIENT FUNCTION:**

Nutrient substance like glucose,amino acids lipids and vitamins derived from digested food are absorbed from gastrointestinal tract and carried by blood to different parts of the body for growth and production of energy.

- **RESPIRATORY FUNCTION:**

Blood carries oxygen from alveoli of lungs to different tissues and carbon dioxide from tissues to alveoli.

- **EXCRETORY FUNCTION:**

Waste products formed from metabolic activities are removed by blood and carried out to the excretory organs like kidneys, skin, liver, etc. for excretion.

- **TRANSPORT OF HORMONES AND ENZYMES:**

Endocrine hormones are released directly into blood .The blood transport these hormones to their target organs or tissues. Blood also transport enzymes.

- **REGULATION OF WATER BALANCE:**

Water content of the blood is freely interchangeable with interstitial fluid. This helps in regulation of water content of the body

- **REGULATION OF ACID BASE BALANCE:**

The plasma proteins and haemoglobin acts as buffers and help in regulation of acid base balance.

- **REGULATION OF BODY TEMPERATURE:**

Blood is responsible for maintaining the thermo regulatory mechanism in the body since the blood has high specific heat.

STORAGE FUNCTION:

Water, glucose , proteins , sodium , potassium are constantly required by the tissue . And these are taken from the blood during the conditions like starvation, fluid loss , electrolyte loss , etc.

DEFENSIVE FUNCTION:

Neutrophils and monocytes in the blood engulf the bacteria by phagocytosis . Lymphoctyes are involved in development of immunity. Eosinophils are responsible for detoxification disintegraton and removal of foreign proteins.

RED BLOOD CELLS

Red blood cells are non nucleated formed elements of blood. Red blood cells are known as erythrocytes. The red colour of RBC is due to the presence of the colouring pigment called haemoglobin . RBC plays a vital role in transport of respiratory gases. RBCs are larger in numbers compared to the other two cells namely white blood cells and platelets.

NORMAL VALUE

The RBC count ranges between 4to5.5 million/cu mm of blood. In adult males it is 5 million/cu mm and in adult females it is 4.5 million/cu mm.

NORMAL SHAPE

RBCs are disc shaped [bioconcave] , the central portion is thinner and periphery is thicker . The bioconcave contour of RBCs has some mechanical and functional advantages.

NORMAL SIZE

Diameter : 7.2μ(6.9-7.4 μ)

Surface area : 120 sq μ

Volume : 85-90 cu μ

HAEMOGLOBIN

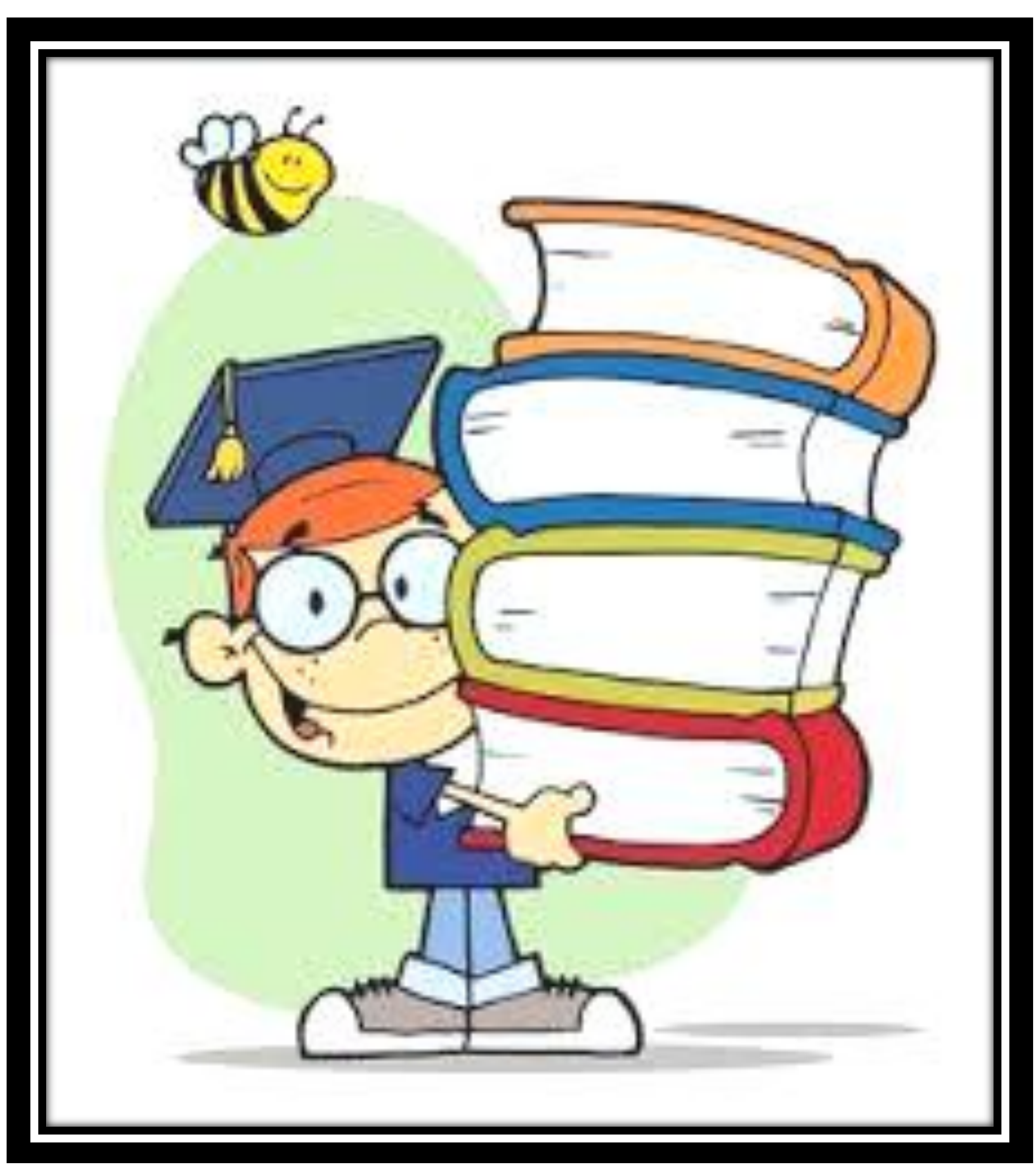
Haemoglobin [Hb] is the iron containing colouring matter of RBC. It is a chromoprotein forming 95% of dry weight of RBC and 30 to 34% of wet weight. The function of haemoglobin is to carry the respiratory gases, and carbon di oxide . It also acts as buffer.

NORMAL HAEMOGLOBIN

Average haemoglobin content in blood is 14 to 16g/dl. However, the value varies depending upon the age and the sex of the individual.

STRUCTURE OF HAEMOGLOBIN

Haemoglobin is a conjugated protein .It consists of a protein combined with an iron containing pigment . The protein part is globin and the iron containing pigment is heme. Iron is converted to stable ferric [Fe^{+++}] state which is attached to N-of the each pyrole ring and N-of the globin molecule.



REVIEW OF LITERATURE

2. Review of Literature

Karwasra Ret al studied the **Safety assessment and attenuation of cisplatin induced nephrotoxicity by tuberous roots of Boerhaaviadiffusa**. The findings of the work indicated that Boerhaavia diffusa was effective in mitigating cisplatin-induced nephrotoxicity in rats and thus, for that the acute and sub-acute toxicity studies conducted to evaluate the safety profile of Boerhaavia diffusa. The no-observed adverse effect level of tuberous roots of Boerhaavia diffusa root extract was 1000 mg/kg.

Saraswati S et al worked on the title,**Punarnavine, an alkaloid from Boerhaavia diffusa exhibits anti-angiogenic activity** via downregulation of VEGF in vitro and in vivo.they reported that Punarnavine, a quinolizidine alkaloid isolated from Boerhaavia diffusa possess analgesic, anti-inflammatory, hepato-protective, immunomodulatory and anti-proliferative properties.

Bairwa K et al, stated that**Rotenoids from Boerhaavia diffusa function as potential anti-inflammatory agents.**The study concluded that the rotenoids exhibited significant anti-inflammatory activity (56.6% at 50 mg/kg) when evaluated in an in vivo carrageenan-induced rat paw model.

Manjrekar PN et al, have done a **Comparative studies of the immunomodulatory activity of Tinospora cordifolia and Tinospora sinensis**. The study was carried out in the following manner. The water and ethanol extracts of stems of Tinospora cordifolia and T. sinensis inhibited immunosuppression produced by cyclophosphamide. Ethanol extracts of stems of both the plants inhibit cyclophosphamide-induced anemia.

Maurya et al worked on the**Constituents of Tinospora sinensis and their antileishmania activity against Leishmania donovani.**Their work proved that there were some compounds isolated from the plants exhibited the highest in vitro antileishmanial activity against Leishmania donovani promastigotes and intracellular amastigotes.

Layeeq Set al processed on **Clinical efficacy of Amalaki Rasayana in the management of Pandu (Iron deficiency anemia)**. They conducted a randomized open clinical trial. Iron deficient anemic patients (n = 25) having Hb <12g% in females and 13g% in males and S.Iron <50mg/dl were selected and divided into two groups. Group A was given 2 g of Amalaki Rasayana thrice a day with unequal quantity of honey and ghee for 45 days, while Group B was given 150 mg ferrous fumarate + 1500 mcg folic acid (standard control) once a day with water for 45 days. Assessment was done on the basis of relief in cardinal symptoms of Pandu and hematological parameters.it was reported that On hematological parameters statistically significant increase was found in mean corpuscular volume and mean corpuscular hemoglobin.

Tahir I et al, worked on the**Evaluation of phytochemicals, antioxidant activity and amelioration of pulmonary fibrosis with Phyllanthus emblica leaves**. They examined the antioxidant potential of a methanol extract of Phyllanthus emblica leaves (PELE) by in vitro methods as well as by an in vivo animal model, along with HPLC-DAD screening for phyto-constituents. It was reported that PELE exhibited an appreciable in vitro antioxidant activity and scavenged the DPPH radical ($IC_{50} = 39.73 \pm 2.12$ μg/mL) and nitric oxide ($IC_{50} = 39.14 \pm 2.31$ μg/mL) while for anti-lipid peroxidation moderate antioxidant activity was noticed.

Thenmozhi AJet al studied on the title,**Tannoid principles of Emblica officinalis attenuated aluminium chloride induced apoptosis** by suppressing oxidative stress and tau pathology via Akt/GSK-3βsignaling pathway. The work indicated the neuroprotective effect of tannoids principles of Emblica officinalis (EoT) against memory loss caused by aluminium chloride ($AlCl_3$) intoxication. It was concluded that administration of EoT nullified the cognitive deficits, biochemical abnormalities and apoptosis induced by $AlCl_3$ treatment. Moreover EoT prevents tau hyperphosphorylation by targeting the GSK-3β/Akt signaling pathway.

Karim NA et al insisted on the topic**Moringa oleifera Lam: Targeting Chemoprevention.** They poposed that Chemopreventive effects of M. oleifera are expected due to the existence of glucosinolate which is reported to have the ability to induce apoptosis in anticancer studies. They also highlighted the advantages of M. oleifera targeting chemoprevention where glucosinolates could help to slow the process of carcinogenesis through several molecular targets. It also includes inhibition of carcinogen activation and induction of carcinogen detoxification, anti-inflammatory, anti-tumor cell proliferation, induction of apoptosis and inhibition of tumor angiogenesis.

Surendra TV et. al., worked on the heading **RSM optimized Moringa oleifera peel extract for green synthesis of M. oleifera capped palladium nanoparticles with antibacterial and hemolytic property.** The work established that M. oleifera capped Pd nanoparticles were synthesized by microwave assisted methanolic extract of M. oleifera peel. The better optimized conditions for the extraction was found as 400W, 25mL of CH3OH at 65°C for 2min. They observed 61.66mg of extract yield from this method. Eco-friendly M. oleifera capped Pd NPs were synthesized using M. oleifera peel extract and confirmed using the different characterization techniques like UV- Vis spectroscopy, XRD, SEM and HR-TEM analysis. They found the size of the M.oleifera capped Pd NPs nanoparticles as 27±2nm and shape of the particles as spherical through the TEM analysis. M.oleifera capped Pd NPs exhibits good antibacterial activity against S. aureus (Staphylococcus aureus) and E. coli (Escherichia coli) bacterial strains and we found the zone inhibition as 0.6 and 0.7mm. The synthesized M. oleifera capped Pd NPs are screened for hemolytic activity and it proved the M. oleifera capped Pd NPs are non-toxic on RBCs cells.

Karthivashan G, et al worked on **The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model.** In the study, they evaluated the nephro-protective potential of Moringa oleifera leaf extract against N-Acetyl-p-Aminophenol, also known as acetaminophen. Moringa Oleifera leaf extract has demonstrated therapeutic effectiveness against acetaminophen induced nephrotoxicity through enhancement of the endogenous antioxidant system and a modulatory effect on specific inflammatory cytokines in kidney tissues.

Dungca NT proved the **Protective effect of the methanolic leaf extract of Eclipta alba** (L.) Hassk. (Asteraceae) against gentamicin-induced nephrotoxicity in Sprague Dawley rats. The extract protected the rat kidneys against gentamicin-induced renal tubular alterations and rises in blood urea nitrogen, serum creatinine, and microprotein levels. Lipid peroxidation and decrement in catalase levels were also ameliorated. The study revealed the protective effect of the methanolic leaf extract of E. alba and suggests that the probable mechanism for the nephroprotection by the extract may be due to its good radical scavenging activity and $Fe(3+)$ ion-reducing ability.

Arya et al studied on the title **Anti-breast tumor activity of Eclipta extract** in-vitro and in-vivo: novel evidence of endoplasmic reticulum specific localization of Hsp60 during apoptosis. They worked on the bio-guided fractionation of Eclipta alba extract and discovered that particularly the chloroform fraction of Ecliptaalba is selectively inducing cytotoxicity to breast cancer cells over non-tumorigenic breast epithelial cells. LC-MS approach identified that luteolin is mainly attributed for its anti-cancer activities. Moreover, oral administration of CFEA not only offers potential anti-breast cancer effects in-vivo but also mitigates tumor induced hepato-renal toxicity. Together, the study offered novel mechanistic insight into the CFEA mediated inhibition of breast cancer and may potentially open up new avenues for further translational research.

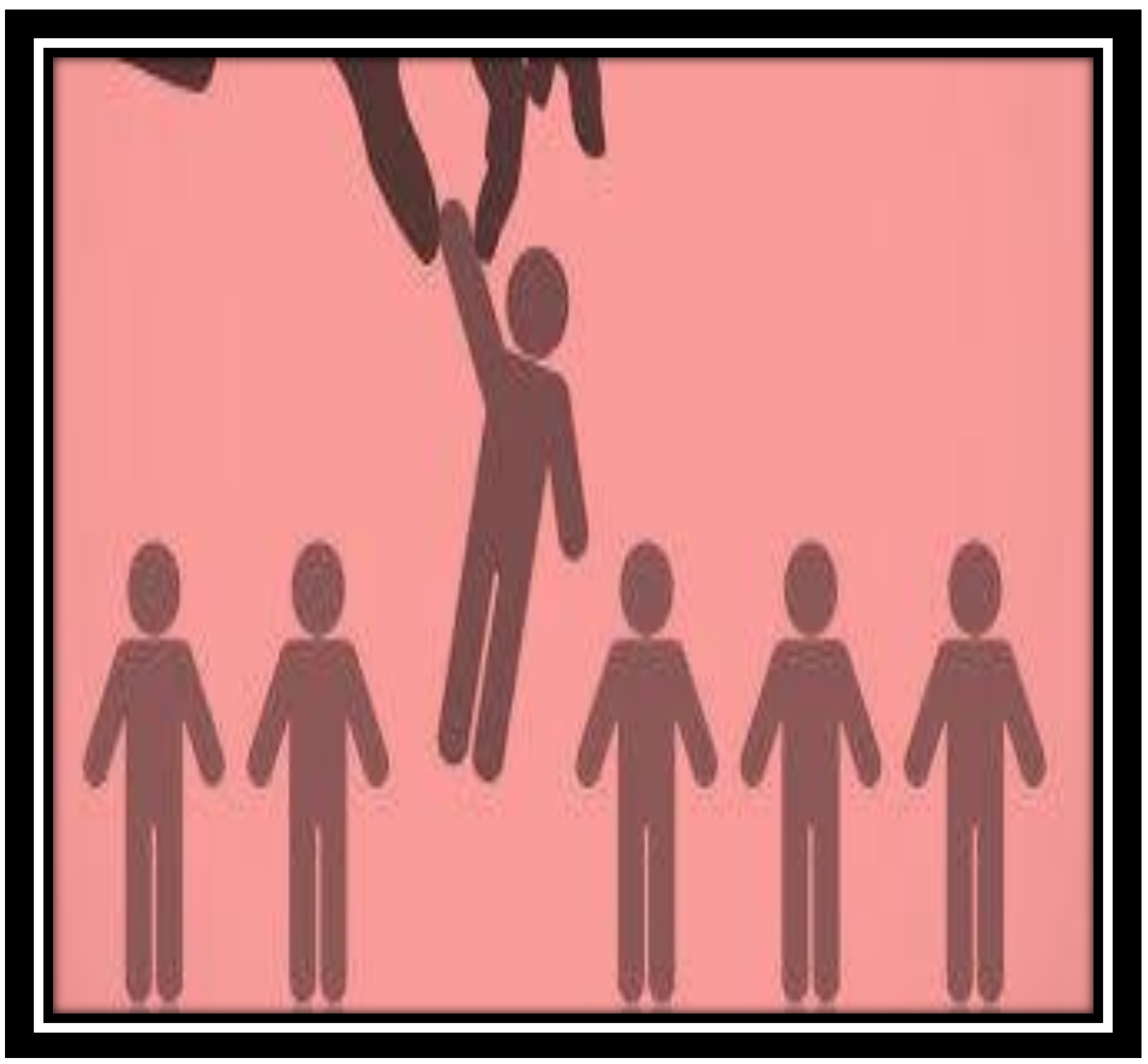
Mahar Set.al., studied on **Haemostatic effect of eclipta alba on albino rabbits.** In the present study ethanolic leaf extract of eclipta alba was evaluated for its haemostatic activity by their ability to affect the screening test or modify the experimentally induced prolongation of test time on the basis of its folklore claim in normal, aspirin and heparin treated rabbits. The parameters recorded were BT, CT, PT and APTT & platelet count in all the three groups. The study was carried out in 3 parts in different doses 200,400,600 & 800mg/kg. Part-A-study of the haemostatic effect of Ethanolic extract of E.alba (EEEA) in graded doses on normal rabbits. Part-B-study of EEEA in aspirin treated rabbits. Part-C-study of EEEA in heparin treated rabbits. Tranexamic acid was used as the standard drug. Eclipta alba demonstrated to have anti-

haemorrhagic activity against snake bite not affecting any coagulation factor. No significant effect on PT, APPT and platelet count was observed. EEEA exhibited definite haemostatic effect in the above 3 sets of study.

Molla SH et.al work revealed **anthelmintic effects of *M. koenigii* on *Haemonchus contortus*.**It was evident from their paralytic condition and/or death at eight hour post exposure in different concentrations (12.5, 25, 50 mg/ml) of aqueous and methanolic extracts which were dose-dependent. Aqueous and methanolic extracts of *M. koenigii* were found to have low percent inhibitory effect on egg hatching but showed significant anthelmintic activity.

16.Nollu.B et.al.,studied the effect of *M.koenigii* leaves in the inhibition of 26S proteasome and its apoptotic action *in vivo* in a xenograft tumor mouse model . Analysis of the extract revealed the presence of flavonoid compounds which act as proteasome inhibitors. The literature reported that : Quercetin, Apigenin, Kaempferol and Rutin; flavonoids present in the leaf extract, dose-dependently inhibited the endogenous 26S proteasome activity in MDA-MB-231 cells. Reduction in tumor growth was associated with a decrease in proteasomal enzyme activities in the treated groups.

Ramasamy et.al., reported that, **the hydro-alcoholic extract of Murraya koenigii leaves possesses significant anti-diarrheal activity** due to its inhibitory effect on gastrointestinal motility, making it useful for a wide number of gastrointestinal diseases.



RATIONALE FOR SELECTION

3. RATIONAL FOR SELECTION

The more technical we grow, the much we get to know about the immense importance of our Indigenous herbs in the treatment of various ailments. Being a student of this era, I was curious in finding out the hidden disorder or disease that needs to be much studied. One such disease, that is prevalent in many, irrespective of age (younger/adults/older) is anaemia**.**Having many inbuilt metabolism to deal with blood and blood loss i.e the menstrual cycle and pregnancy, female are most commonly affected by anaemia.

Herbs are gaining much importance in treating the diseases. The growing herbal market makes the polyherbal formulations an evoking trend! Developing therapeutic agents from natural products has renewed the world wide attention and stimulated new wave of research on the benefits of Herbal Medicine as an effective alternative therapy tool for various illness.When it comes to a polyherbal formulation, the herbs used in it should be popular. They would have gained much trust among the folk and at the same time be economical and affordable.
Taking into consideration the above parameters the following herbs were chosen.

- ★ *Boerhavia diffusa*(Roots)
- ★ *Eclipta prostrate* (Leaves)
- ★ *Murraya koenigii*(Leaves)
- ★ *Moringa oleifera (*Leaves)
- ★ *Phyllanthus embelica*(Fruit pulp)
- ★ *Tinospora sinensis*(Stem)

The hexa herbs chosen have many Etho-medicinal application and traditional claims.
They are employed in numerous ayurvedic formulations and are marketed successfully.
They all have the traditional claim to be helpful in aneamia. Giving them in a combination, would promisingly exhibit synergistic action.

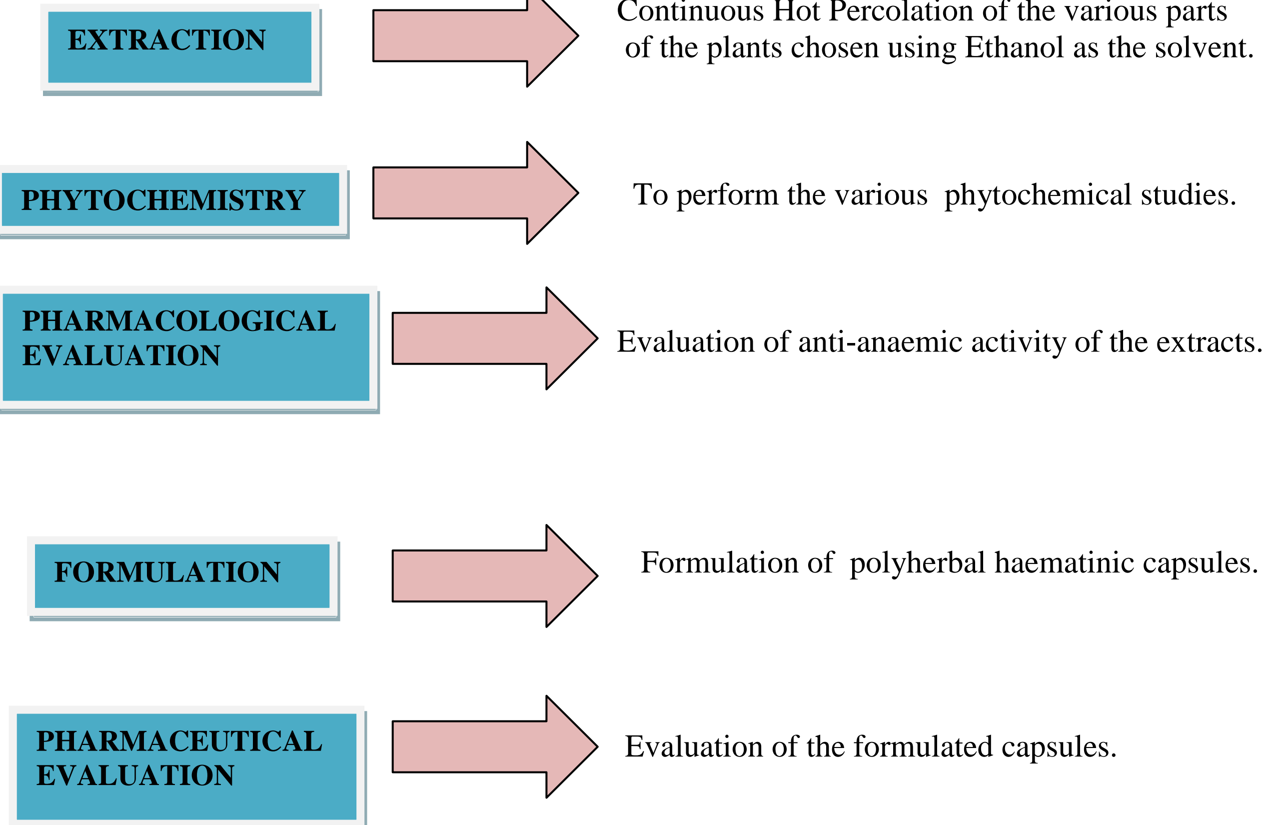
Thus the present study was designed completely, to formulate a polyherbal haematinic capsule and helpout the womenhood live a happy healthy life!

AIM AND OBJECTIVE

4. AIM AND OBJECTIVE

The aim of the present work is to develop a polyherbal haematinic capsules from the selected plant materials and evaluate the same.

The objectives are selection of the plant material followed by,



PLANTS PROFILE

5. PLANT PROFILE

Boerhavia diffusa [28,29]

A very variable, diffusely branched, pubescent or glabrous, prostrate herb, abundantly occurring as a weed throughout India.

Synonym: Spreading Hog weed

Fig.5.1*Boerhavia diffusa*

Common name: Punarnava

Vernacular names

Bengali: Gadhapurna, Punarnaba

Gujarati: Satodi

Hindi: Biskhafra

Kannada: Bala-vadikae

Malayalam: Thumizhazhma

Marathi: Raktavasu

Sanskrit: Punarnavam

Tamil: Mukku-rattai

Telugu: Atikamamidi

DISTRIBUTION

Found throughout India

Fig.5.2*Boerhavia diffusa*

PLANT TAXONOMY[30]

Kingdom :Plantae

Phylum :Angiosperms

Class :Eudicots

Order :Caryophyllales

Family :Nyctaginaceae

Genus : *Boerhavia*

Species : *diffusa*

PLANT DESCRIPTION

ROOT

Stock stout, fusiform,woody

STEMS

Creeping, often purplish, swollen at the nodes, upto 1.2m long

LEAVES

Long-petioled, ovate or oblong – cordate, entire or sinuate, usually whitish and smmoth beneath and rough green on upper surface.

FLOWERS

Red, pink or white, in small umbels arranged in axillary and terminal panicles

FRUITS

Ovate, oblong, pubescent, five-ribbed, viscid, glandular anthocarps

FLOWERING AND FRUITING

Throughout the year

TASTE

Bitter

Fig.5.3 ***Boerhavia diffusa***

Eclipta prostrata **[31,32]**

A small genus of herbs, distributed in the tropical and sub-tropical regions of the world. One species occurs in India.

Synonym : *Eclipta alba*

Fig.5.4 ***Eclipta alba***

Vernacular names

Bengali - Kesuti

Gujarati- Bhangra

Hindi - Bhangra

Kannada- Garagadasoppu

Malayalam- Kyonni

Marathi- Bhringuraja

Sanskrit- Bhringaraja, kesaraja

Tamil- Garuga, kayanthakara

Telugu- Galagara

Fig.5.5 ***Eclipta alba***

DISTRIBUTION

Distributed throughout India

HABITAT

Found in moist areas, waste places and road sides. The plant shows variations in habit in different habitats, in dry and rocky habitats, it reduces in size.

DESCRIPTION

An erect or prostrate, much branched, strigosely hirsuate, annual, often rooting at the nodes.

LEAVES

opposite, sessile, oblong, lanceolate, 1-4 inch long

FLOWERS

Head white, 0.25 to 0.35 inch in diameter

Fig.5.6 *Eclipta alba*

PLANT TAXONOMY[33]

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Genus: *Eclipta*

Species: *prostrata*

FLOWERING AND FRUITING

Throughout the year

TASTE

Bitter

ETHNO-MEDICINAL USE

ROOT

Snake bite, applied externally as antiseptic in ulcer, emetic and purgative.

LEAF

Fever , malaria, conjunctivitis, scorpion sting, gastric problem, skin disease, ulcer, dysentery, blood purifier, diarrhea, dysentery, wound, elephantiasis.

WHOLE PLANT

Anodyne and absorbent, rubbed on gums in tooth ache.

ETHNO-MEDICINAL USE

ROOT

Diarrhea, dysentery, diabetes

LEAF

Fever, headache, piles, joint pain, diabetes, gastric trouble.

STEM

Skin diseases, jaundice, stomachic, asthma, abdominal gripes, diphtheria, cough, leprosy and snake bite, joint diseases, fever, jaundice, dysentery , malaria, diarrhoea, ulcers, swelling.

WHOLE PLANT

Bone fracture, piles, fever, jaundice.

Moringaoleifera [34,35]

A small genus of quick-growing trees distributed in India, Arabia, Asia Minor, Africa. Two species are recorded from India , of which one, M.oleifera, is widely cultivated in the tropics for its edible fruits.

SYNONYM: Moringa pterygosperma

Fig.5.7*Moringaoleifera*

COMMON NAME: Drumstick tree, Radish Tree.

VERNACULAR NAMES

Sanskrit: Shobhanjana

Hindi: Mungna, sainjna, shajna

Bengali: Sajina

Marathi: Achajhada, Shevgi

Gujarati: Midhosaragavo, Saragavo

Telugu: Mulaga, muga, tellamunga

Tamil: Murungai

Kanada: Nugge

Malayalam: Murima, sigru, moringa

Assam: Saijna, sohjna.

PLANT TAXONOMY[36]

Kingdom :Plantae

Phylum: Angiosperms

Class : Mangoliopsida

Order : Brassicales

Family : Moringaceae

Genus :*Moringa*

Species: *oleifera*

DISTRIBUTION

Found wild in the sub-Himalayan tract ,from Chenab eastwards to Sarda, and cultivated all over the plains of India

PLANT DESCRIPTION

A small or medium-sized tree, about 10m. high,.

BARK: Thick, soft, corky, deeply fissured, young parts tomentose.

LEAVES: Tri-pinnate

LEAFLETS: Elliptic

FLOWERS: White, fragrant, in large panicles

PODS: Pendulous, greenish, 22.5-50.0cm or more in length, triangular, ribbed

SEEDS: Trigonous with wings on angles.

Fig.5.8 ***Moringaoleifera*****Flower**

FLOWERING AND FRUITING

Almost throughout the year.

Fig.5.9 ***Moringaoleifera*****Leaf Powder**

ETHNO-MEDICINAL USE

ROOT- Rheumatism, asthma, liver complaint, Paralysis, epilepsies, scorpion bite, snlae bite, tooth ache, astringent

GUM- Ear complaint

BARK- Stomach trouble, fever, eczema, typhoid, boils, toothache, poisonous bites, rheumatism

LEAF- Scurvy, wound , night blindness, diarrhea, dysentery, cough, cold, diseases of liver, spleen.

FLOWERS- Eye ailments

PODS- Eye ailments, diseases of Liver, spleen.

SEED- Indigestion, Seed oil in Gout, Rheumatism.

Murraya koenigii [35,36]

Synonym: Bergera koenigi

Common names: Curry leaf tree.

Fig.5.10*Murraya koenigii*

Vernacular names

Tamil: karuveppilei

Hindi: kathnim, gandhela, barsanga.

Bengali: barsanga, kariaphulli.

Marati: karhinimb, gandla, jhirang.

Gujarati: goranimb, kadhilimbdo

Telugu: karepaku

Kannada: karibevu

Malayalam: kariveppilei

Oriya: barsan, basango

DISTRIBUTION

Distributed from South and East Asia to Australia .

In India cultivated in Tamil Nadu, Maharashtra, and North India.Especially in konkan, W.ghats,

along the foot of the Himalaya from Kumaon to Sikkim.

Fig.5.11*Murraya koenigii*

PLANT TAXONOMY[37]

Kingdom: Plantae

Phylum: Spermatophyta

Class: Magnoliospida

Order: Sapindales

Family: Rutaceae

Genus: *Murraya*

Species: *koenigii*

FLOWERING AND FRUITING

February – April.

TASTE:

PLANT DESCRIPTION

A handsome, aromatic, more or less deciduous shrub or small tree.

Leaves: Imparipinnate,strongly aromatic.

Leaflets: 9-25,ovate, lanceolate, dentate, oblique base, almost glabrous above, pubescent beneath

Flowers: terminal corymbose cymes, white in colour, have characteristic fragrance.

Berries: ellipsoid or sub-globose, purplish black when ripe, 2 seeded.

ETHNO-MEDICINAL USE

ROOT

Tonic, stomachic, carminative, Juice of root used in relieving renal pain

LEAF

used internally in diarrhea, spasmolytic, appetizer, externally for bruises and eruptions, tonic.

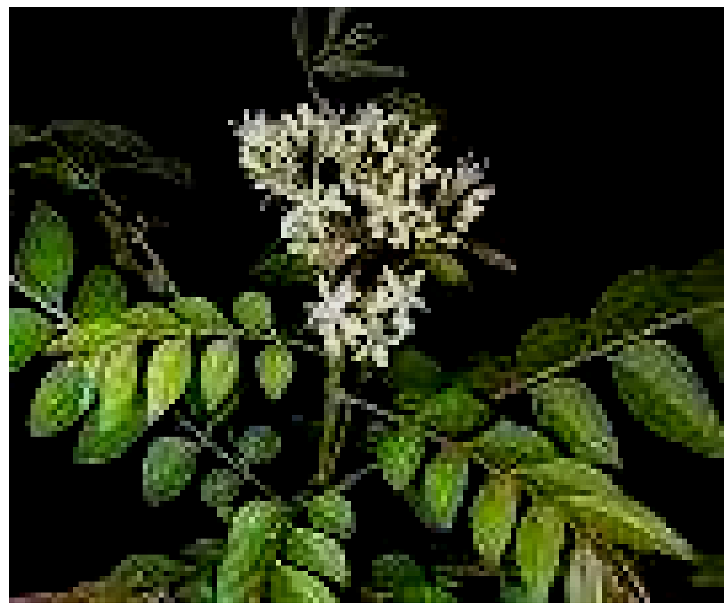


Fig.5.12 ***Murraya koenigii***

Phyllanthus embelica [38,39]

Synonym: Emblica officinalis, Emblic Myrobalan, Indian Gooseberry

Common names: Amla

Fig.5.13 ***Phyllanthus embelica***

Vernacular names

Bengali- Amla

Gujarati- Amali, ambala

Hindi- Amla, amlika, aonla

Kannada- Amalakas

Malayalam- Nelli

Sanskrit- Adiphala, dhatri, amalaka

Tamil-Nelli

Telugu- Amlakamu

DISTRIBUTION

Native of India, Ceylon, Malaya, China

Fig.5.14 ***Phyllanthus embelica***

PLANT TAXONOMY[40]

KINGDOM: Plantae

PHYLUM: Tracheophyta

CLASS: Magnoliopsida

ORDER: Euphorbiales

FAMILY: Euphorbiaceae

GENUS:*Phyllanthus*

SPECIES:*embelica*

FLOWERING AND FRUITING

February - April

TASTE: Sweet, sour, astringent

Fig.5.15 *Phyllanthus embelica* Fruits

ETHNO-MEDICINAL USE

ROOT

Diarrohoea, gonorrhea, jaundice.

LEAF

Conjuctivitis, diaarhoea, dysentery, dyspepsia, inflammation

FRUITS

Anaemia, asthma, bronchitis, colic, cough, diabetes, diarrheoa, dysentery, dyspepsia, fever, flatulence, haemorrhages, inflammation, jaundice, leprosy, leucorrhoea, menorrhagia, peptic ulcer, skin medicine.

SEEDS

Asthma, bronchitis, menstrual trouble.

Tinospora sinenisis **[41,42]**

A genus of deciduous woody climbers, distributed in the tropics of Asia, Africa, and Australia. Three species ouucr in India.

Synonym:*Tinospora malabarica, Tinospora tomentosa*

Fig.5.16 *Tinospora sinenisis*

Common names : Guduchi

Vernacular names

Bengali: Podmo gulancha

Gujarati: Gado

Hindi: Gileo, gulcha

Kannada: Sudarsna-balli

Malayalam : Amrytu

Marathi: Gulvel

Sanskrit: Amrita

Tamil: Seenthil kodi

Telugu: Tippateege

Fig.5.17 *Tinospora sinenisis*

DISTRIBUTION

Throughout tropical India

PLANT TAXONOMY [43]

KINGDOM: Plantae

PHYLUM: Tracheophyta

CLASS: Mangoliopsida

ORDER: Ranunculales

FAMILY:Menispermaceae

GENUS: *Tinospora*

SPECIES: *sinensis*

Fig.5.18 *Tinospora sinenisis*

FLOWERING AND FRUITING

December-June

TASTE: Bitter

ETHNO-MEDICINAL USE

ROOT

Diaarhoea, Diabetes,diabetes

LEAF

Head ache, fever, piles, joint pain, gastric trouble

STEM

Skin disease, jaundice, stomachic, asthma, diphtheria, cough, leprosy, jaundice, ulcers, diarrhea, malaria, snake bite.

WHOLE PLANT

Bone,fracture, piles, fever, jaundice.

Fig.5.19 *Tinospora sinenisis*

PLAN OF WORK

6. PLAN OF WORK

- ✓ PLANT COLLECTION
- ✓ AUTHENTICATION
- ✓ PREPARATION OF HERBARIUM
- ✓ PREPARATION OF EXTRACTS.

PRELIMINARY QUALITY CONTROL OF THE RAW MATERIALS

1)LOSS ON DRYING

2)DETERMINATION OF ASH VALUES

- Total ash
- Water soluble ash
- Acid insoluble ash
- Sulphated ash

3) DETRMINATION OF EXTRACTIVE VALUES

- Water soluble extractive value
- Alcohol soluble extractive value
- Ether soluble extractive value

4) FLUORESCENCE ANALYSIS

5) QUALITATIVE PHYTOCHEMICAL STUDIES OF THE POWDER AND EXTRACTS

CHROMATOGRAPHICANALYSIS

- TLC of all plantsextract
- HPTLC of combinedextract

QUANTITATIVEANALYSIS

- Estimation of Totalalkaloids
- Estimation of Total phenolicconstituents
- Estimation ofFlavonoids
- Estimation of Ironcontent
- Estimation of heavy metals

PRE FORMULATIONAL STUDIES

- Bulk density
- Tapped density
- Compressibility index
- Hausner’s ratio
- Angle of repose.

DEVELOPMENT OF FORMULATION

PHARMACEUTICAL EVALUATION

- Uniform Weight
- Disintergration test
- pH

PHARMACOLOGICALSTUDIES

- Evaluation of Anti-anaemicactivity

METHODOLOGY

7. METHODOLOGY

Polyherbal haematinic capsules , consists of six herbs viz., *Boerhavia diffusa,*(roots) *Eclipta prostrata,*(leaves) *Moringa oleifera,* (leaves) *Murraya koenigii,* (leaves) *Phyllanthus embelica* (fruit pilp), *Tinospora cordifolia.*(stems).

PLANT COLLECTION

As the plants selected were all available in and around the locality, they were collected in person from the respective places mentioned below, during the Months of JUNE-JULY, 2017.

Table 7.1Plant Collection Details

Name of the plant	Place
Boerhavvia diffusa	Dhindivanam, Tamil Nadu
Eclipta prostrata	Dhindivanam, Tamil Nadu
Moringa oleifera	Chennai, Tamil Nadu
Murraya koenigii	Nagapattinam, Tamil Nadu
Phyllanthus embelica	Dhindivanam, Tamil Nadu
Tinospora cordifolia	Dhindivanam, Tamil Nadu

AUTHENTICATION

The collected plants were authenticated in the " SIDDHA CENTRAL RESEARCH INSTITUTE" , Arumbakkam, Chennai-600106, by P.Sathiyarajeswaran, Assistant Director Incharge and Sasikala Ethirajulu, Consultant(Pharmacognosy) .

PLANT MATERIALS

The procured plant materials were washed thrice in running water, and cleaned thoroughly. They were then dried under shade for a week or so. Once they were completely dried, they were ground into coarse powder and stored in air tight containers and preserved for the further processing.

PHYSIO-CHEMICAL CONSTANTS[44-46]

Shade dried powdered plant materials of the plants, *Boerhavia diffusa,*(roots) *Eclipta prostrata,*(leaves) *Moringa oleifera,* (leaves) *Murraya koenigii,* (leaves) *Phyllanthus embelica* (fruit pilp)*, Tinospora cordifolia.*(stems),used for the determination of the physio chemical constants in accordance with the WHO guidelines.

DETRMINATION OF ASH VALUES

Ash values are helpful in determining the quality and purity of a crude drug in the powdered form. The residue remaining after incarnation is the ash content of the drug, which simply represents inorganic salts, naturally occurring drug or adhering to it or delibility added to it, as a form of adulteration.

Ash value of a crude drug is defined as the inorganic residue remaining after incineration, which complies of inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration. Hence it is used for the determination of the quality and purity of the crude drug in the powdered form.

TOTAL ASH:

Total ash method is designed to measure the total amount of material remaining after ignition. They include both physiological ash which is derived from plant tissue itself and non-physiological ash which is the residue of extraneous matter adhering to the plant surface.

Procedure:

Silica crucible was heated to red hot for 30 minutes and cooled in the desiccators Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tarred silica dish at a temperature not exceeding 450^0c until the sample is free from carbon, cooled in desiccators and weighed. The ash obtained was weighed. The percentage of total ash was calculated.

WATER SOLUBLE ASH:

The difference in weight between the total ash and the residue after treatment of the total ash in water.

Procedure:

Total ash obtained is boiled for 5 minutes with 25 ml of water, insoluble matter were collected in a ashless filter paper, washed with hot water and ignite for 15 min at a temperature not exceeding 450^0. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per gram of air-dried material.

$$\text{Water soluble ash} = \frac{\text{Weight of residue obtained}}{\text{Weight of the sample taken}} \times 100$$

ACID INSOLUBLE ASH:

The residue obtained after boiling the total ash with dilute hydrochloric acid, the remaining insoluble matters are ignited and measured. This measures the amount of silica present, especially as sand and siliceous earth.

Procedure:

To the crucible containing total ash of the sample, 25 ml of dilute hydrochloric acid is added. The insoluble matter is collected on an ashless filter paper (Whatman 41) and washed with hot water until the filtrate is neutral. Filter paper containing the insoluble matter to the original crucible, dry on hot plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators for 30 minutes and weighed without delay. Content of acid-insoluble ash with reference to the air dried drug is calculated.

$$\text{Acid insoluble ash} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

SULPHATED ASH

Sulphated ash test is used to measure the amount of residual substance not volatilized from a sample. These test are usually used to determine the content of inorganic substance.

Procedure:

Silica crucible are heated to redness for 10 minutes, allowed to cool in a desiccator and weigh. 2 g of sample were accurately weighed, ignited gently then thoroughly chared. Cool, moistened the residue with 1 ml of sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 ± 25° until all black particles have disappeared. Crucible are allowed to cool, add few drops of sulphuric acid and heat. Ignite as before, allow to cool and weigh. This process is repeated until two successive weighing differ by more than 0.5 mg.

$$\text{Sulphated ash} = \frac{\text{weight of the residue obtained}}{\text{Weight of the sample taken}} \times 100$$

DETERMINATION OF EXTRACTIVE VALUES:

Extractive values are useful for the evaluation of phyto constituents especially when the constituents of a drug cannot be readily estimated by any other means. Further these values indicate the nature of the active constituents present in a crude drug.

Determination of water soluble extractive

5gm of air dried coarsely powdered sample was weighed and macerated with 100ml of chloroform water (95ml distilled water and 5ml chloroform) in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for rest eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and was dried at 105°C for 1 hour in the hot air oven and cooled in desiccators for 30min and weighed. The process was repeated till a constant weight was obtained; the percentage of water soluble extractive value was calculated with reference to the air dried drug.

$$\text{Water soluble extractive value} = \frac{\text{weight of the dried extract}}{\text{weight of the sample taken}} \times 100$$

Determination of alcohol soluble extractive

5gm of the coarsely powdered sample was weighed and macerated with 100ml 90% ethanol in a closed flask for 24 hours. It was shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent and 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. It was dried at 105°C for 1hour in a hot air oven. The dish was cooled in desiccator and weighed. The process was repeated till the constant weight was obtained. The percentage of alcohol soluble extractive value with reference to the air dried drug was calculated.

$$\text{Alcohol soluble extractive} = \frac{\text{weight of the dried extract}}{\text{weight of the sample taken}} \times 100$$

Determination of non volatile ether soluble extractive (fixed oil content)

A suitably weighed quantity of the sample was transferred to an extraction thimble and extracted with solvent ether or petroleum ether (Boiling Point 40 - 60°C) in a Soxhlet for 6 hours. The extract was filtered into a tarred evaporating dish, evaporated and dried at 105°C to constant weight. The percentage of non volatile ether soluble extractive value with reference to the air dried drug was calculated.

$$\text{Non volatile ether soluble extractive} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

Determination of volatile ether soluble extractive

2gm of powdered sample was accurately weighed and extracted with anhydrous diethyl ether in a continuous extractive apparatus for 20hours. The ether solution was transferred to tarred porcelain dish and evaporated spontaneously, dried over phosphorous pent oxide for 18hours and the total ether extract was weighed. The extract was heated gradually and dried at 105°C to constant weight. The loss in weight represents the volatile portion of the extract.

$$\text{Volatile ether soluble extractive} = \frac{\text{Weight of the dried extract}}{\text{Weight of the sample taken}} \times 100$$

DETERMINATION OF MOISTURE CONTENT:

Loss on drying

10 g of the sample substances (without preliminary drying) was taken in a tarred evaporating dish. Use of high speed mill in preparing the samples are avoided. The sample in the tarred evaporating dish were placed in the drying chamber (105°C) for 5 hours and weigh. Drying and weighing is continued every one hour interval until the difference between the two successive weight is not more than 0.25 percent. Constant weight is reached when the two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccators, show not more than 0.001 g difference. Percentage moisture content is compared with respect to the air dried sample.

$$\% \text{ Moisture content} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

PHYTOCHEMICALSTUDIES

The Phytochemical investigation of a plant involves authentication and extraction of plant material; qualitative and quantitative evaluations; separation and isolation of the constituents of interest; characterisation of the isolated compound. Parallel to this may be the assessment of pharmacological activity.

PREPARATION OF EXTRACT [47]

Extraction is the preliminary step involved in the phytochemical studies. Ethanol proves to the universal solvent. As the present study utilizes a combination of herbs, ethanol was opted for the extraction of the active constituents from the individual plants separately. The method of extraction is HOT PERCOLATIONMETHOD.

HOT PERCOLATION METHOD

About 200g of coarsely powdered parts of the plant was extracted with Ethanol at 60-70°C. Extract of individual plants were concentrated using rotary vacuum evaporator. The percentage yield, colour and consistency of all the extracts were noted and were taken up for further detailed phytochemical and pharmacological screening.

PRELIMINARY PHYTOCHEMICAL SCREENING [48]

Qualitative analysis for various phytoconstituents in the dried powder and extracts of the plant parts of *Boerhavia diffusa, Eclipta prostrata, Moringa oleifera, Murraya koenigii, Phyllanthus embelica, Tinospora cordifolia* was carried out using different reagents as mentioned below.

DETECTION OF ALKALOIDS

Dragendroff's Test

The powder/extract was dissolved in 5ml of distilled water, to this 5ml of 2M Hcl was added. Then 1ml of Dragendroff's reagent was added and examined for an immediate formation of an orange red precipitate.

Mayer's Test

The powder/extract was mixed with little amount of dilute Hcl and Mayer's reagent and examined for the formation of white precipitate.

Wagner's Test

The powder/extract was mixed with Wagner's reagent and examined for the formation of reddish brown precipitate.

DETECTION OF GLYCOSIDES

Bontrager's Test

The powdered plant material/extract was boiled with 1ml of sulphuric acid in a test tube for few minutes. The solution was filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of the solvent was separated and shaken with half of its volume of dilute Ammonia. Formation of a rose pink to red colour in the ammoniacal layer indicates the presence of glycosides.

Modified Bontrager's Test

The test material was boiled with 2ml of the dilute sulphuric acid. This was treated with 2ml of 5% freshly prepared aqueous ferric chloride solution for 5 min and shaken with equal volume of chloroform .the lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. Formation of a rose pink to red colour in the ammoniacal layer indicates the presence of glycosides.

Legal's Test

The test material when treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of a pink to blood red colour indicates the presence of cardiac glycosides.

DETECTION OF STEROIDS

Liebermann-Buchards Test

The powdered drug/extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube. If a brown ring is formed at the junction of two layers and upper layer turns green, it shows presence of steroids.

Shinoda Test

To the solution of extract few piece of magnesium turnings and drops of concentrated Hcl were added. If a pink crimson red or occasionally green to blue colour appears after few minutes, this indicates the presence of flavonoids.

Alkaline reagent Test

To the test solution, few drops of sodium hydroxide solution were added. If there is a formation of intense yellow colour which turns to colourless on addition of few drops of dilute acid, this indicates presence of flavonoids.

DETECTION OF CARBOHYDRATES

Molisch's Test

To the test solution, few drops of alcoholic alpha-napthol and few drops of concentrated Sulphuric acid were added through the sides of the test tube. Appearance of purple to violet colour ring at the junction indicates the presence of carbohydrates.

Fehling's Test

The test solution was mixed with Fehling's I and II, heated and examined for the presence of red colouration for the presence of sugar.

DETECTION OF PHENOL

Ferric chloride Test

A small quantity of powdered drug/ extract was dissolved in 2ml distilled water and a few drops of 10% aqueous ferric chloride solution was added and observed for appearance of blue or greencolour.

DETECTION OF PROTIENS

Biuret Test

The sample was treated with 5-8 drops of 1% w/w copper sulphate solution and 1ml of 5% sodium hydroxide. If a violet colour is formed it indicates the presence ofproteins.

DETECTION OF TANNINS

Lead acetate Test

The test solution was mixed with basic lead acetate solution and examined for formation of white precipitate.

Ferric chloride Test

A few drops of 5% aqueous ferric chloride solution was added to 2 ml of aqueous extract of the drug and examined for the presence of bluish black colour.

DETECTION OF SAPONINS

A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for 3minutes. Development of any honey comb like froth was examined.

DETECTION OF GUMS AND MUCILAGE

A small quantity of powdered drug/extract was dissolved in 5 to 10 ml of acetic anhydride by means of heat, cooled and 0.05ml of concentrated Sulphuric acid was added. Formation of bright purplish red colour indicates the presence of gums andmucilage.

DETECTION OF FIXED OILS AND FATS

A small quantity of extract was pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils andfats.

FLUORESCENCE ANALYSIS [48,49]

Fluorescence analysis was carried out in day light and in UV light. The powdered plant material of *Boerhavia diffusa, Eclipta prostrata, Moringa oleifera, Murraya koenigii, Phyllanthus embelica, Tinospora cordifolia* and their extracts was treated with various reagents and solvents to identify the presence of chromophores. The fluorescence was observed in day light and in short and long UV light 254nm and 365nm respectively.

CHROMATOGRAPHIC ANALYSIS [49,50]

THIN LAYER CHROMATOGRAPHY

Principle

It consists of a thin layer of adsorbent coated on a chromatographic plate, the mobile phase (developing solvent) flows against gravitational force by means of capillary action. The separation is mainly on the differential migration that occurs when the solvent flows along the thin layer of stationary phase. The principle involved thin layer chromatography isadsorption.

TLC PLATE PREPARATION

The plates were prepared using Stahl TLC spreader. 40gm of silica gel G was mixed with 85ml of water to prepare homogenous suspension and poured into the spreader. 0.25mm thickness of plates was prepared, air dried until the transparency of the layer disappeared and the plates were then dried at 110°C for 30 minutes and kept in desiccators.

Mobile phase

Single or mixture of solvent was selected based on the phytoconstituents present in the extracts. Factors such as nature of the components, stationary phase, mobile phase, polarity influence the rate of separation of constituents. From various trials, best solvent was selected which showed good separation with maximum number ofcomponents.

HPTLC FINGERPRINT PROFILE[50]

HPTLC is one of the advanced and versatile chromatographic technique which helps in the identification of compound and thereby authentication of purity of herbal drugs. It is very quick process. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of the phytoconstituents which is unique to each plant. The finger print obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extracts.

INSTRUMENT CONDITIONS

SampleUsed : Combined ethanolic extracts of all six plants.

Instrument : CAMAG HPTLC

HPTLCApplicator : CAMAG Linomat 5

<u>SAMPLE APPLICATION</u>

Spray gas : Inert gas

Sample Solvent type : Methanol

Dosage speed : 150 nl/s

Pre Dosage Volume : 0.2 µl

<u>SEQUENCE</u>

Syringe size :100 µl

No of tracks : 3

<u>STATIONARY PHASE</u>

Material : HPTLC Aluminium Sheets Silica Gel 60F 254

Manufacturer : E.MERCK KGaA

<u>MOBILEPHASE</u>

Solvent : TOLUENE:ETHYL ACETATE:METHANOL[2:2:1]

Solvent Front Position: 70.0mm

Volume : 10.0ml

Drying device : Oven

Temperature : 60°C

Time :5 minutes.

<u>SCANNER</u> : CAMAG TLC Scanner

Wavelength	**:366**
Lamp	**:D2 & W**
Measurement Type	**:Remission**
Measurement Mode	**:Absorption**
Optical filter	**:Second order**
Detector mode	**: Automatic**
PM high voltage	**:354 V**

Chromatographic conditions

The estimation has been done using the following chromatographic conditions.

Chromatography was performed on a 10*10cm pre-activated aluminium coated silica gel-Merk 60 F254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat V with inert gas flow CAMAG Switzerland 8mm from the bottom.

QUANTITATIVEANALYSIS

ESTIMATION OF FLAVONOIDS[51]

The total flavonoid content is usually determined spectrophotometrically using Ultra-violet spectroscopy.

Preparation of Standard Stock solution

Accurately weighed 25 mg ofQuercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide(DMSO). The serial dilution (20mcg,40mcg, 60mcg,80 mcg ,100 mcg) were made with dimethyl sulfoxide.

Preparation Of Test Solution

The leaf extract was weighed accurately equal to the weight of Standard Quercetin and transferred to 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg,80 mcg ,100 mcg) were made with dimethyl sulfoxide.

PROCEDURE

From the prepared solution of standard and test solutions 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10 minute at ambient temperature. After 10 minute, measure the absorbance spectrophotometrically at 435 nm with the standard and test sample solutions.

ESTIMATION OF PHENOLIC CONSTITUENTS[51 -53]

Total phenolic content of the extracts were determined using Folin –Ciocalteu's assay. 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted Folin Ciocalteu's reagent and 2.5ml of 7.5% sodium carbonate. After incubation at 40°c for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrophotometer. Three replicates were made for each test sample. Gallic acid was used as a standard and total phenolic content of the extract was expressed in mg of Gallic acid equivalents (mg GAE/g extract)

ESTIMATION OF IRON[55]

Method: Flame Absorption

WaveLength(nm):248.30

INSTRUMENT PARAMETERS

Slit(nm):0.2

High Voltage(V):275.70

Lamp Current(mA):3.00

Types of Flame: Air-C2H2

Fuel Gas Flow Rate(L/min):1.50

Fuel Gas Pressure(MPa):0.10

Combustion-Supporting Gas: Air

Combustion-Supporting Gas Flow Rate(L/min):5.00

Combustion-Supporting Gas Pressure(MPa):0.20

Burner Height(mm):8.0

Preparation of Iron standard stock solution:

About 100 mg of iron powder was accurately weighed and transferred to a1000ml volumetric flask. It is dissolved in 25 ml of 6N hydrochloric acid and diluted with water to make up the volume.

Preparation of Standard solution:

2.0ml of Iron standard stock solution was transferred to separate 100ml volumetric flask. Contents of each flask was diluted with water and made up to volume and mixed to obtain solutions having known concentrations of about 2.0mg of iron per ml.

Preparation of sample:

1 gram of sample was transferred to a crucible and kept inside a muffle furnace maintained at about 550° for 6 to 12 hours and cooled. About 60ml of hydrochloric acid was added and boiled gently on a hot plate or steam bath for 30 minute intermittently rinsing the inner surface of the crucible with 6N hydrochloric acid. It was cooled, the contents of the crucible was quantitatively transferred to a 100ml volumetric flask. The crucible was rinsed with small portions of 6 N hydrochloric acid and the rinings were added to the flask. Diluted with water to volume, mixed and filtered, discarded the first 5 ml of the filtrate. This solution was diluted quantitatively and stepwise if necessary, with 0.125N hydrochloric acid.

Procedure:

The absorbance of the Standard preparations and the assay preparation was determined at the iron emission line at 248.3nm with an atomic absorption spectrophotometer (Spectrophotometry and Light-Scattering á 851ñ) equipped with an iron hollow-cathode lamp and an air-acetylene flame, using 0.125 N hydrochloric acid

as the blank.

A graph was plotted using absorbance of standard Vs concentration. From this the concentration of the sample was determined.

QUANTITATIVE ESTIMATION OF HEAVYMETALS[56]

Instrumentation parameters:

The quantitative analysis of heavy metals was done by the inductive coupled plasma-optical emission spectroscopy method.

Instrument model:

PE optima 5300 DV ICP-OES; optical system Dual view-axial or radial.

Detector system:

Charge coupled detector, (UV-visible detector which is maintaining at -40°C) to detect the intensity of the emission line.

Light source:

The Torch is positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Standard alumina injector: 2.0mm inner diameter

Spray chamber: Scott type

Nebulizer: cross flow gem type

Preparation of sample by acid digestion method:

50mg of the powder was treated with a mixture of sulphuric acid:water in the ratio of 4:1 in a kjeldahl flask and heated continuously till the solution was colourless. The sample mixture was then transferred in a 25ml volumetric flask and made upto the volume with distilled water. Blank solution was prepared as above without sample.

The standard of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

PHARMACOLOGICALSTUDIES

EVALUATION OF ANTI-ANAEMIC ACTIVITY

Experimental animals

Healthy wistar albino rats of either sex weighing 150-220g were procured from Animal Experimental Laboratory, Madras Medical College, Chennai-03. The study was approved by the Institutional Animal Ethical Committee of Madras medical College Chennai. Which is certified by the committee for the purpose of control and supervision of experiments of animals, India (CPCSEA).**The Approval number:IAEC/MMC/O5/2016 Dated: 21.11.2016**

The animals were kept individually in a clean and dry metallic cages and maintained in a well-ventilated animal house at a room temperature of 22±2° and for 12 hours light and 12 hours dark cycle. The animals were fed with standard pellet diet and water *ad libitum*.

Acute toxicity studies[57, 58, 59,60,61,62,63]

The acute toxicity studies have already been done as per OECD guide lines

423 for the individual plants. They are safe upto 2000-4000mg/kg. Since the present study deals with combined formulation, acute toxicity studies for the combined extracts were done .

Anti-anaemic activity

Vit.B12 syrup as standard and phenyl hydrazine to induce anaemia, all the chemicals used in the study were of analytical grade.

Phenyl hydrazine induced anaemic model [64]

This is one of the methods used in evaluating anti-anaemic activity. Anaemia was induced in adult wistar albino rats of either sex by oral administration of phenyl hydrazine 10mg/kg for eight days. The rats with haemoglobin concentration lower than the normal (13g/dl) were recruited for the study.

Procedure

The recruited animals were divided into 5 groups each containing 6 rats; they are treated with phenylhydrazine to induce anaemia from 1 to 8 days, except positive group. The standard group receives vit.B12 as standard, group IV and V animals are treated with 200mg/kg and 400mg/kg of polyherbal extractfrom 9th day to 60th day respectively. Blood is collected on initial day after induction of anaemia and 60th day after completion of treatment. Haematological measurements are made using suitable chemical, electrical and microscopical methods. Anti-anaemic activity of the polyherbal extract was calculated by statisticalmethods.

Table 7.1 : EXPERIMENTAL DESIGN

S.No	Group	No.of animals	Name of the group	Treatment
1	I	6	Positive control	Receives vehicle for 60 days
2	II	6	Negative control	Treatment with PHZ 10mg/kg from day 1 to day 8 orally
3	III	6	Standard	Treatment with vit. B12 from 9th day to 60th day orally
4	IV	6	Test dose 1	Treatment with test dose 1(200mg/kg) from 9th - 60th day Orally
5	V	6	Test dose 2	Treatment with test dose 2 (400mg/kg) from 9th – 60th day Orally
Total		30 animals		Duration of the study is 60 days

Evaluation of Anti-anaemic activity-Haematological parameters [65]

The complete blood count (CBC) was measured before the induction of anaemia, after the induction of anaemia and after drug treatment using Sysmex Automated haematology Analyser. The white blood cells, red blood cells, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration are counted using the direct current detection method with coincidence correction and flow cytometer. The haematocrit was done directly based on the red cell count and volume detection of each individual RBC. Haemoglobin analysis was conducted using Non-cyanidemethod.

Statistical analysis

The data was subjected to analysis of variance (ANOVA). P values < 0.05 was considered significant.

FORMULATION OF CAPSULES

Prior to formulation process the ethanolic extracts were subjected to freeze drying process .The extracts were dried for a period of time according to their rate of drying . The lyophiliser was made utilized in the Pharmaceutics Laboratory, of our college.

The dried extracts were weighed accurately to 2 gram individually. The weights of the chosen 6 plants extracts get summed up to 12gram of the extracts. Magnesium carbonate and Calcium Carbonate each 4.8 gram were weighed and mixed with the extracts. They act as adsorbents. They were mixed by Trituration method. After trituration, the mixed powder blend was passed through sieve no.26. The fine powder so obtained was subjected to preformulation studies.

PREFORMULATION STUDIES[66]

Prior to formulation, it is essential that fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning is known as Preformulation. It aims to optimize the process of turning a drug into a drug product. During preformulation the physiochemical properties of the drug candidate are determined.

DETERMINATION OF THE GRANULES PARAMETERS

- BULK DENSITY
- TAPPED DENSITY
- COMPRESSIBILITY INDEX
- HAUSNER'S RATIO
- ANGLE OF REPOSE

BULK DENSITY

The bulk density of a powder is dependent on particle packing and changes as the powder consolidates. A consolidated powder is likely to have a greater arch strength than a less consolidated one and may therefore be more resistant to powder flow.

PROCEDURE

Weighed amount of the powder (m i.e mass of the powder) is transferred to a measuring cyclinder and its volume V is measured. From the values, the bulk density of the powder is calculated using the following formula:

$$\text{BULK DENSITY} = \frac{\text{MASS OF POWDER (m)}}{\text{BULK VOLUME (V)}}$$

FIGURE 7.1, 7.2:MEASUREMENT OF TAPPED DENSITY AND ANGLE OF REPOSE.

TAPPED DENSITY

Weighed amount of the powder (m i.e mass of the powder) is transferred to a measuring cylinder and its volume V is measured. The cyclinder is tapped mechanically. The volume of 200 tappings is noted. This is called the tapped volume.

$$\text{TAPPED DENSITY} = \frac{\text{MASS OF POWDER (m)}}{\text{TAPPED VOLUME}(V_f)}$$

COMPRESSIBILITY INDEX

It is an indirect method to determine the flow of powders. As it was developed by a scientist called Carr, henced named after the Carr's index. It is defined as the direct measure of the potential powder or arch or bridge strength and stability. It is calculated by the equation

$$\%\ \text{COMPRESSIBILITY} = \frac{\text{TAPPED DENSITY} - \text{BULK DENSITY}}{\text{TAPPED DENSITY}} \times 100$$

HAUSNER'S RATIO

Hausner found that the ratio (V_o/V_f) was related quantitatively to interparticulate friction.

$$\text{HAUSNER'S RATIO} = \frac{\text{TAPPED DENSITY}}{\text{BULK DENSITY}} \times 100$$

ANGLE OF REPOSE

As there is a relationship between interparticulate cohesion and Angle of Repose, it proves to be a quantifying method of powder flow. There are many different methods of determining it .They are as Fixed height cone, Fixed base cone , Titlting table, Rotating Cyclinder. In my present study I performed the Fixed height Cone method. Angle of repose was calculated from the formula:

$$\text{ANGLE OF REPOSE} = \tan^{-1}(h/r)$$

h=height of the pile

r=radius of the circle

Table 7.2: General flow characterstics

FLOW PROPERTY	ANGLE OF REPOSE	CARR'S INDEX	HAUSNER'S RATIO
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1,18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1,26-1.34
Poor	46-55	26-31	1.35-1.45
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

FORMULATION OF CAPSULES[67-72]

TABLE7.3:LIST OF MATERIALS USED IN THE FORMULATION

S.NO	INGREDIENT	USE
1.	*Boerhavvia diffusa*	Active ingredient
2.	*Eclipta prostrate*	Active ingredient
3.	*Moringa oleifera*	Active ingredient
4.	*Murraya koenigii*	Active ingredient
5.	*Phyllanthus embelica*	Active ingredient
6.	*Tinospora cordifolia*	Active ingredient
7.	Magnesium oxide	Adsorbent
8.	Calcium Carbonate	Adsorbent
9.	Lactose	Diluents
10.	Magnesium stearate	Lubricant
11.	Sodium Starch Glycolate	Disintergrant

SELECTION OF CAPSULE SIZE[73]

The volume of material to be filled into the capsule should be determined. Generally, capsules of sizes “0” to “4” were readily available in the market and the relationship between the capsule size and related body volume to be known at the development stage. For pharmaceutical products it is unusual to use a size larger than “0” because of the difficulties in the swallowing larger size capsules, what size capsules, whilst size “5” is rarely used due to the difficulties in the automatic filling process. Capsule of size “0” were selected to fill the polyherbal formulation.

FILLING OF CAPSULES

The dried powder extracts, along with the adsorbent were taken. Lactose, 1.2 gram, was added as diluents. Magnesium stearate , 1gram was weighed and included as the lubricant. Sodium starch glycolate , weighed accurately to 1 gram was added as it aids the disintergration process.

Figure 7.2: POLYHERBAL HAEMATINIC CAPSULES

EVALUATION OF FINISHED PRODUCT[75]

The developed polyherbal were evaluated for its Description, uniformity of weight, disintergration time, moisture content, pH. Determination of uniformity of weight, disintergration time, moisture content was performed as per Indian Pharmacopoeial procedures.

DESCRIPTION

The general appearance of capsule , its visual identity and overall elegance is essential for consumer acceptance. The colour, shape, odour, surface texture, and legibility of any identifying marking are all noted for the capsules prepared.

AVERAGE WEIGHT AND UNIFORMITY OF WEIGHT

20 individual capsule were selected at random and this content was weighed and their average weight was calculated. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table and none deviate more than twice that percentage.

Table 7.4:Specification of Average and Uniformity of weight

S.No	DOSAGE FORM	AVERAGE WEIGHT	% DEVIATION
1.	Capsules	<300mg >300mg	10% 7.5%

DISINTERGRATION TIME

This test is done to measure the time taken by the drug to disintergrate in the body. This is done to determine whether the capsule disintergrates within the prescribed time when placed in a liquid medium under the prescribed experimental conditions. Each capsule was added to each of the six tubes of the basket and a disc was added to each of the tube. The tubes were dipped in0.1N Hydrochloric acid maintained at 37°C.

pH

The pH value of a solution was determined potentiometrically by means of a digital pH meter. The pH meter was caliberated using buffers of 4, 9 and 7 pH. 1g of capsule content,was taken and dissolved in 100ml demineralized water. The electrodes were immersed in the solution and the pH is measured.

RESULTS AND DISCUSSION

8. RESULTS AND DISCUSSION

8.1. PHYSIO-CHEMICAL CONSTANTS

The various ash values for the powders used in the formulation are tabulated in table

Table 8.1: ASH VALUES OF THE POWDERS

S.No	NAME OF THE PLANT	TOTAL ASH(%W/W)	WATER SOLUBLE ASH (%W/W)	ACID INSOLUBLE ASH (%W/W)	SULPHATED ASH (%W/W)
1	***Boerhavia diffusa***	4.54±0.34	1.23±0.67	0.91±0.31	4.07±0.13
2	***Eclipta prostrata***	2.68± 0.47	0.97±0.11	0.76±0.13	2.12±0.17
3	***Moringa oloeifera***	2.56±0.17	0.65± 0.16	0.81±0.16	2.37±0.11
4	***Murraya koenigii***	3.12±0.35	0.69±0.14	0.97±0.12	3.04±0.14
5	***Phyllanthus embelica***	3.36 ±0.12	0.77±0.05	0.82±0.17	3.29±0.23
6	***Tinospora sinensis***	4.78± 0.76	0.63±0.14	0.75±0.09	4.01±0.15

The extractive values of the powders were found to be as tabulated in table .

Table 8.2: EXTRACTIVE VALUES OF THE POWDERS

S.No	NAME OF THE PLANT	Water soluble extractive	Alcohol soluble extractive	Ether soluble extractive	Non volatile Ether soluble extractive
1	***Boerhavia diffusa***	17.56±1.12	20.12±0.13	3.56±0.67	2.12±0.12
2	***Eclipta prostrata***	13.43±0.78	24.67±0.34	4.70±1.56	2.09±0.56
3	***Moringa oloeifera***	18.67±0.95	21.31±0.45	3.23±1.27	2.78±0.43
4	***Murraya koenigii***	10.78±1.56	22.21±0.19	4.51±1.43	2.31±0.13
5	***Phyllanthus embelica***	16.37±1.17	25.89±0.24	2.23±1.09	2.09±0.76
6	***Tinospora sinensis***	15.95±0.92	20.78±0.52	4.76±1.17	2.13±0.32

The results of the loss on drying process are summarized in the table .

Table 8.3:LOSS ON DRYING VALUES OF THE POWDERS

S.NO	NAME OF THE PLANT	LOSS ON DRYING
1	***Boerhavia diffusa***	4.38±0.75
2	***Eclipta prostrate***	3.12±0.68
3	***Moringa oloeifera***	4.23±1.25
4	***Murraya koenigii***	3.45±1.12
5	***Phyllanthus embelica***	4.34±0.89
6	***Tinospora sinensis***	4.45 ±1.09

PHYTOCHEMICAL STUDIES

Preparation of extracts

The shade dried crude dried drugs of *Boerhavia diffusa, Eclipta prostrata, Moringa oleifera, Murraya koenigii, Phyllanthus embelica, Tinospora cordifolia* was extracted in soxhlet extractor with ethanol . All the extracts were concentrated using rotary vacuum evaporator. The percentage yield was calculated for every extract in terms of dried weight of plant material. The colour and consistency of the concentrated extracts are given in table no.8.4

Table 8.4 : Percentage yield of extracts

S.NO	PLANT NAME	METHOD OF EXTRACTION	PHYSICAL NATURE	COLOUR	YIELD (%W/W)
1.	***Boerhavia diffusa***	Continuous Hot percolation method using Soxhlet apparatus	Semisolid	Dark brown	2.16
2.	***Eclipta Prostrate***		Semisolid	Black green	3.89
3.	***Moringa oleifera***		Semisolid	Dark green	5.78
4.	***Murraya koenigii***		Semisolid	Black green	4.34
5.	***Phyllanthus embelica***		Semisolid	Dark brown	3.12
6.	***Tinospora sinensis***		Semisolid	Dark brown	2.97

QUALITATIVE ANALYSIS

Qualitative chemical tests

The coarse powder of the six plants and their ethanolic extracts were subjected to qualitative phytochemical analysis to identify the various phytoconstituents present in it, as per the standard procedures. The results are given in the table no.8.5

Table 8.5 : Preliminary phytochemical analysis of powder

Chemical constituents	*B.diffusa*	*E.prostrata*	*M.oleifera*	*M.koenigii*	*P.embelica*	*T.sinensis*
Steroids	+	+	+	+	+	+
Glycosides	+	_	+	+	_	_
Saponins	_	+	_	_	+	_
Flavonoids	+	+	+	+	+	+
Tannins	_	+	+	_	+	_
Proteins	+	_	+	+	+	_
Alkaloids	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Fats and oils	_	+	+	+	+	_

+ indicates presence , - indicates absence

Table 8.6: Preliminary phytochemical analysis of ethanolic extract

Chemical constituents	*B.diffusa*	*E.prostrata*	*M.oleifera*	*M.koenigii*	*P.embelica*	*T.sinensis*
Steroids	+	+	+	+	+	+
Glycosides	+	_	+	+	_	_
Saponins	_	+	_	_	+	_
Flavonoids	+	+	+	+	+	+
Tannins	_	+	+	_	+	_
Proteins	+	_	+	+	+	_
Alkaloids	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Fats and oils	_	+	+	+	+	_

+ indicates presence , - indicates absence

Qualitative preliminary phytochemical analysis was performed with different respective chemical detecting agent to detect the phytoconstituents and their presence in each extract and powder.

FLUORESCENCE ANALYSIS

Fluorescence analysis for the extracts and the powdered drugs were carried out with various reagents to identify the presence of chromophores. The importance of fluorescence analysis is that UV light shows the fluorescent nature of the compound whereas fluorescence cannot be observed in day light. Hence it is performed according to the standard procedures. The results are shown in Tables 8.7 to 8.13

Table 8.7 : Fluorescence analysis of powder of *Boerhavia diffusa*

Substance	Day light	Short UV	Long UV
Powder	Pale brown	Brown	Brown
Powder+ H_2O	Puff brown	Black	Puff brown
Powder +1N HCl	Brown	Black	Brown
Powder +1N HnO_3	Brown	Black	Brown
Powder + acetic acid	Pale yellow	Brown	Yellow
Powder +1N NaOH	Pale yellow	Brown	Yellow
Powder +alc.NaOH	Dark Yellow	Brown	Dark Yellow
Powder +1N KOH	Dark Yellow	Brown	Dark Yellow
Powder +alc.KOH	Yellow	Brown	Dark Yellow
Powder+H_2So_4	Dark brown	Black	Dark brown
Powder+NH_4	Brown	Black	Brown
Powder+I_2	Dark brown	Black	Dark brown
Powder+$FeCl_3$	Black	Black	Black
Powder+ethanol	Pale brown	Black	Black

Table 8.8. Fluorescence analysis of powder *Eclipta prostrata*

Substance	Day light	Short UV	Long UV
Powder	Green	Black	greenish brown
Powder+ H_2O	Green	Black	greenish brown
Powder +1N HCl	Dark Green	Black	Orange green
Powder +1N HnO_3	Dark Green	Black	Orange
Powder + acetic acid	Pale Green	Dark green	Greenish yellow
Powder +1N NaOH	Pale Green	Black	Pale green
Powder +alc.NaOH	Pale Green	Black	Pale green
Powder +1N KOH	Pale Green	Black	Pale green
Powder +alc.KOH	Pale Green	Black	Pale green
Powder+H_2So_4	Black	Black	Black
Powder+NH_4	Dark Green	Black	Green
Powder+I_2	Reddish Brown	Black	Brown
Powder+$FeCl_3$	Dark Green	Black	Brown
Powder+ethanol	Pale Green	Black	Greenish brown

Table 8.9: Fluorescence analysis of powder of *Moringa oleifera*

Substance	Day light	Short UV	Long UV
Powder	Green	Black	greenish brown
Powder+ H_2O	Green	Black	greenish brown
Powder +1N HCl	Dark Green	Black	Orange green
Powder +1N HnO_3	Dark Green	Black	Orange
Powder + acetic acid	Pale Green	Dark green	Greenish yellow
Powder +1N NaOH	Pale Green	Black	Pale green
Powder +alc.NaOH	Pale Green	Black	Pale green
Powder +1N KOH	Pale Green	Black	Pale green
Powder +alc.KOH	Pale Green	Black	Pale green
Powder+H_2So_4	Black	Black	Black
Powder+NH_4	Dark Green	Black	Green
Powder+I_2	Reddish Brown	Black	Brown
Powder+$FeCl_3$	Dark Green	Black	Brown
Powder+ethanol	Pale Green	Black	Greenish brown

Table8.9 :Fluorescence analysis of powder Murraya koenigii

Substance	Day light	Short UV	Long UV
Powder	Green	Black	greenish brown
Powder+ H_2O	Green	Black	greenish brown
Powder +1N HCl	Dark Green	Black	Orange green
Powder +1N HnO_3	Dark Green	Black	Orange
Powder + acetic acid	Pale Green	Dark green	Greenish yellow
Powder +1N NaOH	Pale Green	Black	Pale green
Powder +alc.NaOH	Pale Green	Black	Pale green
Powder +1N KOH	Pale Green	Black	Pale green
Powder +alc.KOH	Pale Green	Black	Pale green
Powder+H_2So_4	Black	Black	Black
Powder+NH_4	Dark Green	Black	Green
Powder+I_2	Reddish Brown	Black	Brown
Powder+$FeCl_3$	Dark Green	Black	Brown
Powder+ethanol	Pale Green	Black	Greenish brown

Table 8.10 : Fluorescene analysis of powder of *Phyllanthus embelica*

Substance	Day light	Short UV	Long UV
Powder	Brown	Black	Brown
Powder+ H_2O	Pale brown	Black	Puff brown
Powder +1N HCl	Brown	Black	Brown
Powder +1N HnO_3	Pale brown	Brown	Brown
Powder + acetic acid	Yellowish brown	Black	Yellow
Powder +1N NaOH	Yellowish brown	Black	Yellow
Powder +alc.NaOH	Pale brown	Black	Dark Yellow
Powder +1N KOH	Pale brown	Black	Dark Yellow
Powder +alc.KOH	Pale brown	Black	Dark Yellow
Powder+H_2So_4	Black	Black	Dark brown
Powder+NH_4	Brown	Black	Brown
Powder+I_2	Reddish brown	Black	Dark brown
Powder+$FeCl_3$	Blackish green	Black	Black
Powder+ethanol	Pale brown	Brown	Black

Table8.11 : Fluoresence analysis of *Tinospora sinensis*

Substance	Day light	Short UV	Long UV
Powder	Pale brown	Black	Brown
Powder+ H_2O	Puff brown	Black	Puff brown
Powder +1N HCl	Brown	Black	Brown
Powder +1N HnO_3	Brown	Brown	Brown
Powder + acetic acid	Pale brown	Black	Yellow
Powder +1N NaOH	Pale brown	Black	Yellow
Powder +alc.NaOH	Brown	Black	Dark Yellow
Powder +1N KOH	Pale brown	Black	Dark Yellow
Powder +alc.KOH	Pale brown	Black	Dark Yellow
Powder+H_2So_4	Black	Black	Dark brown
Powder+NH_4	Brown	Black	Brown
Powder+I_2	Reddish brown	Black	Dark brown
Powder+$FeCl_3$	Black	Black	Black
Powder+ethanol	Pale brown	Brown	Black

Table no. 8.12: Fluorescence analysis of extracts

Plants	Day light	Short UV	Long UV
Boerhavia diffusa	Brown	Black	Brown
Eclipta prostrata	Dark Green	Black	Dark Green
Moringa oleifera	Dark Green	Black	Dark Green
Murraya koenigii	Dark Green	Black	Dark Green
Phyllanthus embelica	Brown	Black	Brown
Tinospora sinensis	Brown	Black	Brown

No fluorescence was observed for the powder as well as extracts indicating the absence of chromophore in the plant.

CHROMATOGRAPHIC ANALYSIS

Thin layer chromatography:

The ethanolic extract of all the plants were subjected to thin layer chromatographic studies using various solvent systems. Several mobile phases were tried for the separation of maximum components by trial and error method. The solvent system selected was ethyl acetate: formic acid: acetic acid: water (5:3:1:1), ethyl acetate: n- butanol:ethanol:water (4:1:0.1:5) and ethanol: glacial acetic acid: formic acid:water(3:0.9:0.9:0.5). R_f values were noted down for each selected extracts after elution by using different detecting agents such as Dragendroff's,

Ninhydrin, Libberman Burchard, Con.Sulphuric acid & Ferric chloride.

Table 8.13 : R_f values of the extract

S.No	Name of the plant	R_f value
1.	*Boerhavia diffusa*	0.57
2.	*Eclipta prostrate*	0.76
3.	*Moringa oleifera*	0.34
4.	*Murraya koenigii*	0.68
5	*Phyllanthus embelica*	0.65
6	*Tinospora sinensis*	0.49

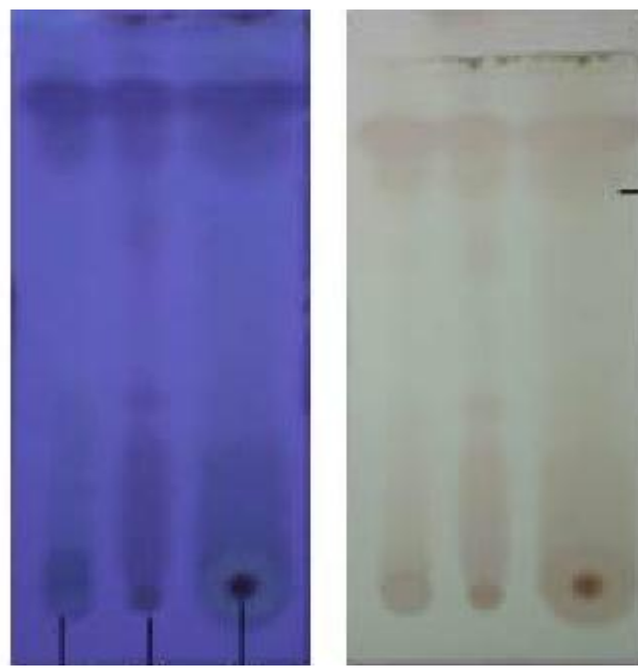


Figure 8.1 : TLC of *Moringa oleifera*

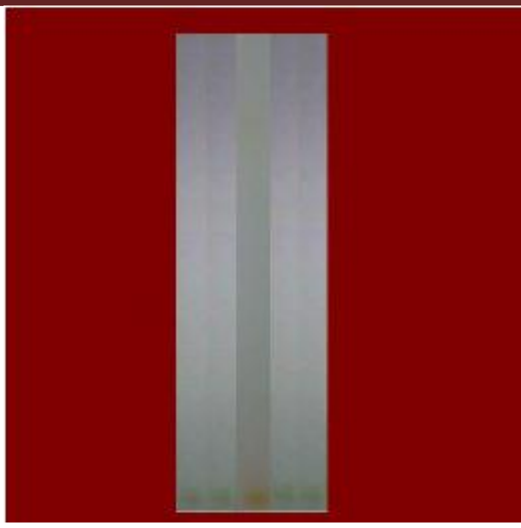


Figure8.2 : TLC of *Tinospora Cordifolia*

Figure 8.3: TLC of *Boerhavia diffusa*Figure8.4 : TLC of *Eclipta prostrata*

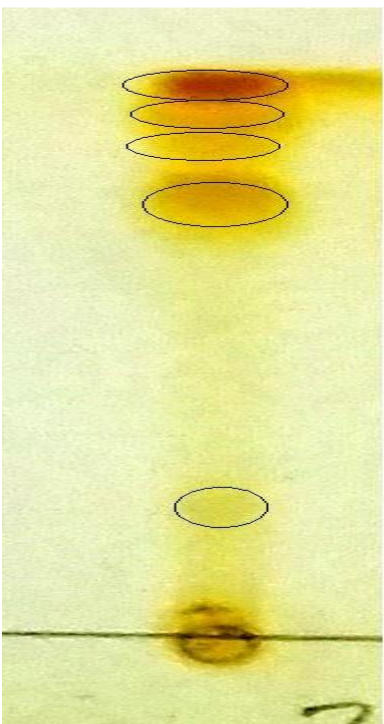
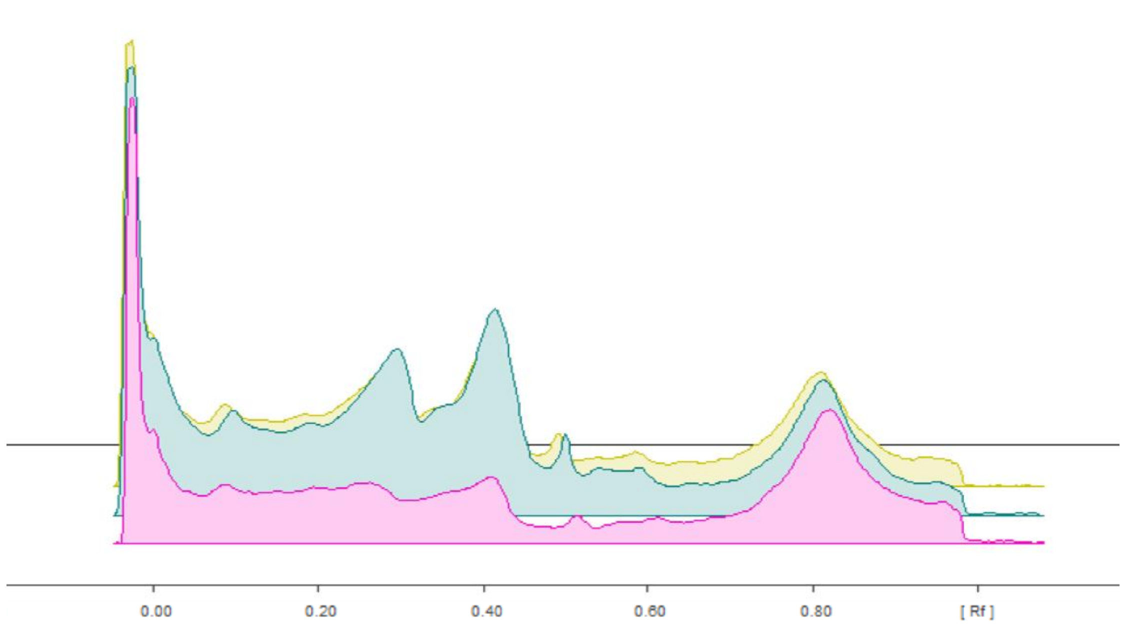
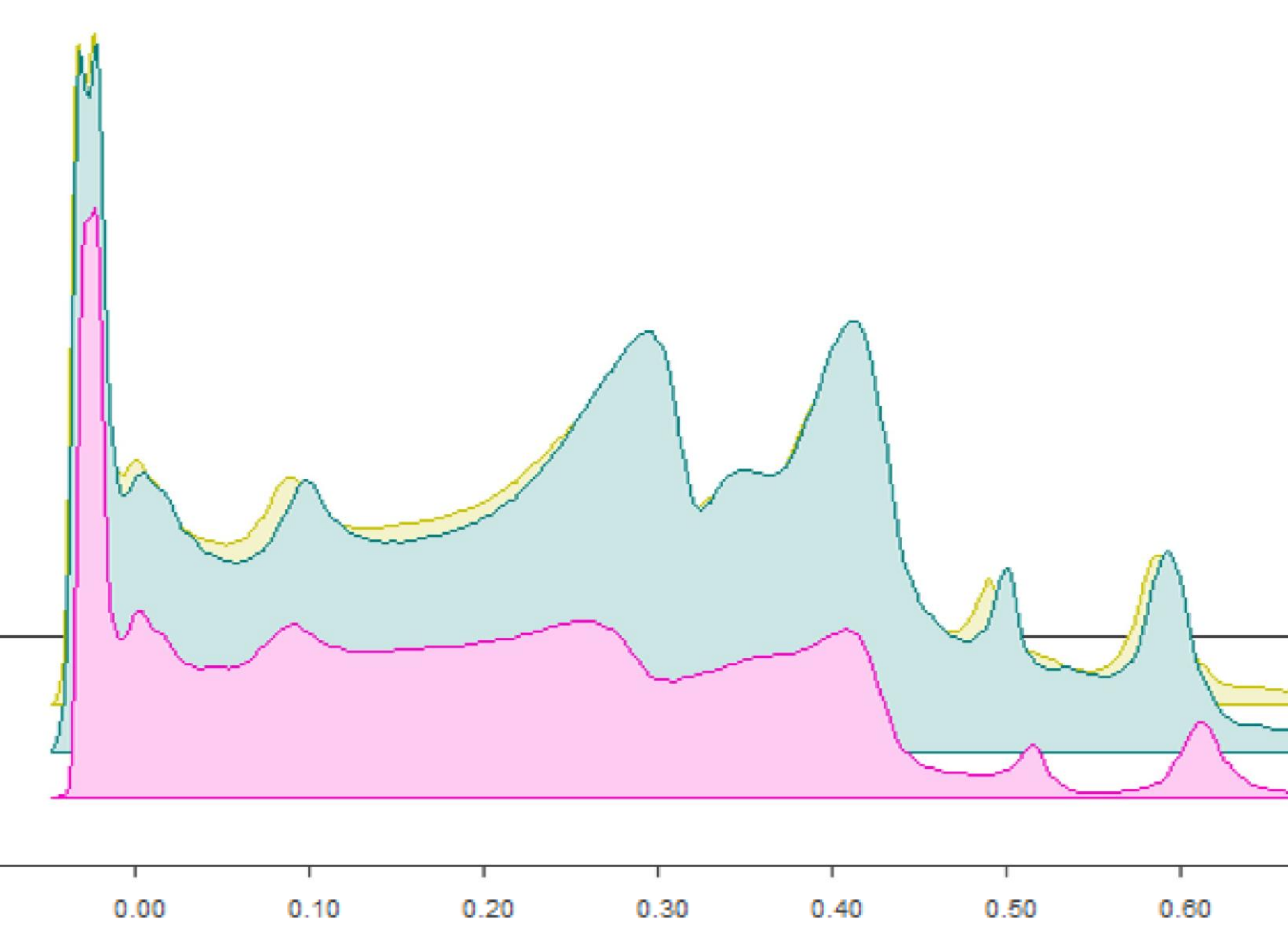


Figure 8.5TLC of *Phyllanthus embelica*

Figure8.6 : TLC of *Murraya koenigii*

FIG8.7 :HPTLC FINGER PRINTING OF THE POLYHERBAL EXTRACT AT 254nm





HPTLC FINGER PRINTING OF THE POLYHERBAL EXTRACT AT 366nm

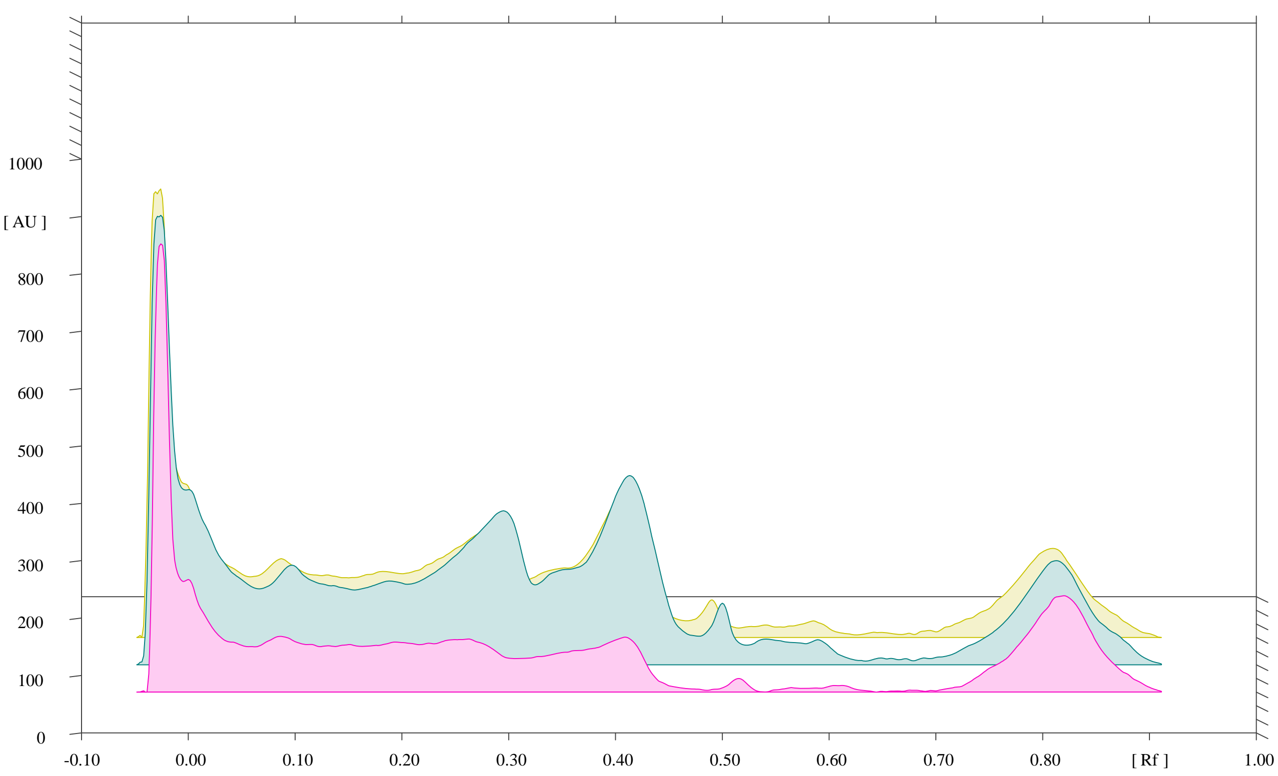


Figure8.8 : HPTLC finger printing – All Tracks

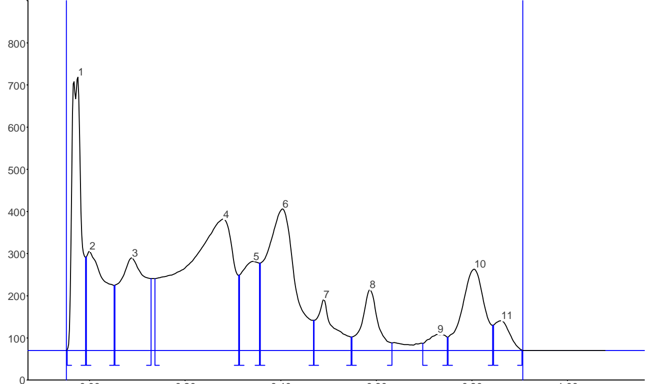


FIG8.9 :FINGER PRINT 1

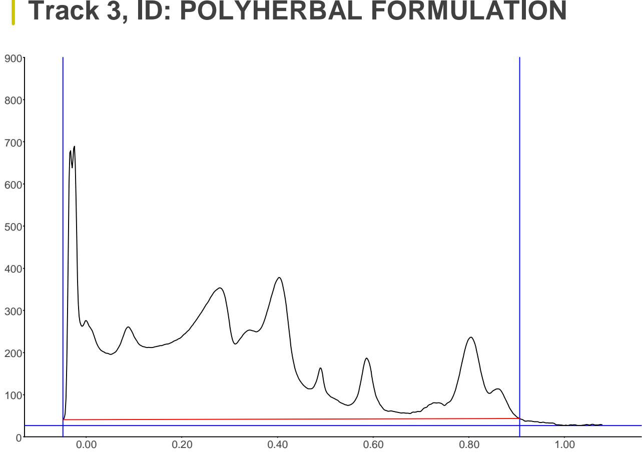


Fig 8.10: Finger print 2

Table 8.14 : HPTLC DATA

Start Start Max Max Max End End Area
Peak Rf Height Rf Height % Rf Height Area % Assigned substance

	Start		Start							
Peak	**Rf**	Start Height	Rf	Start Height	%	Rf	Height	**Area**	%	**Assigned** Substance
1	0.05	1.6	-0.02	648.9	25.63	-0.01	221.6	9139.8	10.67	unknown *
2	0.01	222.7	0.00	235.4	9.30	0.05	154.8	6997.7	8.17	unknown *
3	0.05	155.2	0.09	219.9	8.68	0.13	171.0	8886.5	10.38	unknown *
4	0.14	170.7	0.28	312.0	12.32	0.31	178.6	24886.6	29.06	unknown *
5	0.31	179.4	0.34	211.6	8.36	0.36	207.5	5446.5	6.36	unknown *
6	0.36	207.6	0.40	336.2	13.28	0.47	71.8	14497.5	16.93	unknown *
7	0.47	71.8	0.49	121.1	4.78	0.55	32.4	3149.2	3.68	unknown *
8	0.55	32.4	0.59	144.4	5.70	0.63	18.3	3493.3	4.08	unknown *
9	0.70	17.2	0.73	38.3	1.51	0.75	31.8	1047.8	1.22	unknown *
10	0.75	32.1	0.81	193.4	7.64	0.85	60.0	6483.6	7.57	unknown *
11	0.85	60.7	0.86	70.8	2.80	0.91	1.0	1615.7	1.89	unknown *

The HPTLC finger printing of the polyherbal ethanolic extracts showed the above tabulated R_f values with corresponding peak area.

HPTLC fingerprint is one of the versatile tools for qualitative and quantitative analysis of active constituents of multicomponent sample and also a diagnostic method to find out the adulterants to check purity.

ESTIMATION OF IRON

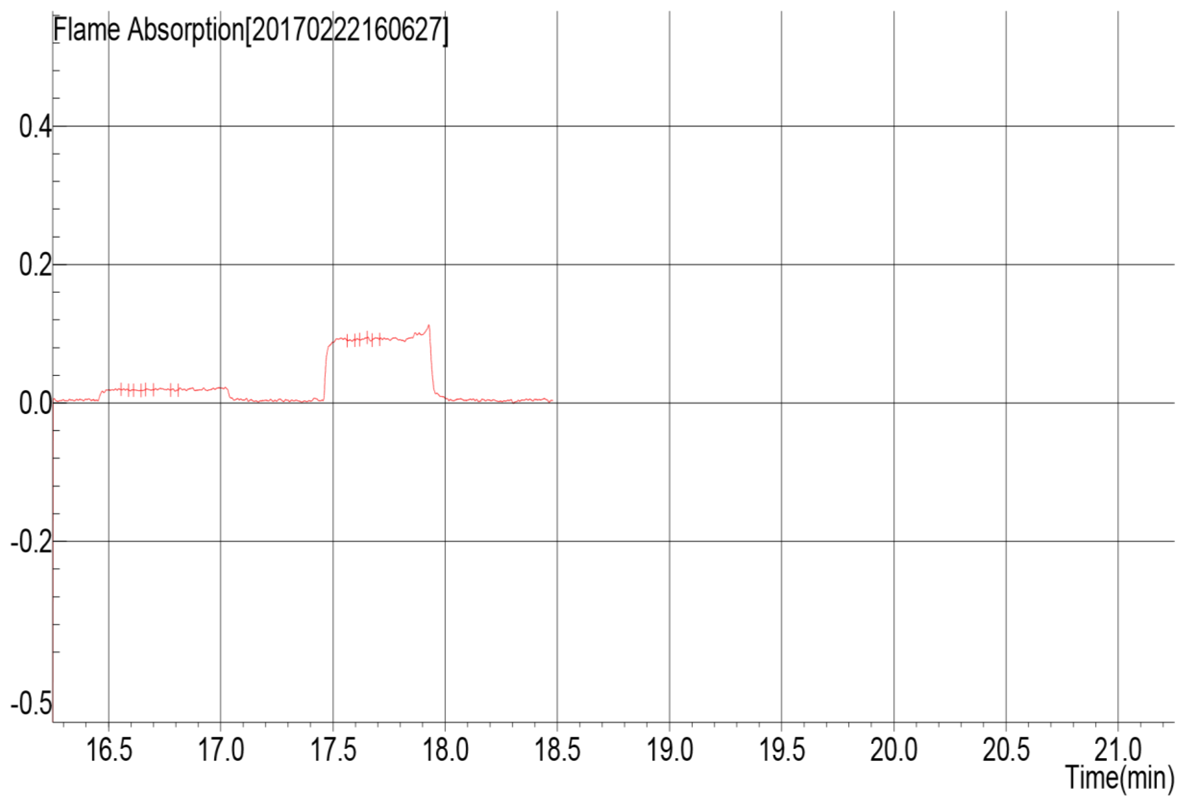


FIGURE 8.11: IRON ESTIMATION

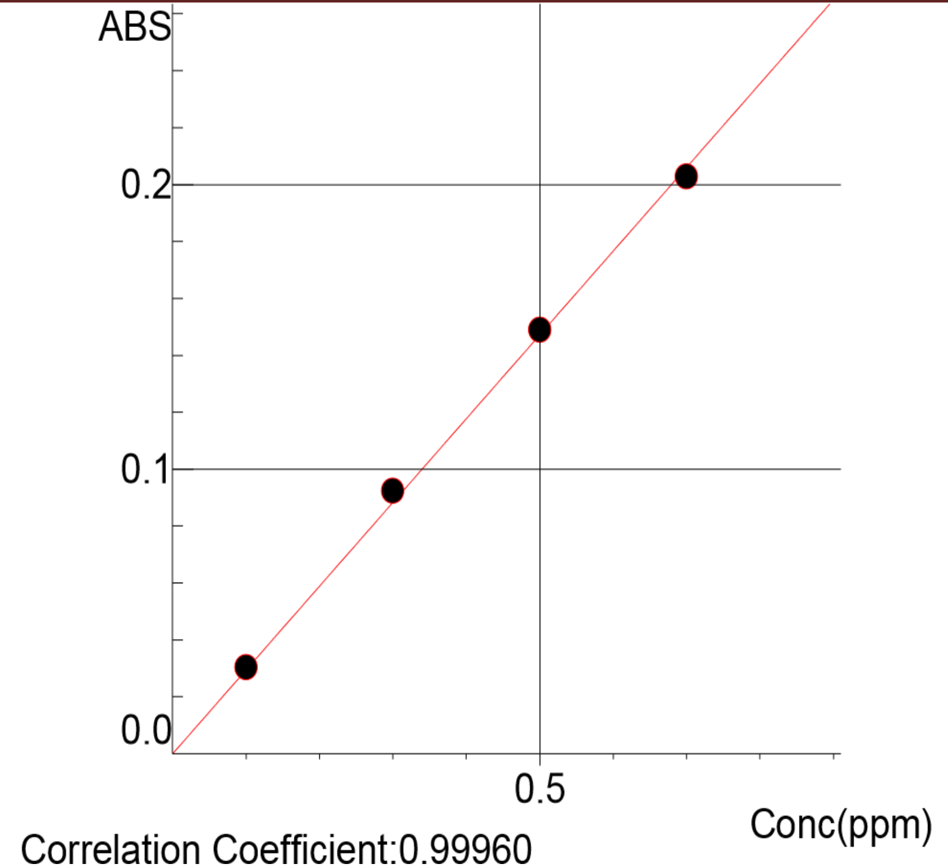


FIGURE 8.12: IRON ESTIMATION

Table8.15: Observation table for Iron estimation

Name	Meas ures	ABS	Conc(ppm)	SD	RSD(%)	Result* (Mg)
Calibration Blank	1	0.0029				
	2	0.0022				
	3	0.0022				
		0.0024	--	0.0004	16.9500	
STD1	1	0.0305				
	2	0.0302				
	3	0.0304				
		0.0304	0.1000	0.0001	0.4585	
STD2	1	0.0932				
	2	0.0929				
	3	0.0911				
		0.0924	0.3000	0.0011	1.2428	
STD3	1	0.1488				
	2	0.1481				
	3	0.1497				
		0.1489	0.5000	0.0008	0.5405	
STD4	1	0.2032				
	2	0.2028				
	3	0.2021				
		0.2027	0.7000	0.0006	0.2835	
Murraya koexigii	1	0.0168				
	2	0.0164				
	3	0.0158				
		0.0164	0.0557	0.0005	3.1675	0.0557()
Moringa oleifera	1	0.0134				
	2	0.0135				
	3	0.0144				
		0.0138	0.0469	0.0006	4.0801	0.0469()
Phyllanthus emblica	1	0.0171				
	2	0.0177				
	3	0.0177				
		0.0175	0.0595	0.0004	2.0168	0.0595()
Eclipta prostate	1	0.0183				
	2	0.0184				
	3	0.0182				
		0.0183	0.0622	0.0001	0.6384	0.0622()
Tinospora sinensis	1	0.0478				
	2	0.0480				
	3	0.0485				
		0.0481	0.1634	0.0003	0.7169	0.1634()
Boerrhavia diffusa	1	0.0266				
	2	0.0257				
	3	0.0265				
		0.0263	0.0894	0.0005	1.8780	0.0894()

S.No	Name of the plants(Ethanolic Extracts)	Concentration of Iron (ppm)
1.	*Murraya koenigii*	0.0557
2.	*Moringa oleifera*	0.0469
3.	*Phyllanthus embelica*	0.0595
4.	*Eclipta prostrata*	0.0622
5.	*Tinospora sinensis*	0.1634
6.	*Boerhavia diffusa*	0.0894

Table 8.16: **Results for Iron estimation of ethanolic extracts**.

The above table explains clearly that the chosen plants contain Iron in considerable high amounts. Thus it throws a light on the fact that, they can be used as the ingredients for the poly herbal formulation to treat anemia.

Estimation of heavy metals

Heavy metals in the sample was digested by wet digestion or dry digestion or high pressure microwave digestion and the amount of heavy metals were determined, i.e. arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) by using graphite furnace atomic absorption spectrophotometer (GF-AAS) and flow injection analysis system-atomic absorption spectrophotometer (FIAS-AAS) and the values were compared with the WHOstandards.

Table No.8 .17: Estimation of heavy metals

Element	Mercury	Arsenic	Lead	Cadmium
Boerhavia diffusa	Nil	Nil	0.21	Nil
Eclipta prostrate	Nil	Nil	0.20	Nil
Moringa oleifera	Nil	Nil	0.22	Nil
Murraya koenigii	Nil	Nil	0.21	Nil
Phyllanthus embelica	Nil	Nil	0.19	Nil
Tinospora sinensis	Nil	Nil	0.22	Nil
Specification as per WHO Guidelines	Not more than 0.5ppm	Not more than 5.0ppm	Not more than 10ppm	Not more than 0.3ppm

The estimation of heavy metals in the sample revealed heavy metals are within the prescribed limits. It is safe and does not cause any harm on consumption

QUANTITATIVE ESTIMATION

Table 8.18: **Spectrophotometric absorbance of Standard and Sample**

S.No	Concentration of standard solution(µg/ml)	Absorbance(435nm)
1.	20	0.135
2.	40	0.175
3.	60	0.213
4.	80	0.306
5.	100	0.347
6.	*Boerhavia diffusa*	0.178
7.	*Eclipta prostrata*	0.165
8.	*Moringa oleifera*	0.159
9.	*Murraya koenigii*	0.167
10.	*Phyllanthus embelica*	0.198
11.	*Tinospora sinensis*	0.156

Standard Calibration Curve for Quercetin

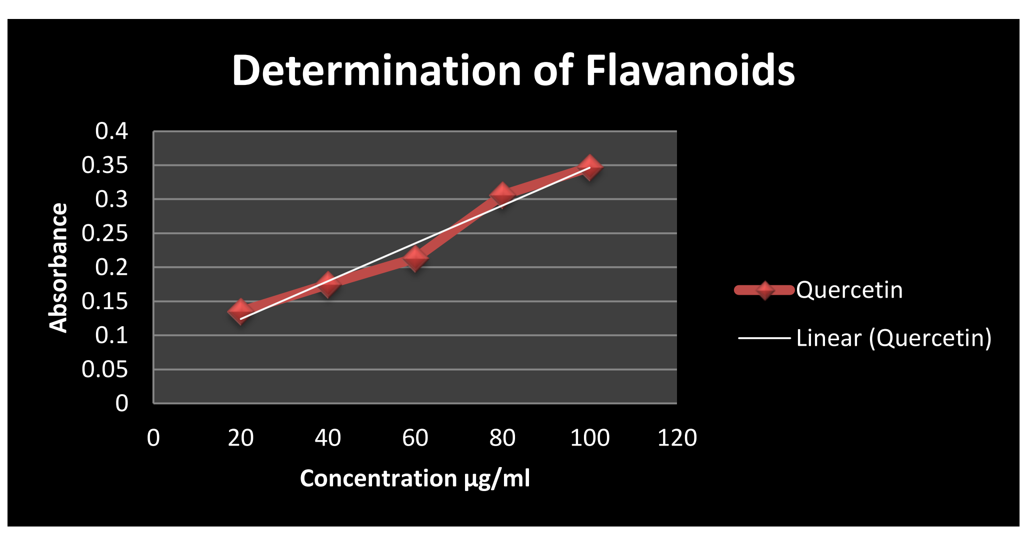


FIGURE 8.13 : DETERMINATION OF FLAVANOIDS

From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of flavonoid present in 1gm of the extract was calculated by applying the dilution factor. The concentration of each extract obtained

TABLE 8.19:PERCENTAGE YIELD OF TOTAL FLAVONOID

S.NO	SAMPLE	CONCENTRATION OBTAINED (mg/gm)	PERCENTAGE OF FLAVONOIDS PRESENT
1.	*Boerhavia diffusa*	43.43	4.3
2.	*Eclipta prostrate*	39.85	4
3.	*Moringa oleifera*	33.72	3.4
4.	*Murraya koenigii*	36.12	3.7
5.	*Phyllanthus embelica*	47.67	5.1
6.	*Tinospora sinensis*	37.32	3.8

TOTAL PHENOLIC CONTENT

Total phenolic content of the individual extract was determined and compared with that of standard.

TABLE 8.20: **Spectrophotometric absorbance of Standard and Sample**

S.No	Concentration of standard solution(µg/ml)	Absorbance(435nm)
1.	20	0.156
2.	40	0.194
3.	60	0.304
4.	80	0.390
5.	*Boerhavia diffusa*	0.266
6.	*Eclipta prostrata*	0.231
7.	*Moringa oleifera*	0.216
8.	*Murraya koenigii*	0.242
9.	*Phyllanthus embelica*	0.278
10.	*Tinospora sinensis*	0.215

Standard Calibration Curve for Gallic acid

To determine the accuracy of the phenolic compound by plotted the standard absorbance obtained from spectrophotometrically. The calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80mcg) of Gallic acid Standard stock solution, the absorbance plotted against concentration

FIGURE 8.14 : **STANDARD CALIBRATION CURVE FOR GALLIC ACID**

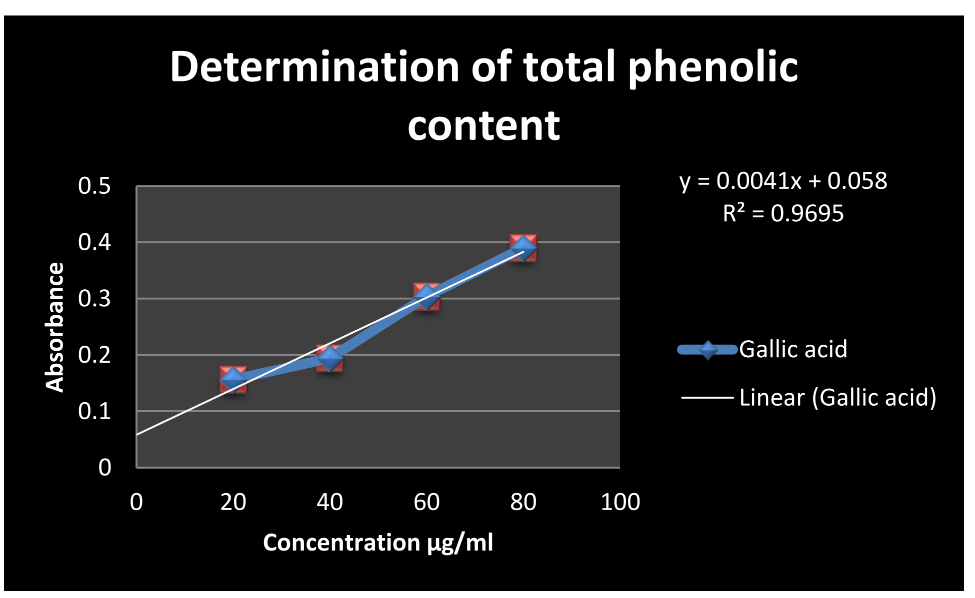


TABLE 8.21:PERCENTAGE OF PHENOLIC CONTENT PRESENT IN EACH EXTRACT

S.NO	Sample	Concentration Obtained (mg/gm)	Percentage of phenolic content Present(%w/w)
1.	*Boerhavia diffusa*	204.33	20.4
2.	*Eclipta prostrata*	168.27	16.8
3.	*Moringa oleifera*	108.17	10.8
4.	*Murraya koenigii*	153.34	14.5
5.	*Phyllanthus embelica*	234.19	22.1
6.	*Tinospora sinensis*	178.41	17.5

PHARMACOLOGICAL ACTIVITY

ACUTE TOXICITY STUDIES

The polyherbal extract was found safe. No abnormalities were observed in the body weight, behavior and blood parameters. No morbidity or mortality was observed. The extracts were safe upto to the dose 2000mg/kg.

EVALUATION OF ANTI-ANAEMIC ACTIVITY

Effect of the combined ethanolic extracts (200mg/kg and 400mg/kg) on the haematological parameters compared with that of the standard in following tables and graphs.

Table 8.22 : **Effect of Polyherbal extract on RBC count**

S. NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	7.3± 0.45**	7.09± 0.65*	7.29± 0.57***	7.39± 0.53***	7.4± 0.44***
2	INDUCED	6.37± 0.43**	5.69± 0.69*	5.47± 0.47***	4.64± 0.55***	4.86± 0.74***
3	TREATMENT	7.37± 0.27**	6.75± 0.59*	6.91± 0.58***	7.63± 0.63***	7.77± 0.81***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA)

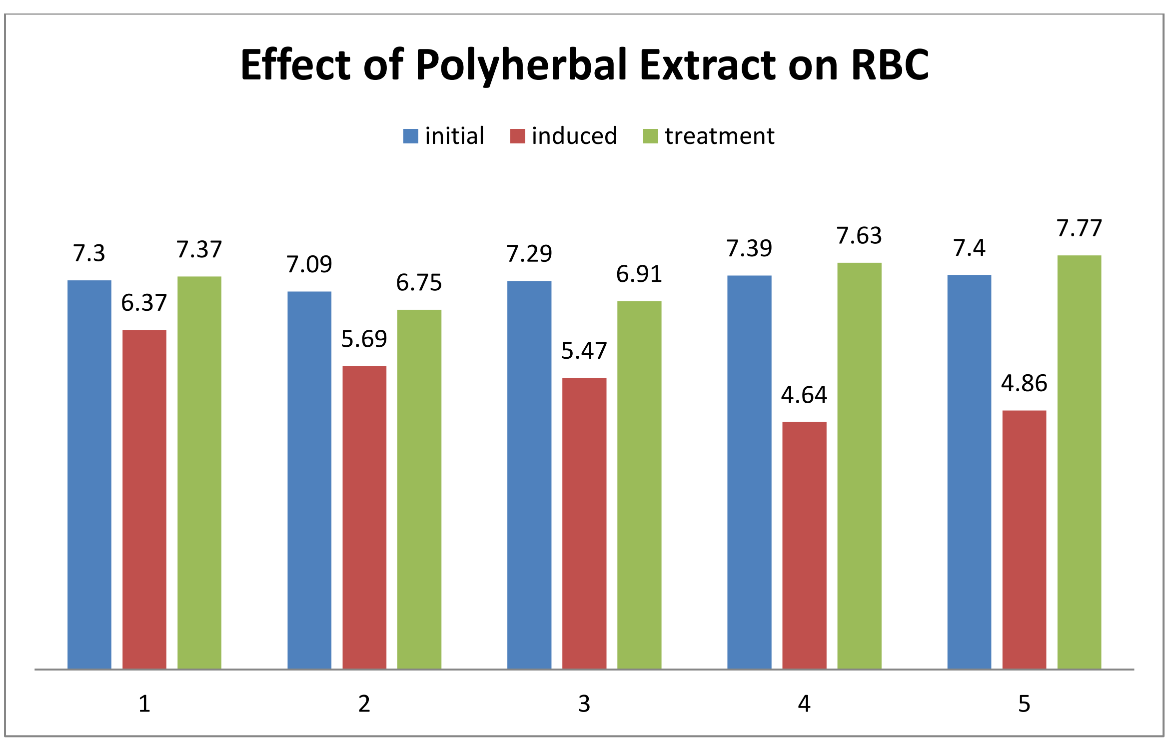


FIGURE 8. 15:**Effect of Polyherbal extract on RBC count**

TABLE 8.23 : Effect of Polyherbal extract on Haemoglobin count

S. NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	14.28± 0.53**	14.61± 0.74*	14.51± 0.36***	14.28± 0.53***	14.45± 0.75***
2	INDUCED	7.45± 0.57**	6.566± 0.49*	6.138± 0.26***	5.703± 0.46***	7.618± 0.55***
3	TREATMENT	14.81± 0.61**	6.58± 0.36*	15.79± 0.60***	17.69 ± 1.50***	18.31± 1.54***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA).

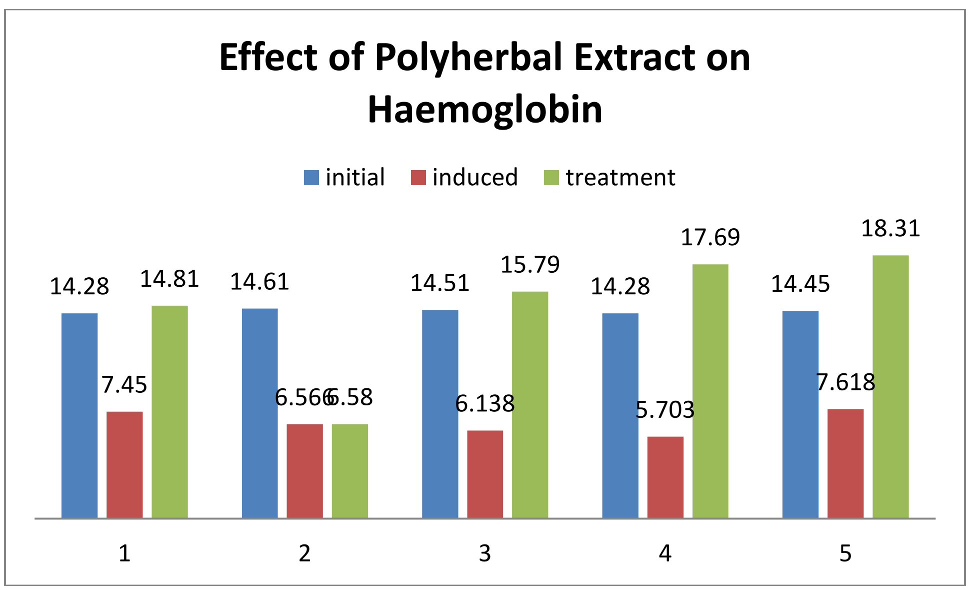


FIGURE 8.16 : Effect of Polyherbal extract on Haemoglobin count

Table 8.24 : Effect of Polyherbal extract on Haematocrit

S. NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	42.85± 2.15**	42.86± 1.75*	43.266± 5.40***	42.13± 1.66***	42.77± 4.84***
2	INDUCED	41.31± 4.48**	30.1± 1.51*	35.38± 3.44***	34.97± 2.86***	37.73± 4.34***
3	TREATMENT	42.99± 4.73**	29.8± 1.72*	44.39± 4.16***	47.031± 5.48***	48.49± 2.48***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA).

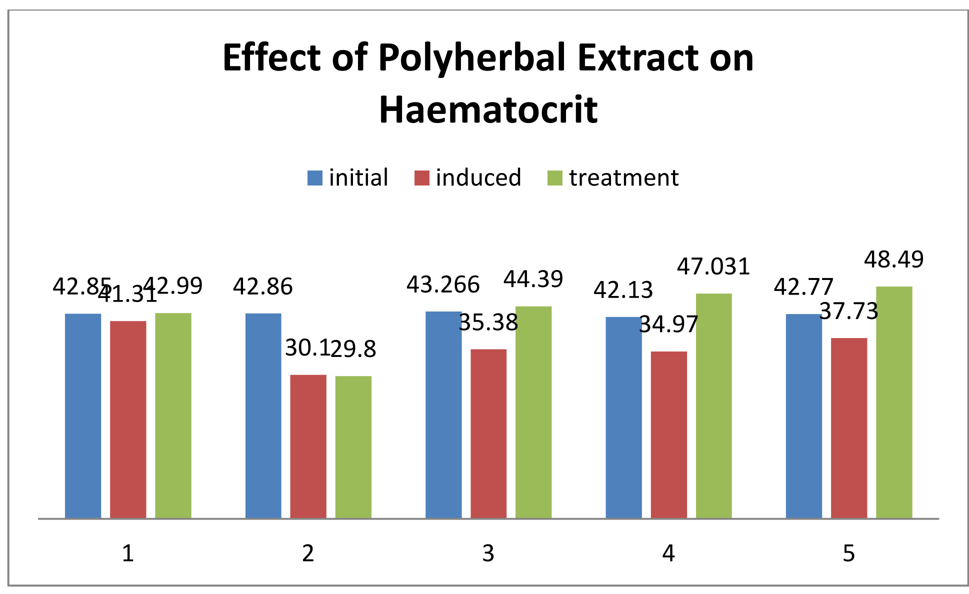


FIGURE 8.17: **Effect of Polyherbal extract on Haematocrit**

Table 8.25 : Effect of Polyherbal extract on MCV

S.NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	61.7± 1.90**	60.7± 1.86*	62.56± 1.53***	61.63± 1.97***	62.68± 1.83***
2	INDUCED	61.73± 1.089**	32.75± 3.62*	32.43± 2.92***	32.53± 2.71***	33.08± 4.03***
3	TREATMENT	64.1± 1.04**	30.71± 6.097*	43.83± 3.34***	56.65± 4.65***	58.33± 6.45***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA).

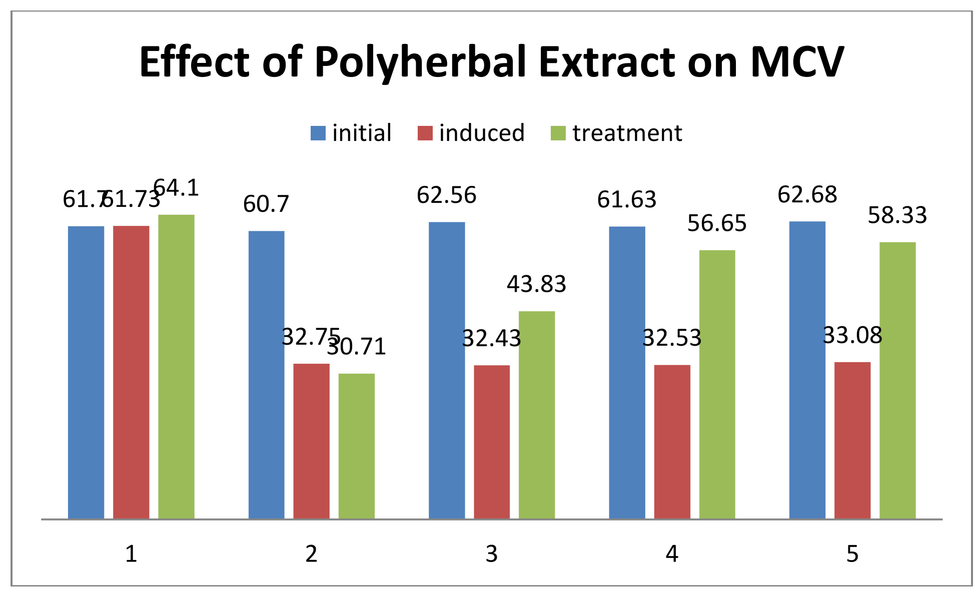


Table8.26 : Effect of Polyherbal extract on MCH

S.NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	18.76± 1.33**	17.85± 0.74*	18.36± 1.69***	17.71± 1.29***	18.45± 1.33***
2	INDUCED	18.86± 1.16**	14.31± 1.39*	15.23± 0.81***	13.35± 0.87***	13.75± 0.85***
3	TREATMENT	18.85± 1.06**	13.25± 1.07*	16.75± 0.83***	18.66± 1.675***	19.38± 1.40***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA).

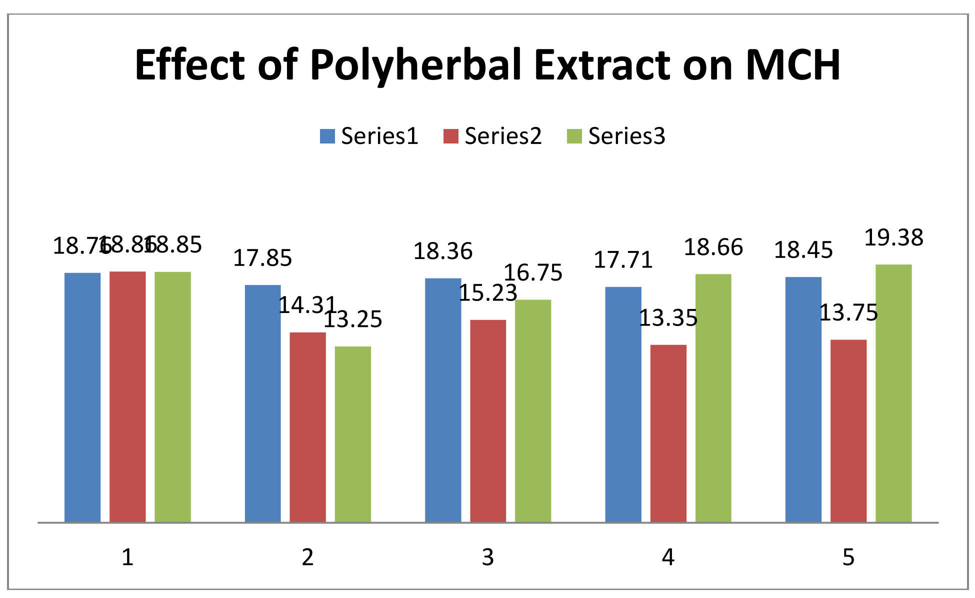
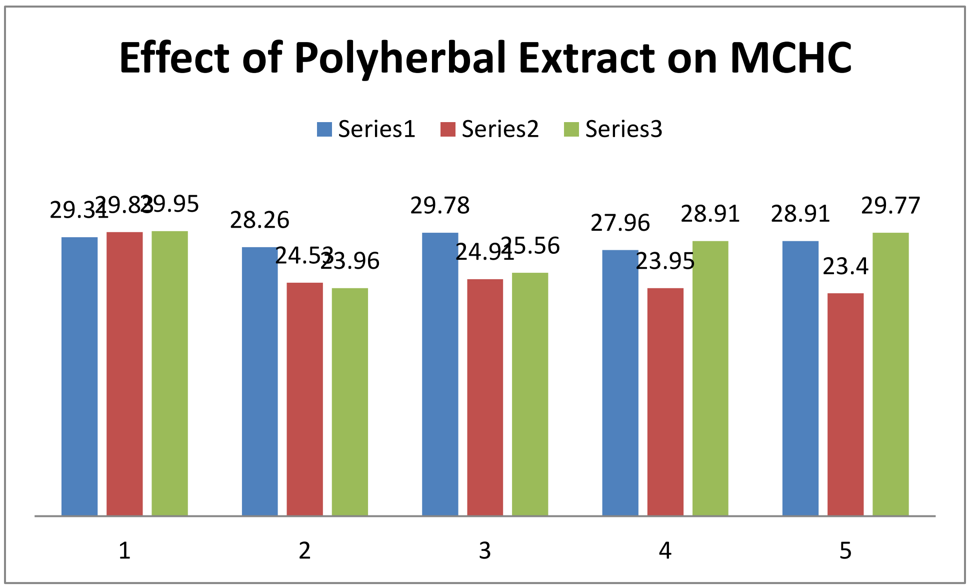


FIGURE 8.18: Effect of Polyherbal extract on MCH

Table 8.27 : Effect of Polyherbal extract on MCHC

S.NO	PARAMETERS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	INITIAL	29.31± 1.72**	28.26± 1.63*	29.78± 1.26***	27.96± 1.56***	28.91± 1.8***
2	INDUCED	29.83± 1.021**	24.53± 1.69*	24.91± 2.221***	23.95± 1.35***	23.4± 1.42***
3	TREATMENT	29.95± 1.005**	23.96± 1.42*	25.56± 2.26***	28.91± 2.07***	29.77± 4.14***

*P< 0.005, **p< 0.01, ***p< 0.0001 as compared to positive control and negative control .The data was analysed using one way Analysis of Variance (ANOVA).



PREFORMULATION STUDIES

The preformulation studies revealed that the powder flow determining parameters like bulk density, tapped density, Carr's Index, Angle of Repose, Hausner's ratio were all within the standard flow characterstics. The polyherbal extract powder had good flow peoperty.

PHARMACEUTICAL EVALUATION OF POLYHERBAL CAPSULES

The formulated capsules were evaluated for the appearance, uniformity in weight and disintegration time.

Table 8.30 : ORGANOLEPTIC CHARACTERS

S.NO	NAME OF THE TEST	OBSERVATIONS
1.	Description	Greenish black powder contained in Blue cap/ transparent body “0” size capsule
2.	Colour	Greenish black powder
3.	Odour	Characterstic odour
4.	Taste	Bitter

TABLE 8.31 : PHYSICAL PARAMETERS

S.NO	NAME OF THE TEST	OBSERVATIONS
1.	pH(1% Aqueous Solution)	7.33±0.21
2.	Moisture Content	3.2% ± 0.14
3.	Average Weight	253mg ± 5.65
4.	Uniformity of weight	RSD : 3.8
5.	Disintegration time	2’32 ± 0.34

Summary and Conclusion

SUMMARY AND CONCLUSION

There are several synthetic drugs available and are still on the way being discovered by many young research fellows of our kind, but still the affinity for herbal drugs hasn't reduced. People feel secure in using the plant based drugs for their chronic diseases.

People still rely on nature which has blessed human kind with abundant medicinal plants for prevention and cure of disease.

The prevalence of anaemia is increasing worldwide. Though medicines are available for the treatment of anaemia in allopathic system of medicine, its compliance is an issue due to sideeffects.

Therefore this study is an attempt to formulate and evaluate the folklore claims of six indigenous herbs viz., Boerhavia diffusa, Eclipta prostrata, Moringa oleifera, Murraya koenigii,Phyllanthus embelica, Tinospora sinensis,as capsules for the treatment of anaemia.

The selected plant powders were subjected to preliminary evaluation. The physio-chemical constants like ash values, extractive values, loss on drying, were performed. The results obtained proved the procured raw materials were of good standard.

Phytochemical analysis was performed for the powder as well as the ethanolic extracts of the plants. It showed the presence of major phyto constituents like alkaloids, flavanoids, terpenoids, steroids ,carbohydrates, proteins.

Chromatograhical studies revealed the various R_f values, which ensures the presence of the various phytoconstituents.

The dried extracts were transformed to powder, and subjected to pre-formulation studies and it proved the powder had good flow characterstics.

Hence it was filled in capsules and evaluated for the parameters like,appearance, uniformity in weight, disintergration time. The results were good.

The *in-vivo* Anti-anaemic activity was carried out by phenyl hydrazine induced anaemic model using the polyherbal extract. Two doses of the polyherbal extract i.e 200mg/kg and 400mg/kg orally were used for anti-anaemic evaluation. The studies indicates that polyherbal extract at the dose of 400mg/kg has better anti-anaemic activity. The haematological studies also showed increase in RBC and haemoglobin content and the overall improvement in blood quality. These changes are markedly good at higher testdose.

Hence, from these studies it is concluded that the formulation proves effective in the treatment of anemia and therefore is haematinic in nature!

The future studies are recommemded in the stability determination areas for the formulated polyherbal capsules. It also includes the clinical trials,to prove the haematinic activity of the capsules in human subjects.

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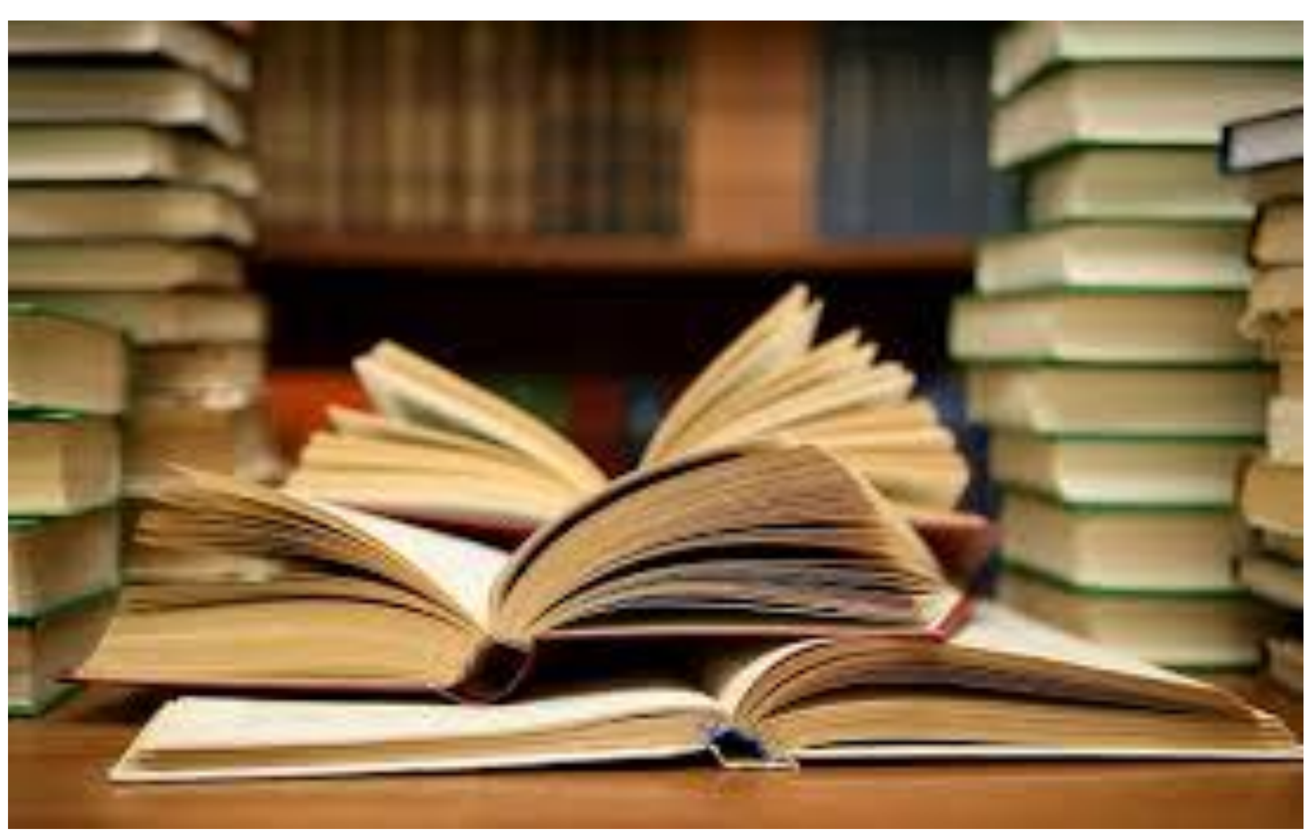

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