CHAPTER I. INTRODUCTION

1.1 Herbal Formulations

Herbs and products containing herb(s) have been in trade and commerce and are currently used for a variety of purposes.[1] The WHO defines an herb as being fresh or dried, fragmented or powdered plant material, which can be used in this crude state or further processed and formulated to become the final herbal product. Treatment of herbs by squeezing, steaming, roasting, decocting or infusing in water, extracting with alcohol, or sweetening and baking with honey can create „herbal products” such as juices, tinctures, decoctions, infusions, gums, fixed oils, essential oils, and resins. These may be used medically or as the starting material for additional processing and as food ingredients. Depending on the sophistication of the „herbal preparation,” these products may be subject to any number of physical, chemical, or biological processes, including pulverization, extraction, distillation, expression, fractionation, purification, concentration, or fermentation. Formulation of the „final product” may require mixing one or more plant preparations with minerals or animal products and constituents isolated from herbal materials or synthetic compounds. These phytotherapeutic formulations may also be referred to as drugs or botanicals, or when taken orally to provide health benefits, they may be called dietary supplements or even food ingredients in some cases.[2]

Herbal medicines are plant derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or animal origin. Herbal medicine preparations are developed and created drugs by the modern pharmaceutical industry. Nowadays, they are manufactured and sold most widely on the pharmaceutical market for curing diseases and promoting public health in India.[3]

Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world’s 12 biodiversity centres with the presence of over 45000 different plant species. India”s diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda sir
*Ayurveda* system of medicine uses about 700 species, *Unani* 700, *Siddha* 600, Amchi 600 and modern medicine around 30 species.[4]

India is sitting on a gold mine of well-recorded and traditionally well-practised knowledge of herbal medicine. This Country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. There are very few medicinal herbs of commercial importance which are not found in this country. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the Third World countries.[5]

**Indian traditional medicine**

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002). WHO has also issued Guidelines for the Assessment of Herbal Medicines (WHO, 1996). These guidelines defined the basic criteria for the evaluation of quality, safety and efficacy of herbal medicines with the goal of assisting national regulatory authorities, scientific organizations and manufacturers in assessing documentation, submissions and dossiers in respect of such products. It was recommended that such assessments take into account long-term use in the country (over at least several decades), any description in the medical and pharmaceutical literature or similar sources or documentation of knowledge on the application of a herbal medicine, and marketing authorizations for similar products. Although prolonged and apparently uneventful use of a substance usually offers testimony of its safety, investigation of the potential toxicity of naturally occurring substances may reveal previously unsuspected problems. It was also recommended that regulatory authorities have the authority to respond promptly to new information on toxicity by withdrawing or limiting the licenses of registered products containing suspect substances, or by reclassifying the substances to limit their use to medical prescription. The guidelines stressed the need for assessment of efficacy including the determination of pharmacological and clinical effects of the active ingredients, and labeling which includes a quantitative list of active ingredient(s), dosage, and contraindications. [6]

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and less:
chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by ecofriendly processes and will bring economic prosperity to the masses growing these raw materials. [7]

During the latter part of the 20th century herbalism has become main stream worldwide. This is due in part to the recognition of the value of traditional and indigenous pharmacopeias, the incorporation of some derived from these sources into pharmaceuticals, the need to make health care affordable for all, and the perception that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived. For a variety of reasons more individuals nowadays prefer to take personal control over their health, not only in the prevention of diseases but also to treat them. This is particularly true for a wide variety of chronic or incurable diseases (cancer, diabetes, arthritis) or acute illnesses readily treated at home (common cold etc.) In this respect many individuals have become disenchanted with the worth of allopathic treatments, and the adverse effects that can be anticipated. [8]

1.2 Preference of herbal drugs in modern societies

Recent years have witnessed a renewed interest in plants as pharmaceuticals in the western world. In the global context, herbal medicines flourish as the method of therapy of choice in many parts of the world. In recent years, the increasing demand for herbal medicines is being fueled by a growing consumer interest in natural products. Now it is finding new popularity as an alternative conventional medicine even in the industrialized countries and the adoption of crude extracts of plants for self-medication by the general public is in the increase. [9]

1.3 Categorization of herbal medicines:

1.3.1 Traditional herbal medicines

These are defined as herbal medicines (single or mixture of herbs) that have been widely used, supported by well-established safety and efficacy data, or have been used within the local community for a minimum period of 15 years. This category would also include traditional medicine formulations to which minor changes have been made. Herbal medicines that are not indigenous to the Eastern Mediterranean Region, e.g. ginseng, could also be included if t
used within the region and if sufficient knowledge about their safety and efficacy exists.

1.3.2 New herbal medicines

Herbal medicines (single or mixture of herbs) can be considered “new herbal medicines” if never used within the community or region, used for only a short period of time, used to a very small extent (few uses in a small number of patients), or used in a new combination of herbal substances never combined before.

1.3.3 Phytotherapeutic agents or phytomedicines

Phytotherapeutic agents or phytomedicines; are standardized herbal preparations consisting of complex mixtures of one or more plants, which are used in most countries for management of various diseases. According to the WHO definition, herbal drugs contain as active ingredients plant part or plant materials in the crude or processed state plus certain excipients, i.e. solvents, diluents or preservatives. The active principles responsible for their pharmacological actions are not usually known.[10]

1.4 Quality – A Major Concern

Quality of herbs has become a major concern following reports of heavy metals in Indian herbs. Adulteration of plants is a serious problem. Some of the common adulterants are: botanicals, toxic metals, microorganisms, microbial toxins, pesticides, and fumigation agents. One study showed that 64% of Herbal Medicinal Products (HMP) samples collected in India contained significant amounts of lead (64% mercury, 41% arsenic and 9% cadmium). A recent Harvard Medical School study reported that 14 (20%) of 70 HMPs contained heavy metals. However, this problem is not unique to ayurvedic medicine. Other traditional medicines – Chinese, Middle East and South American-have also been implicated. Such contamination can lead to serious harm to patients taking such remedies and could also interfere with the assessment of safety in a clinical trial. Quality has to be assured at all stages – herbal raw materials, processing of herbals and finished herbal medicines. [11]
1.5. Safety:

Doubts on safety of the herbal drugs is one of the most convenient weapon that comes into play in any event of fall out of ill effects of the herbal drugs, even when it could be due to misuse or unauthorized use/prescription. Citation of historical or traditional use is often cited as the basis of safety. However, in modern perspectives such a reference can only be of collateral corroboration and appears to lack any significance standalone. Rationally, the safety aspects have been less rigorously attended and emphasized in the literature. The therapeutic references on the herb have over-shadowed any effect as the therapeutic action appears to be the key focus and toxicity appears to have gone barley beyond tolerating (via co-administration of another herb) or specific linkage of the herb with food or drinks. Thus, a growing library of the epidemiological, toxicity data, co-effects, secondary effects etc. need to be appended to the information on historical/traditional use of the herb. Also, such growing database information would keep the potential consumers well educated and informed about the herb and forms an important platform for academic and investigative discussion. Particularly, sharing, documentation and publication of the negative (adverse effect) or null data is most important for rational conclusions to be drawn for the therapeutic action of the herbs. Similarly, a good placebo system needs to be evolved for herbs and herbal products for corroboration of the claimed benefits.[12]

![Graph showing frequency of occurrence of herbs in herbal formulation in India](image)

Fig.1.1. Frequency of occurrence of herbs in herbal formulation in India
1.6. W.H.O. Guidelines for Quality Standardized Herbal Formulations

a. Quality control of crude drugs material, plant preparations and finished products.
b. Stability assessment and shelf life.
c. Safety assessment; documentation of safety based on experience or Toxicological studies.
d. Assessment of efficacy by ethnomedical information and biological activity evaluations. The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC and GC).

The standardization of crude drug materials includes the following steps:

1. Authentication (Stage of collection, parts of the plant collected, regional status, botanical identity like phytomorphology, microscopical and histological analysis, taxonomical identity, etc.)
2. Foreign matter (herbs collected should be free from soil, insect parts or animal excreta, etc.)
3. Organoleptic evaluation (sensory characters – taste, appearance, odor, feel of the drug, etc.)
4. Tissues of diagnostic importance present in the drug powder.
5. Ash values and extractive values.
6. Volatile matter
7. Moisture content determination
8. Chromatographic and spectroscopic evaluation. TLC, HPTLC, HPLC methods will provide qualitative and semi quantitative information about the main active constituents present in the crude drug as chemical markers in the TLC fingerprint evaluation of herbals (FEH). The quality of the drug can also be assessed on the basis of the chromatographic fingerprint.
9. Determination of heavy metals – e.g. cadmium, lead, arsenic, etc.
10. Pesticide residue – WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin cause serious side-effects in human beings if the crude drugs are mixed with these agents.
11. Microbial contamination – usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits c
clearly throws light towards the harvesting and production practices. The substance known as aflatoxins will produce serious side-effects if consumed along with the crude drugs.[13]

Standardization of herbal formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phytochemical, and standardization, and In-vitro, In-vivo parameters. The quality assessment of herbal formulations is of paramount importance in order to justify their acceptability in modern system of medicine. One of the major problems faced by the herbal industry is the unavailability of rigid quality control profiles for herbal materials and their formulations. In India, the department of Ayush, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurvedic preparations. The subject of herbal drug standardization is massively wide and deep. There is so much to know and so many seemingly contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental function. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization.
1.7. Physical/physicochemical standardization

In the standardization of herbal material, physical and physico-chemical factors play an important role in the establishment of purity and quality. Ash values testify for the presence or absence of foreign matter like silica; extractive values indicate the extractable matters in a solvent and is an indication of possible exhausted material.

Advantages of Herbal Medicine

- They have large amount of use.
- They have better patient tolerance as well as acceptance.
- The medicinal plants have renewable source of cheaper medicines.
- Improvements in the quality, efficacy and safety of herbal medicines with the development of science and technology.
• Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
• they are cheap in cost.
• they are not harmful.
• they are more effective than any synthetic drug.
• Throughout the world herbal medicines have provided many of the most potent medicines to the vast arsenal of drugs available to modern medical science, both in crude form. [14]

Physicochemical parameters were determined as per guidelines of WHO. Raw materials were evaluated for total ash value, acid insoluble ash, moisture, alcohol soluble extractive value, water soluble extractive value, pH (1% w/v) and the finished product was evaluated by weight variation, pH (2% w/v), total ash value, acid insoluble ash, moisture, alcohol soluble extractive value, water soluble extractive value, disintegration time, dissolution using standard pharmacopoeia methods. Tannin content was estimated by gravimetric method, Vitamin C by UV Spectrophotometer and Gallic Acid content by High Performance Liquid Chromatography in both raw materials and finished product. [14]

The concept of polyherbalism is peculiar to Ayurveda although it is difficult to explain in term of modern parameter. Sarandghar Samhita highlights the concept of synergism behind polyherbal formulations. Ayurveda has fundamental aspects for drug formulation. The herbs are selected according to disease other herbs are used to prevent side effect arising from chief herb. It is evident that there are many herbal formulations of varying potency since these preparation act by different mechanism, it is theoretically possible that different combination of these extract will do better job in reducing blood glucose. In the traditional system of plant medicine it is usual to use plant formulation and combined extract of plant are used as a drug of choice rather than individual ones, to get the benefit of synergism and to find suitable antidiabetic and antioxidant combination therapy. [15]

Physical tests are performed to establish and determine quality and purity of a crude drug. Physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The study involves determination of physicochemical characters [16-20] viz.

1.7.1 Ash values- Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. S
variables like calcium oxalate, silica, carbonate, content of the crude drug affects total ash value. Ash value parameters include total ash value, acid-insoluble ash value and water soluble ash values. Acid insoluble ash value is used to determine the silica impurities, while the significance of total ash value lies in the determination of excess calcium oxalate or calcium carbonate crystals present. Water soluble ash is good indicator of either previous extraction of water soluble salts in drugs or incorrect preparation.

1.7.2 Extractive values- These are also useful for the evaluation of a crude drug and at the same time give idea about the nature of the chemical constituents present, which is helpful for the estimation of specific constituents, soluble in that particular solvent used for extraction. For this purpose we have to determine alcohol-soluble and water soluble extractives. Water soluble extractive value gives idea about presence of tannins, sugars, plant acids, mucilage and other water soluble phytochemicals. It also indicates about drug quality, adulteration and or incorrect processing. The alcohol soluble extractives are also indicative of the same purpose and at the same time are best to determine the resin content of a drug.

1.7.3 Determination of ash
The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash and water-soluble ash. The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth.

Water-soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

The residue remaining after incineration is the ash content of the drug which simply represents inorganic salts, naturally in drug or adhering to it or deliberately added to it as a form of adulteration. Physiological ash is the total ash of the drug is inclusive of physiological as well as non physiological ash. Physiological ash is derived from the plant tissues, while non-physiological ash consist of residue of the extraneous matter (such as soil, sand etc) adhering to the herb itself. Many a tim
admixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with the different inorganic contents for determining ash the powdered drug is incinerated so as to burn out all organic matter. Ash value is a criterion to judge the identity or purity of crude drugs. Total ash usually consists of carbonate, oxides, phosphate, silicates and silica. Acid insoluble ash, which is a part of total ash insoluble in dilute HCL, is also recommended for certain drugs. Adhering dirt and sand may be determined by acid insoluble ash.

1.7.4 Determination of Extractives:-
The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of content of drugs various solvents are used for determination of extraction. The solvent used for extraction is in a position to dissolve appreciable quantities of substances desired.

1. Water soluble extracts:- This method is applied to drugs contain water soluble active constituents of crude drugs such as tannins, sugars, plant acids, mucilage, glycosides etc.

2. Alcohol soluble extracts:- Alcohol is an ideal solvent for extraction of various chemicals like tannins, resins etc. Therefore this method is frequently employed to determine the approximate resin content of the drug. It is also used as an official method for assay in case of Myrrh and Asafoetida. Generally 95% ethyl alcohol is used for determination of alcohol soluble extractives. In some cases diluted alcohol may be used depending upon solubility of constituents of drugs.[21]

1.8. Potential Toxic contaminants in herbal formulation

A World Health Organization survey indicated that about 70–80% of the world populations rely on non-conventional medicine mainly of herbal sources in their primary healthcare. In recent years, we have witnessed the increasing growth in popularity of over-the-counter (OTC) health foods, nutraceuticals, and medicinal products from plants or other natural sources in developed countries. This indirectly indicates that the public is not satisfied with their orthodox medical (OM) treatment. Such increase in popularity has also brought concerns and fears over the professionalism of practitioners, and quality, efficacy and safety of their treatment methods and products from herbal and natural sources available in the market. Over the past decade several news-catching episodes in developed communities indicated adverse effects, sometimes life threatening, allegedly arisen consequential to taking of OTC herbal products or traditional medicines from various ethni
products may be contaminated with excessive or banned pesticides, microbial contaminants, heavy metals, chemical toxins, and for adulterated with orthodox drugs.

Excessive or banned pesticides, heavy metals and microbial contaminants may be related to the source of these herbal materials, if they are grown under contaminated environment or during collection of these plant materials. Chemical toxins may come from unfavorable or wrong storage conditions or chemical treatment due to storage. The presence of orthodox drugs can be related to unprofessional practice of manufacturers. Some of these environment related factors can be controlled by implementing standard operating procedures (SOP) leading to Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources. The public’s belief that herbal and natural products are safer than synthetic medicines can only be ascertained by imposing regulatory standards on these products that should be manufactured using this Good Practices.[22]

1.8.1 Sources of toxic chemicals and contaminations in herbal products.

The practices of most ethnic herbal medicine include the use crude or raw herbs that are collected from the wild or from cultivated fields and their prepared or ready-made (formulated mixture of herbal or other natural materials) products Toxic contaminants may come from Environments and conditions that the medicinal plants are grown or collected The conditions under which they are dried and processed, the storage conditions and conditions during transport. The manufacturing processes when the ready-made medicinal products are produced. [22]

1.9. Contamination of herbal formulation

Contaminants from the source materials such as microbes, microbial toxins, environmental pollutants, or heavy metals should be checked in herbal and traditional medicines. Estimation of the contaminants should be done with safety level identification and control. Stability of the finished product in various conditions to determine its shelf life is important for marketing of the product. The manufacture of the finished products should be in accordance with the good manufacturing practices (GMPs), with post-marketing quality assurance surveillance Evaluation of the toxicity and adverse drug reaction of the herbal preparation has been a neglected area, as herbs are considered natural products and, therefore safe. This lack of information makes it difficult to compare the benefit-risk profile of herbal medicines. Despite the
comparison of traditional medicines with modern drugs with comparative efficacy has not been conducted for most of the drugs. [23]

1.10. WHO Guidelines for Potential contaminants in Herbal Formulations

- Determination of heavy metals – e.g. cadmium, lead, arsenic, etc. WHO, (1998) mentions maximum permissible limits in raw materials only for arsenic, cadmium, and lead, which amount to 1.0, 0.3, and 10 ppm, respectively.

- Pesticide residue – WHO and FAO (Food and Agricultural Organization) set limits of pesticides, which are usually present in the herbs. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, aldrin, and endosulfan cause serious side-effects in human beings if the crude drugs are mixed with these agents.

- Microbial contamination – usually medicinal plants containing bacteria and molds are coming from soil and atmosphere. Analysis of the limits of E. coli and molds clearly throws light towards the harvesting and production practices.

- Limits for Microbial Contamination

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Finished product</th>
<th>Raw materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>$10^1$</td>
<td>$10^4$</td>
</tr>
<tr>
<td>Salmonella</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total aerobic bacteria</td>
<td>$10^5$</td>
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</tr>
<tr>
<td>Enterobacteria</td>
<td>$10^3$</td>
<td>-</td>
</tr>
</tbody>
</table>

- Aflatoxins should be completely removed or should not be present.

- Radioactive contamination – Microbial growth in herbals are usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of WHO.[24]

There have been reports of acute and chronic intoxicati
exhibit organ specific toxicity, hence the delay in manifestation of toxic effects. Lack of standardization is a major concern regarding use of medicinal herbal medicines. Herbal medicines are complex mixtures in which the active ingredient may not be known or may be only a small percent of the total product. Some are believed to achieve their beneficial effects through the combined actions of several ingredients. Little is known about chronic toxicities that might be associated with their prolonged use.

Poor quality of herbal drugs may also be due to contamination with bacteria and fungi, microbial metabolites (i.e. aflatoxins), pesticides or heavy metals residues. Lead and mercury have been frequently found in Ayurvedic preparations: a case of lead poisoning is reported in a woman after consuming an herbal preparation to treat asthenia. Besides coming from air and soil pollution or manufacturing processes, heavy metals can be intentionally added in some Asian herbal preparations due to their alleged pharmacological properties. Many of the crude herbal drugs marketed in Italy come from extra-European countries and the Asiatic market is one of the most present ones. For the import of crude herbal material no quality control procedures are required in Italy; so a thorough purity check of marketed herbal drugs is of particular interest. Considering the growing interest in Western countries for herbal products of Chinese origin, it was decided to perform a set of purity assays on ten Chinese crude herbal drugs chosen among the most used in Italy. Samples were screened for contamination by foreign matter, inorganic residues, heavy metals and microorganisms. [25]

Today, to meet the needs of expanding herbal markets, many popular herbs are no longer totally wild-crafted. Depending on national laws, regulatory policies may control the amounts to be harvested from the wild, how they might be propagated for commercial harvest, or if a restriction of export of live material is allowed. Applying resource management to preserve rare or threatened medicinal species is a matter of concern for many countries where such taxa exist, and there is a need to rigidly enforce national regulations and international policies to ensure future availability. Guidelines for the Conservation of Medicinal Plants formulated by the WHO, the International Union for Conservation of Nature and Natural Resources (IUCN), and the World Wide Fund for Nature (WWF) (WHO/IUCN/WWF, 1993) is regularly updated (e.g., July 28, 2004) and forms the basis for providing a framework for the conservation and sustainable use of plants in medicine.[26]
1.11. Toxicological standardization

Herbal medications are claimed and widely believed to be beneficial; however, there have been reports of acute and chronic intoxications resulting from their use. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies. A common misperception is that medicaments of natural substances cannot be present in toxic concentrations in a variety of herbal preparations and dietary supplements.

The clinical manifestations of metal poisoning have been well characterized. Heavy metal poisoning has decreased because of improved industrial hygiene and environmental controls so that the signs and symptoms of such poisoning are likely to go unrecognized. If metal poisoning is identified, the true source may be wrongly associated with environmental occupational exposures, not medicament. Failure to establish the true cause of exposure means that the patient continues taking the metal-containing medication. Thus, the screening of traditional remedies for efficacy and safety has been recommended to protect public health. Heavy metals are a known contaminant or adulterant of many traditional remedies. The Asian and Indian traditional remedies have been reported to contain high levels of arsenic, lead and mercury and high level of lead respectively. [27]

1.11.1 Heavy Metal Content of Ayurvedic Herbal Medicine Products

20% of 70 Ayurvedic herbal medicinal products contained potentially harmful levels of toxic heavy metals (American Medical Association 2004) Ayurvedic medicines theory attributes an important therapeutic role to metals such as mercury; arsenic and Lead 35 to 40% of the medicines in the Ayurvedic formulary intentionally contain at least one metal.[28]

1.12. Arsenic

Arsenic is a highly toxic, naturally occurring grayish-white element used as a poison in pesticides and herbicides. Arsenic is also found as an ingredient in pigments and wood preservatives. Arsenic contained in wolmanized lumber will not release toxic compounds unless burned. Arsenic can be harmful through inhalation, absorption through skin and mucous membranes, skin contact, and ingestion.

Arsenic is found in the natural environment in some abundance in the Earth’s crust and in small quantities in rock, soil, water and air. It is preminerals. About one third of the arsenic in the atmosphere
sources, such as volcanoes, and the rest comes from man-made sources. Due to natural geological contamination, high levels of arsenic can be found in drinking water that has come from deep drilled wells. This is particularly true for Bangladesh. Industrial processes such as mining, smelting and coal-fired power plants all contribute to the presence of arsenic in air, water and soil. Environmental contamination also occurs because it is used in agricultural pesticides and in chemicals for timber preservation.

Arsenic occurs in different forms and some is transported between different parts of the environment where it may change its form. Arsenic in weathered rock or soil can be picked up and moved by the wind and water. Many arsenic compounds bind to soil and only move short distances when water percolates down through the soil. If arsenic is released into the atmosphere by industrial processes or volcanic activity, it attaches to particles that are dispersed by the wind and fall back to the ground. Microbes in soil and sediment also release substances containing arsenic into the atmosphere. These are then converted to other arsenic compounds that settle back onto the ground.[29]

One is a rigorous procedure that has been employed in natural product chemistry and is appropriate for the identification of compounds of previously unknown structure. Arsenic species are separated from a large quantity of starting material, purified and isolated, and their structures determined by X-ray crystallography, NMR spectroscopy, IR spectroscopy, mass spectrometry, UV-visible spectroscopy and elemental analysis. The method affords an unequivocal identification, but requires a rather large amount of arsenic compound and, usually, much time. This type of approach has been used to provide The other method is to combine a separation method with selective and sensitive detection methods. A typical method is a chromatographic separation with atomic absorption or emission spectrometric detection. These methods are selective and sensitive if appropriate combinations are made, and are suitable for both quantitative and qualitative analysis if standard arsenic compounds are available. [30]

1.12.1 Human Health Effects of Arsenic

Arsenic may serve a useful function in the body, but only at very low levels. If there is a useful role, the amounts found naturally in foods are enough or more than is needed. Excessive exposure is clearly harmful. At very high dosages arsenic causes immediate (acute) effects including nausea, vomiting, and diarrhea. Ingestion of two grams or more may be lethal in a very short time. More commc
involves much less than this, and may not cause any immediate or observable effects. Arsenic at low doses over a long period of time is known to cause skin changes that may lead to skin cancer. More recently arsenic has been found to cause other kinds of cancer including lung, colon and bladder cancers. It is classified as a Class A (known) human carcinogen by the US Environmental Protection Agency, and has also been associated with harmful effects on the heart and the circulatory system chronic exposure of animals to arsenic in water may also be harmful to them. Prolonged use of arsenic-rich water for irrigation can result in elevated soil arsenic levels that may become harmful to plants, animals and human beings in the area. [31]

1.13 Cadmium

Cadmium is a toxic heavy metal, well known for its occupational health risk, and cadmium (as a pollutant of air and water) is an increasing public health concern. Inhalation of cadmium fumes or dust is the primary cause of cadmium exposure. Contamination of ground water (wells) and food are the other predominant sources of environmental pollution Cadmium accumulates in the human body a half-life every year. Though it is recognized as a neurotoxic and nephrotoxic metal in developed countries, there is not much awareness of its toxicity in developing countries. [32]

1.13.1 Human Health Effects of Cadmium

The kidney is the critical target organ for the general population as well as for occupationally exposed populations. Cadmium is known to accumulate in the human kidney for a relatively long time, from 20 to 30 years, and, at high doses, is also known to produce health effects on the respiratory system and has been associated with bone disease. Most of the available epidemiological information on cadmium has been obtained from occupationally exposed workers or on Japanese populations in highly contaminated areas. Most studies have centered on the detection of early signs of kidney dysfunction and lung impairment in the occupational setting, and, in Japan, on the detection and screening for bone disease in general populations exposed to cadmium-contaminated rice. More recently, the possible role of cadmium in human carcinogenesis has also been studied in some detail. [33]

1.14. Lead

Lead is a ubiquitous toxicant. Lead poisoning is an insidious disease that can result in developmental delays, behavioral disorders and irreversible brain damage. The major signs and symptoms of lead poisoning are pallor, gingival lead line, gastrointestinal disorder, and anemia, renal and neurological s
neuropathy, ataxia and memory loss) in adults. Chronic exposure to lead is associated
with renal dysfunction whilst, chronic lead toxicity will also lead to sterility in adults.
Coincidentally, lead poisoning from traditional remedies mainly used for enhancing
sexual performance has been reported and lead has adverse effects on the male
reproductive system. [34]

![Fig.1.2. Level of Lead in marketed herbal formulations](image)

**1.14.1 Human Health Effects of Lead**

Lead is well known to inhibit the biosynthesis of heme, and consequently of
hemoglobin and decrease the life span of circulating red blood cells. Iron deficiency
and Pb toxicity can be synergistic and potentially devastating, upto 50% more Pb may
be absorbed in children with iron deficiency. The developing fetus is at maximum risk
of lead toxicity. Exposure of pregnant women can transfer significant amount of this
metal to the developing fetus which may result in premature birth, low birth weight or
even abortion. Infants born to mothers exposed to high level of Pb show significant
signs of neurological deficits. In countries, where a major proportion of people are
prone to anemia due to a variety of reasons, Pb exposure can be more serious. [35]

**1.14.2 Clinical manifestation**

Lead has been shown to have much clinical and biological manifestation. Increased levels of serum erythrocyte protoporphyrin and increased urinary excretion
of coproporphyrin and d–aminolaevulinic acid are observed when lead concentrations
are elevated. Inhibition of the enzymes d–aminolaevulinic acid dehydratase and di
hydrobioptrerin reductase are observed at lower levels. The effects of lead on the
hemopoietic system result in decreased haemoglobin Synthesis and anemia has been
observed in children at Pb concentrations above 1.92 mmol/litre (40 mg/dL). For
neurological, metabolic and behavioural reason, children are n
effects of lead than adults. Lead has been shown to be associated with impaired neurobehavioral functioning in children. Lead is known to cause proximal renal tubular damage, characterized by generalized aminoaciduria, hypophosphatemia with relative hyperphosphaturia and glycosuria accompanied by nuclear the reproductive effects of lead in the male are limited to sperm morphology and count. In the female, some adverse pregnancy outcomes have been attributed to lead.

Lead is well known to inhibit the biosynthesis of heme, and consequently of hemoglobin and to decrease the life span of circulating red blood cells.[36]

Iron deficiency and Pb toxicity can be synergistic and potentially devastating, upto 50% more Pb may be absorbed in children with iron deficiency. The developing fetus is at maximum risk of lead toxicity. Exposure of pregnant women can transfer significant amount of this metal to the developing fetus which may result in premature birth, low birth weight or even abortion. Infants born to mothers exposed to high level of Pb show significant signs of neurological deficits. In countries, where a major proportion of people are prone to anemia due to a variety of reasons, Pb exposure can be more serious. [37]

The most common methods currently used for analysis of Pb in biological and environmental samples are flame atomic absorption spectrophotometry (AAS) and graphite furnace atomic absorption spectrophotometry (GFAAS), anode stippling voltametry (ASV), inductively coupled plasma atomic emission spectroscopy (ICP/AES), and inductively coupled plasma mass spectrometry (ICP/MS). For samples analysed by these methods, detection limits of 0.12mmoles Pb/l blood (2.49 mg/dL) can be achieved. [38]

1.15. Microbial Contamination

The microbial quality of pharmaceuticals is influenced by the environment and quality of the raw materials used during formulation. Some infectious outbreaks have been associated with the use of heavily contaminated raw materials of natural origin. The incidence of micro flora in non-sterile medicines generally is indicated by the nature of the ingredients the quality of the vehicle and the care and attitude of personnel involved in their handling. [39]

Raw materials of herbal formulation are frequently carrier of numerous possibly pathogenic microorganisms which may cause serious infection. The permitted numbers of apathogenic bacteria in oral medicaments (10^3 bacteria/g) is very much lower than foodstuff. The limit in natural starting material should be 10^4 bacteria/g and 100 mould or yeast cell/g. The type and frequenc
on the product. Some pharmacopoeial monograph demand absence of one or more indicator micro organism such as E.coli, salmonella species, Staphylococcus aureus and pseudomonas aeruginosa. Human or animal fecal contamination is always possible when the herbs have been harvested from small rural farms still using as fertilizer either „night soil“ or animal dung or when the workers themselves prepare the products under unsanitary conditions. Although survival of bacterial enteropathogens for any length of time is unlikely in dried material, these and other organisms have the potential of causing GI tract and nosocomial infections. [40]

The WHO has specified total microbial contamination limits for contamination crude plant materials the limit adopted for untreated plant material harvested under acceptable hygienic condition. [41]

1.15.1 Pseudomonas aeruginosa

This rod-shaped bacterium, Pseudomonas aeruginosa, is normally found in water, soil and other places that contain moisture. It is a pathogen that takes advantage of the weakened immune system of an ill person and causes different infections. Thus, these types of pathogen are called 'opportunistic pathogen'.

1.15.1.1. Health effect of Pseudomonas aeruginosa Infection

The symptoms of pseudomonas aeruginosa infection, depends on the part of the body that is infected. Fever, muscle and joint pain are symptoms of pseudomonas bacteremia. The following list gives the respective symptoms of each infection,

- Bone infection: Swollen infected part, redness.
- Ear infection: Pain in the ear, reduced ability to hear, facial paralysis.
- Eye infection: Pain in the eye, reduced vision, swollen eyelids.
- Cystic fibrosis: Cough, reduced appetite, fast breathing, enlargement of abdomen.
- Skin infections: Ulcer that can result in bleeding.

1.15.2. Escherichia coli

E. coli are a group of bacteria that can cause a variety of illnesses in humans, including, respiratory illnesses and other problems. There are also many types of E. coli bacteria that are harmless.

One type of E. coli that often causes illness and outbreaks in humans is known as E. Coli O157. There are other strains of E. coli that cause illness as well, but O157 is the most notorious.
1.15.2.1. Health effect of E. coli infection

Symptoms of an intestinal E. coli infection vary by person but can include:

- Stomach cramps
- Diarrhea (usually bloody)
- Vomiting
- Low-grade fever (typically not over 101)

These symptoms usually resolve on their own within 5 to 7 days. E. coli infections are mild in most people, but they can be serious or even life threatening in others. Symptoms of an E. coli infection typically start 3 to 5 days after exposure to the bacteria, but they can occur as early as one day or as late as 10 days after exposure.

1.15.3. Staphylococcal infection:

Staphylococcus aureus S. aureus is a spherical bacterium (coccus) which on microscopic examination appears in pairs, short chains.

1.15.3.1. Health effect of Staphylococcus aureus

The signs and symptoms for Microbial contamination frequently involves in herbal products since all products come from plants. Therefore, microbial contents in herbal products should be evaluated. According to Thai Pharmacopoeia, microbial limitation for nonsterile edible products such as powders, tablets, and capsules should be as follows: For 1 gram or 1 milliliter; total aerobic bacterial count must not exceed $5.0 \times 10^5$ colonies, Enterobacteriaceae count must not exceed $5.0 \times 10^3$ colonies, total yeasts and molds count must not be more than $5.0 \times 10^3$ colonies, Escherichia coli (E. coli) must not be presented more than 50 colonies, while Staphylococcus aureus (S. aureus), Salmonella spp. and Clostridium spp. must not be presented at all in the sample of 10 grams or10 milliliters.[42]

Medicinal plants may be associated with a broad variety of microbial contaminants, represented by bacteria, fungi, and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Herbal drugs normally carry a number of bacteria and molds, often originating in the soil. Poor methods of harvesting, cleaning, drying, handling, and storage may also cause additional contamination, as may be the case with *Escherichia coli* or *Salmonella* spp. While a large range of bacteria and fungi are from naturally occurring micro flora, aerobic spore-forming bacteria frequently predominate. 

The current habit of using herbal remedies to maintain or enhance good health or prevent certain conditions from occurring has resulted in r
consequences. Because they are often promoted as safe and efficacious, the consumer is usually unaware that these practices can be harmful, particularly if they are taken for indeterminate periods. In many cases, their endorsed uses may fundamentally divert from how they were valued traditionally, so safety parameters are unknown. [43]

1.16. Instrumentation: Atomic Absorption Spectrophometer

Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state.

Fig. 1.3 Atomic Absorption spectrophotometer

In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples used in pharmacology, biophysics and toxicology research. [44]

Atomic absorption spectrometry has many uses in different areas of chemistry such as:

- Clinical analysis: Analyzing metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue, semen
- Pharmaceuticals: In some pharmaceutical manufacturing processes, minute quantities of a catalyst that remain in the final drug product
- Water analysis: Analyzing water for its metal content

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by the sample, and compares them to the wavelengths which originally passed through the sample. A signal processor then integrates the cl absorbed, which appear in the readout as peaks of energy al
wavelengths. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element. When an electron moves from one energy level to another within the atom, a photon is emitted with energy E. Atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy, due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. The concentration is calculated based on the Beer-Lambert law. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions. The concentration is usually determined from a calibration curve, obtained using standards of known concentration. However, applying the Beer-Lambert law directly in AAS is difficult due to: variations in atomization efficiency from the sample matrix, non-uniformity of concentration and path length of analyte atoms (in graphite furnace AA). [45]
1.2. AIM:

Nowadays the use of herbal medicines is increased because of serious adverse reactions shown by the allopathic medicines due to this number of various manufacturing units are opened in recent years manufacturing the herbal formulations. It is not necessary that the raw materials which are used in manufacturing of herbal formulation are every time obtained from same geographical source. Hence it cannot be assume that the products are of same quality.

The present work is aimed to study Organoleptic, physiochemical parameters and to determine the potential contaminants in herbal formulation which are widely used by general public as these are the OTC products. People having bad lifestyles suffering from Diabetic mellitus, liver disease and anemia the Ayurvedic practioners are prescribing herbal formulations' for those patients widely. The presence potential contaminants will have adverse effect on the body of human being as such herbal formulations are used for longer duration as it may results in commutation of contaminants. So it is very necessary to determine potential contaminants from such herbal for formulations

Objectives:

- To study the organoleptic parameters in the marketed herbal Formulations belonging to categories Anti-diabetic, Anti-oxidant, Haematinic and Hepatoprotective.

- To study the physiochemical parameters in the marketed herbal Formulation belonging to categories Anti-diabetic, Anti-oxidant, Haematinic and Hepatoprotective by reported method.

- To establish the presence of Toxicological parameters (potential contaminants) like microbial content and heavy metals in marketed herbal Formulation belonging to categories Anti-diabetic, Anti-oxidant, Haematinic and Hepatoprotective by reported method.

- Comparative study of potential contaminants in marketed herbal Formulations.
1.3. Plan of Work

- The 10 marketed herbal formulations company belonging to each category like Anti-diabetic, Anti-oxidant, Haematinic and Hepatoprotective. manufactured by different companies were procured from local market of Yavatmal, India.

- The selected marketed herbal formulations were standardized for Organoleptic, physiochemical and Toxicological parameters.

- The comparative study for physiochemical parameters, Microbial content and Heavy metal contents were carried out.
2.3 Marketed Herbal formulations profile.

The marketed formulations (10 from each) were belonging to categories like Anti-diabetic, Anti-oxidants, Haematinic and Hepatoprotective selected and subjected to standardization for various parameters. The formulations' were manufactured by different manufacturer were given code before standardization.

2.3.1 About Antidiabetic churna

Antidiabetic churna is one of the well-known powdered preparations of Indian system of medicine being used in Ayurveda. This is well known phytomedicines is made in combination with the following composition

- Neem (Azadirachta Indica) : 20%,
- Karela (Momordica Charantia) : 15%
- Jamun (Eugenis Jambolana) : 20%,
- Amla (Emblica Officinalis) : 10%
- Gurmer (Gymnema Sylvestra) : 10%
- Glo : 7%,
- Paner Dodi : 7%,
- Methi (Trigohella Foenum) qs

This proportion as reported in Ayurvedic Formulary of India (AFI). Anti Diabetic Powder is widely consumed by people affected from Diabetes. Anti Diabetic Powder includes pure extracts of jamun, neem, and karela. It is indicated to reduce sugar level in patients suffering from Diabetes Mellitus avoiding the future complications of Diabetes.[46]

Azadirachta indica, also known as Neem, Nimtree, and Indian Lilac is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta, and is native to India and the Indian subcontinent including Nepal, Pakistan, Bangladesh and Sri Lanka. Typically growing in tropical and semi-tropical regions. Neem trees now also grow in islands in the southern part of Iran. Its fruits and seeds are the source of neem oil.

Neem leaves are dried in India and placed in cupboards to prevent insects eating the clothes and also while storing rice in tins. Neem leaves are dried and burnt in the
tropical regions to keep away mosquitoes. These leaves are also used in many Indian festivals like Ugadi.[47]

Scientific classification
Kingdom: Plantae
Division: Magnoliophyta
Order: Sapindales
Family: Meliaceae
Genus: Azadirachta
Species: A. indica

Momordica charantia often called bitter melon, bitter gourd or bitter squash in English, has many other local names. Goya from the indigenous language of Okinawa and karavella from Sanskrit are also used by English-language speakers. It is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is extremely bitter. Its many varieties differ substantially in the shape and bitterness of the fruit. Bitter melon is generally consumed cooked in the green or early yellowing stage. The young shoots and leaves of the bitter melon may also be eaten as greens. Bitter melon is often used in Chinese cooking for its bitter flavor, typically instir-fries (often with pork and douchi), soups, and herbal teas. It has also been used in place of hops as the bittering ingredient in some Chinese-and Okinawan beers.

Scientific classification
Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Rosids
Order: Cucurbitales
Family: Cucurbitaceae
Genus: Momordica
Species: M. charantia [48]

Syzygium cumini, jambul, jambolan, jamblang, or jamun, is an evergreen tropical tree in the flowering plant family Myrtaceae. Syzygium cumini is native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, Malaysia, the Philippines, and Indonesia. The name of the fruit is sometimes mistranslated as blackberry, which is a different fruit in an unrelated family.
The tree was introduced to Florida, USA in 1911 by the USDA, and is also now commonly grown in Suriname and Trinidad and Tobago. In Brazil, where it was introduced from India during Portuguese colonization, it has dispersed spontaneously in the wild in some places, as its fruits are eagerly sought by various native birds such as thrushes, tanagers and the Great Kiskadee. This species is considered an invasive in Hawaii, USA. It is also illegal to grow, plant or transplant in Sanibel, Florida.

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Rosids
Order: Myrales
Family: Myrtaceae
Genus: Syzygium
Species: S. cumini
Phyllanthus emblica, also known as emblic, emblic myrobalan, Indian gooseberry, or Dhatrik (in Maithili), or amla from Sanskrit amalika, Tamil Suaklu in Zomi is a deciduous tree of the family Phyllanthaceae. It is known for its edible fruit of the same name.

Scientific classification
Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Rosids
Order: Malpighiales
Family: Phyllanthaceae
Tribe: Phyllantheae
Subtribe: Flueggeinae
Genus: Phyllanthus
Species: P. emblica

Medicinal uses
In traditional Indian medicine, dried and fresh fruits of the plant are used. All parts of the plant are used in various Ayurvedic/Unani medicine (Jawarish amla) herbal preparations, including the fruit, seed, leaves, root, bark and flowers. According to Ayurveda, amla fruit is sour (amlà) and astringent.
(rasa), with sweet (madhura), bitter (tikta) and pungent (katu) secondary tastes (anurasas). Its qualities (gunas) are light (laghu) and dry (ruksha), the postdigestive effect (vipaka) is sweet (madhura), and its energy (vitya) is cooling (shita).

Gymnema sylvestre is an herb native to the tropical forests of southern and central India and Sri Lanka. Chewing the leaves suppresses the sensation of sweet. This effect is attributed to the eponymous gymnemic acids. G. sylvestre has been used in herbal as a treatment for diabetes for nearly two millennia, and though there is insufficient scientific evidence to draw definitive conclusions about its efficacy two small clinical trials have shown gymnema to reduce glycosylated hemoglobin levels.

Scientific classification

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Asterids
Order: Gentianales
Family: Asclepiadaceae
Genus: Gymnema
Species: G. sylvestre

While it is still being studied, and the effects of the herb are not entirely known. Gymnema reduces the taste of sugar when it is placed in the mouth. From extract of the leaves were isolated glycosides known as gymnemic acids, which exhibit anti-sweet activity. This effect lasts up to about 2 hours. Some postulate that the herb may block sugar receptors on the tongue. This effect was observed in isolated rat neurons. The active ingredients are thought to be the family of compounds related to gymnemic acid: purified gymnemic acids are widely used as experimental reagents in taste physiology and have also an anti-diabetic effect in animal models, reduce intestinal transport of maltose in rats when combined with acarbose, and reduce absorption of free oleic acid in rats. Historically, the leaves were used for stomach ailments, constipation, water retention, and liver disease; however, these claims are not supported by scientific studies.[49]

Fenugreek (Trigonella foenum-graecum) is an annual plant in the family Fabaceae with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semi-arid crop, and its seeds are a common ingredient in dishes from the Indian Subcontinent.
Scientific classification
Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Rosids
Order: Fabales
Family: Fabaceae
Genus: Trigonella
Species: T. foenum-graecum

Fenugreek is used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell.
Cuboid-shaped, yellow-to-amber colored fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, daals, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavor. [50]

2.3.2 About Anti-oxidant Herbal Formulations
Withania somnifera Linn, also known as Ashwagandha, Indian ginseng, Winter cherry, Ajagandha, Kanaje Hindi, Amukkuram in Malayalam and Samm Al Ferakh, is a plant in Solanaceae or nightshade family. The plant is said to have a potential property of pacifying „Vata” in herbal drugs compared therapeutic value of its roots with Panax ginseng. The main constituents of Ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudotropine, cuscohygrine, anferine and anhydrine. Ashwagandha is reported to have anti-carcinogenic effects in animal and cell cultures and it makes the anus tingle by decreasing the expression of nuclear factor-kappa B, suppressing intercellular tumor necrosis factor, and potentiating apoptotic signaling cancerous cell lines. [51]

2.3.3 About Haematinic Herbal formulations
A medicine that increases the hemoglobin content of the blood and used to treat iron-deficiency anemia. The herbal formulations selected for study were as follows,
Each tablet contains: Pravalpishti, Agasthibhasma, Andatwakpishti and amalaki (Embellica officinalis) 50mg each, Mandoorbhasma 15mg, Suvarna makshik Bhasma 10mg, Binders and Excipients q.s.

Praval Pishti is an Ayurvedic medicine, prepared from Coral. It is used in Ayurvedic treatment of cough, cold, Pitta related diseases etc. This medicine should only be taken strictly under medical supervision

Praval Pishti Benefits: It is used in the treatment of cough, cold excessive burning sensation. It improves immunity. It acts as cardiac tonic.

Effect on Tridosha - Balances Pitta and Kapha.

Andatwakpishti

• Anaemias of nutritional, iron deficiency, pregnancy, mal absorption, lactation and menstruation, vitamin C deficiency, Rickets, weight loss, general debility in any age group


Suvarna makshik Bhasma

Suvarna Makshik Bhasma is an Ayurvedic medicine, prepared from an ore of Copper and Iron Pyrite. It is used in Ayurvedic treatment of diabetes, piles, skin diseases etc. This medicine should only be taken strictly under medical supervision

• It has bitter, sweet principles, aphrodisiac and anti aging properties.
• It is useful in the treatment of diabetes, helminthiasis (Intestinal worms), eye diseases, urinary tract disorders, hemorrhoids, skin diseases, anorexia, insomnia, inflammation, poison, etc.

Swarna Makshik Bhasma Uses:

• It has bitter, sweet principles, aphrodisiac and anti aging properties.
• It is useful in the treatment of diabetes, helminthiasis (Intestinal worms), eye diseases, urinary tract disorders, hemorrhoids, skin diseases, anorexia, insomnia, inflammation, poison, etc.[52]
2.3.4 About Hepatoprotective formulations

Hepatoprotective formulations ie churna of 10 different marketed brands were selected for study. It consist of Carduus marianus, Chelidonium majus, Taraxacum officinale, hionanthus virginica, Quassia amara, Heparbovinum, Ceanothus americanus, Colocynthis, Leptandra virginica, Natrum sulphuricum, Nux vomica, Phosphorus, Teucrium marum Carduus marianus

Silybum marianum has other common names include cardus marianus, milk thistle, blessed milk thistle, Marian Thistle, Mary Thistle, Saint Mary's Thistle, Mediterranean milk thistle, variegated thistle and Scotch thistle. This species is an annual or biennial plant of the Asteraceae family. This fairly typical thistle has red to purple flowers and shiny pale green leaves with white veins. Originally a native of Southern Europe through to Asia, it is now found throughout the world.

Uses

Though its efficacy in treating diseases is still unknown, Silybum marianum is sometimes prescribed by herbalists to help treat liver diseases (cirrhosis, jaundice and hepatitis). Silibinin (syn. silybin, sylimarmin may have hepatoprotective (antihepatotoxic) properties that protect liver cells against toxins. Both in vitro and animal research suggest that silibinin has hepatoprotective (antihepatotoxic) properties that protect liver cells against toxins. [53,54,55]

Chelidonium majus,

Chelidonium majus (greater celandine; tetterwort, although tetterwort also refers to Sanguinaria canadensis, nipplewort, swallowwort is a herbaceousperennial plant, the only species in the genus Chelidonium. It is native to Europeand western Asia and introduced widely in North America. While the greater celandine belongs to the poppy family, the lesser celandine belongs to the buttercup family.

Uses

The aerial parts and roots of greater celandine are used in herbalism. The above-ground parts are gathered during the flowering season and dried at high temperatures. The root is harvested in autumn between August and October and dried. The fresh rhizome is also used. Celandine has a hot and bitter taste. The latex has a narcotic fragrance. Preparations are made from alcoholic and hot aqueous extractions (tea). The related plant bloodroot has similar chemical composition and uses as greater celandine. [56]
**Taraxacum officinale**

Taraxacum officinale, the common dandelion (often simply called "dandelion"), is a flowering herbaceous perennial plant of the family Asteraceae (Compositae). It can be found growing in temperate regions of the world, in lawns, on roadsides, on disturbed banks and shores of water ways, and other areas with moist soils. T. officinale is considered a weed, especially in lawns and along roadsides, but it is sometimes used as a medical herb and in food preparation. Common dandelion is well known for its yellow flower heads that turn into round balls of silver tufted fruits that disperse in the wind called "blowballs" or "clocks" While the dandelion is considered a weed by many gardeners and lawn owners, the plant has several culinary and medicinal uses. The specific name officinalis refers to its value as a medicinal herb, and is derived from the word officina, later officina, meaning a workshop or pharmacy. The flowers are used to make dandelion wine, the greens are used in salads, the roots have been used to make a coffee substitute (when baked and ground into powder) and the plant was used by Native Americans as a food and medicine. [57]