Unraveling Siddha Medicinal Formulation – Aya Chendooram

By

ABINAYA.A

(15PCH001)

A Dissertation Submitted to

Avinashilingam Institute for Home Science and Higher Education for Women

(Estd.u/s of UGC Act 1956)

Coimbatore-641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Chemistry

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LIST OF ABBREVIATION

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AIDS	Acquired Immune Deficiency Syndrome
AKC	AyaKandhaChenduram
AYUSH	Ayurveda, Yoga And Naturopathy, Unani, Siddha And Homoeopathy
BG	BALCK GARLIC
CHN	Carbon , Hydrogen and Nitrogen Analyzer
COMET	Single Cell Gel Electrophoresis Assay
DLS	Dynamic light scattering
DMSO	Dimethylsulfoxide
DNA	Deoxyribo Nucleic Acid
DPPH	1,1-Diphenyl-2-Picryl-Hydrazyl
DSC-TGA	Differential Scanning Calorimetry And Thermo Gravimetric Analysis
EDARF	Energy Dispersive X-Ray Fluorescence
EDAX	Energy Dispersive X-Ray Analysis
FRAP	Ferric Ion Reducing Antioxidant Potential
FTIR	Fourier Transform Infra-Red Spectroscopy
GC	Gas chromatography
GC-MS	Gas Chromatography And Mass Spectroscopy
GP	GandhgaParpam
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
HR SEM	High Resolution Scanning Electron Microscope

HRESIMS	High-Resolution Electrospray Ionization Mass Spectrometry
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
IR	Infrared Spectroscopy
MIC	Minimal Inhibition Concentration
NMR	Nuclear Magnetic Resonance
ORAC	Oxygen Radical Absorbance Capacity
Ppm	Parts Per Million
QDG	Quercetin 3,4‘-*O*-Diglucoside
QMG	Quercetin 4‘-*O*-Monoglucoside
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TGA	Thermo- Gravimetric Analysis
TLC	Thin Layer Chromatography
XPS	X-Ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence Spectroscopy

Introduction

1. INTRODUCTION

1.1 Metals in Medicine

Metal ions are required for many critical functions in humans. Scarcity of some metal ions can lead to disease. Well-known examples include pernicious anaemia resulting from iron deficiency, growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency. The ability to recognize, to understand at the molecular level, and to treat diseases caused by inadequate metal-ion function constitutes an important aspect of medicinal bioinorganic chemistry.

Two major drugs based on metals that have no known natural biological function, Pt (cisplatin) and Au (auranofin), are widely used for the treatment of genitourinary and head and neck tumours and of rheumatoid arthritis, respectively. In addition, compounds of radioactive metal ions such as 99mTc and complexes of paramagnetic metals such as Gd(III) are now in widespread use as imaging agents for the diagnosis of disease. Until recently, studies of the toxic effects of metals and their removal, sometimes categorized under "environmental chemistry," have been empirical, with little insight at the molecular level. (**Stephen J. Lippard *et al*., 2009).**

In the long term, “individualized” medical treatments with optimized drugs and clinical regimes tailored to the individual can be envisaged. These might involve cocktails of metallo-drugs aimed at different pathways and targets. Harnessing this knowledge and responding to and taking advantage of this new environment require teams committed to integrating chemistry and biology research and knowledge. These advances will revolutionize the field of metallodrugs. (**Michael J. Hannon 2010).**

1.2 Importance of Iron

Of the more 100 chemical elements known to scientists today, only a relatively small number of these elements are found in the human body. In fact, only 24 different elements are thought to be essential for humans. The largest elemental components of the body, by mass, are oxygen (65%), carbon (18%), hydrogen (10%), and nitrogen (3%).

The other elements in the body, such as calcium, phosphorus, iron, and copper, are known to physiologists as **mineral elements** and **trace elements**. Although these elements make up a much smaller percentage of the mass of the body, they are vital to the body's proper functioning. They must be present in the body in the proper amounts, and they must also be available to react with other elements to form critical molecules and participate in important chemical reactions. In which iron is one of the important elements necessary for the metabolism of the human body. Although iron comprises only 0.008% of the body's mass (approximately 6 g for a 160-lb (75-kg) adult male), it is necessary for our survival.

The word iron is derived from the Latin word "Ferrum". It is found in two forms, essential iron for normal function of the body and the reserve for times of needs. The essential iron is mostly haemoproteins and is present in haemoglobin or erythron and is the major part of the body iron. In addition to hemoglobin**,** other important proteins in the body contain heme groups including **myoglobin,** which takes oxygen from hemoglobin and delivers it to muscle cells, and the **cytochromes,** which are important for generating energy. The other important position of essential iron is in enzymes required for mitochondrial function, DNA synthesis and cell division. Furthermore, iron is used to produce the connective tissues in our body, some of the neurotransmitters in our brain, and to maintain the immune system. (**Rachel Casiday et al., 2007).**

The storage of iron compounds, in the form of ferritin and haemosiderin are present mainly in the reticulo- endothelial system. Ferritin is the protein within the body that stores iron and releases it through channels in a controlled fashion. The unique structure of ferritin forms a spherical shell in which the iron is "stored" as Fe(III) in a crystalline mineral. Ferritin consists of 24 peptide subunits that form two types of channels where these subunits intersect: the 3-fold channel is polar, and the 4-fold channel is nonpolar. The residues that line the channels determine the polarity of the channel. When the Fe (III) in the crystalline mineral is reduced to Fe (II), the iron becomes solvated and ferritin releases the solvated iron, $Fe(H_2O)_6^{2+,}$ through the 3-fold polar channel. Hence, ferritin can control the amount of available iron in the body, preventing iron disorders like anaemia and iron overload.

Because iron plays such a crucial role in the body, it is important for us to maintain an adequate supply of iron. Our bodies continually lose iron through everyday processes such as urination, defecation, sweating, and sloughing off skin cells. Bleeding contributes to further loss of iron from the body. To compensate for these losses and to maintain an adequate supply of iron, we should consume approximately 18 mg of iron daily. Certain conditions, including heavy bleeding and pregnancy further increase the requirement for iron consumption.

When the body's supply of available iron is too low, a condition known as **iron deficiency** results. People with iron deficiency cannot produce an adequate amount of hemoglobin to meet their body's oxygen-transport needs. When the deficiency becomes severe such that there are too few circulating red blood cells or the hemoglobin content of these cells is very low, the condition is diagnosed as **iron-deficiency anaemia**. The most common symptoms of iron-deficiency anaemia are tiredness and weakness due to the inadequate oxygen supply to the body's cells, and paleness in the hands and eyelids due to the decreased levels of oxygenated hemoglobin, which is red-coloured. **Iron-deficiency anaemia can be treated with iron supplements.** It is also possible to have too much iron deposited in the body tissues. This condition is known as **iron overload.** If the iron overload becomes severe (usually when the total amount of iron in the body exceeds 15 g), the condition is diagnosed as **hemochromatosis.** Hemochromatosis can result in serious damage to the body's tissues, including cirrhosis of the liver, heart failure, diabetes, abdominal pain, and arthritis. A recessive genetic mutation can put some people, particularly those of Irish or Celtic descent, at a higher risk for developing hemochromatosis. Treatment for the condition consists of removing blood from the patient to decrease the amount of iron in the body. So the body should need adequate amount of iron. Good dietary sources of iron include red meat, liver, egg yolk, beans, nuts, orange juice and fortified cereals. (**Rachel Casiday *et al*., *2007).***

Anemia results from insufficient oxygen supply, often because of a decrease in hemoglobin (Hb) blood levels that results from metal deficiency, namely, the need for an adequate supply of essential metals in food. Another cause of anemia exists in individuals who have a mutant variety of hemoglobin, HbS, in which valine has been

substituted for glutamic acid in the sixth position of the *f3* subunits. Interestingly, extensive studies have shown that this phenomenon, which leads to sickling of the red blood cells, does not result from failure of the protein to bind heme or from changes in the O_2 binding constant of the iron atom. Rather, deoxy HbS polymerizes into soluble, ordered fibrous structures that lower the ability of blood to carry oxygen effectively to the tissues. These results illustrate the importance of structural features remote from the metal-binding domain in determining the functional characteristics of a metalloprotein. **(Stephen J. Lippard *et al*., 2009).**

Iron supplements are used in all chronic diseases and iron deficiency anemia as diagnosed by modern medicine. Iron is the most highly utilized transition metal in human health. The widespread problem of iron deficiency anemia in developing countries and in developed countries and in defined segment of more advanced societies elevated iron's status as an empowering nutrient. (**Kapila Amit *et al.*, 2015).** The Siddha, Unani and Ayurvedic system of medicine have been using iron in different diseases from 2nd century BC.

1.3 Herbal Medicine

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Throughout the history of mankind, many infectious diseases have been treated with herbals.

Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments **(Chopra et al., 1956)**. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments **(Rabe and Staden, 1997)**. In which siddha is the one of the traditional medicine to cure the diseases from the nature**. (Sheetal Verma *et al*., 2008).**

In recent years more people throughout world are turning to use medicinal plant products in healthcare system. Worldwide need of alternative medicine has resulted in growth of natural product markets and interest in traditional systems of medicine. **(Pravin H. Nikam *et al.*, 2012).**

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources**. (Sheetal Verma *et al.*, 2008).**

Herbal drug technology is used for converting botanicals into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. In order to prove constant composition of herbal preparations, adequate analytical methods have to be applied such as photometric analysis, thin layer chromatography [TLC], high performance liquid chromatography [HPLC], and gas chromatography [GC], DNA Fingerprinting. **(Pravin H. Nikam *et al*., *2012*).**

1.4 Siddha System

Siddha system of medicine is one of the oldest traditional systems in the world. The term Siddha means achievements and *Siddhars* are those who have achieved perfection in the medicine. This system exists in south India especially in Tamil Nadu .Siddha system is an old age medicinal heritage established by Siddhars who were supernatural sages. Varmam, Alchemistry, Yogic practices are some of unique specialties in Siddha system**. (R.Manickavasagam *et al*., 2016).**

In Siddha system, diagnosis is performed by examination of eight important sites/features: pulse, eyes, voice, touch, colour, tongue, faces and urine. The treatment in Siddha system of medicine lays emphasis of pediatrics, toxicology and ophthalmology among plants, animals and minerals that constitute the Siddha drugs.

Preparation based on minerals and metals are used majority in preference to preparations from plants.

The body should be preserved from decomposing by materials that should not decompose easily. So the Siddhars used metallic preparations apart from herbs. They convert the inorganic substances into atomic and ionic form which can be easily absorbed by the body when it is ground with herbal juices and put on fire. It exerts only therapeutic properties without leaving metallic traces**. (Malathi V *et al*., 2015).** Though metals are used in Siddha medicines, they are subjected to rigorous treatments involving different steps before being converted to a therapeutic form.

1.4.1 Concept of Drugs

In Siddha medicine the use of metals and minerals are more predominant in comparison to other Indian traditional medicine systems. In the usage of metals, minerals and other chemicals, this system was far more advanced than Ayurveda. The drugs used by the Siddhars could be classified into three groups:

1. *Mooligai/Thavaram* (herbal product),
2. *Thathu* (inorganic substances) and
3. *Jeevam or Sangamam* (animal products). (**Shukla *et al* ., 2011)**

The *Thathu* drugs are further classified as:

- ✓ *Uppu (Lavanam)*- drugs that are dissolved in water and get decrepitated when put into the fire giving rise to vapor.
- ✓ *Pashanam*: drugs that are water insoluble but give off vapors when put in to fire.
- ✓ *Uparasam*: Similar to pashanam chemically but have different actions.
- ✓ *Ratnas and uparatnas*, which include drugs based on precious and semi-precious stones.
- ✓ *Loham* - metals and metal alloys that do not dissolve in water but melt when put in to fire and solidify on cooling.
- ✓ *Rasam*: drugs that are soft, sublime when put in to fire changing into small crystals or amorphous powders.

- ✓ *Gandhakam:* sulphur is insoluble in water and burns off when put into fire. From the above basic drugs compound preparations are derived. From the animal kingdom thirty-five products have been included in the materia medica. (**Ravishankar *et al*., 2007).**

According to their mode of application the Siddha medicine could be categorized into two classes:

1. Internal medicine and
2. External medicine.

Internal medicine was used through the oral route and further classified in to 32 categories based on their form, methods of preparation, shelf life, etc.
External medicine includes certain forms of drugs and also certain applications like nasal, eye and ear drops and also certain procedures like leech application.

The internal medicines are 32 in number. They include medicines with short life period to medicines which could be used even for hundreds of years. The metallic preparations that could be preserved and used for longer duration are considered as higher medicines. They act even in very small quantity and are capable of curing chronic illness. Some of the important internal medicines are:

- ➢ Surasam - These are extracts of leaves, roots, barks, flowers, rhizomes and fruits, etc, which are boiled so that their water content is greatly reduced. Their duration of life is 3 hours. Example: ginger juice surasa.

- ➢ Charu - They are extracts of leaves, roots, barks, flowers, etc, obtained by grinding them, or obtained by adding some astringent substances or by direct heat application.

- ➢ Kudineer - These are decoctions prepared by adding water to dry herbs, or fresh ones and the boiling them so that the water content is greatly reduced to 1/16th or 1/8 of the water added. Sometimes, some substances are not directly added to the water but instead they are kept in a clean white cloth, tied and immersed in the water. They also could be used for 3 hours.

- Karkam - These are pastes obtained by grinding wet drugs or by adding water decoctions to dry powders. The kalvam or the pestle and mortar should be cleaned when the drugs are ground in it. It should be ensured that all drugs added to it are properly mixed. Its life period is 3 hours.

- Choornam - The dried drugs are taken separately, purified, either by frying or otherwise and made into fine powders and sieved in fine cloth and mixed with other powder of the drug. The purification of the choornam is made by baking it either in water or in milk. The life period is 3 months.

- Manappagu - Some herbal drugs are made in to decoctions separately, or fruits juices are taken and boiled adding sugar or sugar crystals, till an aromatic smell appears, some drugs could also be added at this stage. This is the syrup form of a drug that has a life period of 6 months.

- Rasayanam - The raw drugs are made in to fine powder, sugar and ghee are added to it and taken in a semi-solid form.

- Ennai - although it indicates gingely oil generally, it includes all oily substances of seeds, climbers, barks, tubers, etc. It is classified in to 12 by its origin and five by its mode of application. Life period is 1 year.

- Mathirai - The raw drugs are ground by adding juices, decoctions, ginger juice or breast milk and made in to small pills according to its dose and dried. Life period is 1 year.

- Mezhugu - They are of two types:
    a. Obtained by grinding drugs.
    b. Obtained by heating them by adding oily substances.

Obtained by grinding drugs: Mercurial compounds are ground separately or with other raw drugs adding juices or honey, in to a semi-solid form.

Obtained by heating them by adding oily substances: Mercurial drugs or pashana drugs are heated by slowly adding oily substances or juices and made in to a semi-solid form and then ground well. Life period is 5 years.

- Kuzhambu - Some juices or single juice kept in a vessel and raw drugs are added to it in fine powder form, then heated and taken in a semi-liquid form. Life period is 5 years.

- Chendooram - Metallic substances or toxic salts are made into red colored powders, by the process of either burning them or frying them or exposing to the sunlight or keeping them in specialized pudas by adding decoctions, cheyaneers, dravagams, etc. Life period is 75 years.

- Neer or Parpam - Loghas, Uparasas or Pashanas are made in to white powders by the puda process, burning them, frying them, blowing them by adding juices, cheyaneers, dravagas, etc. It is to be remembered, the calcination process or the parpa of the gold alone is yellow in color unlike other parpas. Life period is 100 years. Puda is the process in which the drugs was kept in a shallow earthern plate, and covered by an identical plate. The mouth is closed by clay paste clothes, in seven layers and kept in a pit and cooked using a given number of cow dung cakes.

- Kalanghu - Mercurial compounds are kept in pudas after burning them with cheyaneer dravagam or juice and blown till they become beads, then gold and zinc are added to it. Life period is 100 years.

- Chunnam - Mercury or Pashana or Logha either individually or combined are ground in Kalvas by adding juices, dravagam or cheyaneer dried, kept in moosai, blown and made in to white powders. They become red when turmeric powder is added to it because of the presence of Lime in it (calcium). Life period is 500 years.

- Karpam - Some herbal medicines are taken in prescribed doses with specific instructions over a period of time, similarly logha, uparasa preparations are also taken.

- Gurukuligai - The sublime mercury is made in to beads in its amalgam forms. Mercury is used in five forms such as *rasam* (mercury), *lingam* (red sulfide of mercury), *veram* (mercury perchloride), *pooram* (mercury subchloride) and *rasa-chinduram* (red oxide of mercury).They are known as *panchasutha*. (**Shukla *et al*., 2011).**

Siddha system of medicine is a complex system of science as it has included in the works of medicine, an extensive set of pharmacopoeia and Alchemy. Siddha system has applied its own fundamental principles in pharmaceuticals; various types of internal medicines and external therapies are in practice, with specialization in iatro-chemistry well before the development of modern science.

The advantages of metal and mineral based medicines in Siddha are as follows

- Mild dose is enough
- They are very effective for treating dreadful diseases
- The action is very speedy
- Wide range of diseases could be treated with the same medicine by changing or without changing the vehicle
- Shelf life is very high
- Unlike herbal drugs, no problem of chemical changes occurs due to geo-climatic conditions
- They are less expansive.

Even if metal based Siddha medicine causes ill effects due to improper administration diet habits and medical advice, appropriate antidotes are available to nullify the effect. (**Shibi.I.G *et al*., 2012).**

According to modern Siddha medicine different metals have different healing effects. Mercury is antibacterial and antisyphilitic; sulphur is used against scabies and

skin diseases, rheumatoid arthritis, spasmodic asthma, jaundice, blood poisoning, and internally as a stool softener; gold is effective against rheumatoid arthritis, and as a nervine tonic, an antidote, and a sexual stimulant; arsenic cures all fevers, asthma, and anaemia; copper is used to treat leprosy, skin diseases, and to improve the blood; and iron is effective against anaemia, jaundice, and as a general tonic for toning the body.

1.4.2 Chendooram

Metallic substances or toxic salts are made into red colored powders, by the process of either burning them or frying them or exposing to the sunlight or keeping them in specialized pudas by adding decoctions, cheyaneers, dravagams, etc. Life period is 75 years. (**Shukla, *et al*., 2011).**

Generally, the method of preparation of metal based Siddha medicines involves conversion of minerals or metals into the oxide or sulphide form by various herbal treatments followed by repeated high temperature calcinations and grinding cycles. The chendooras thus obtained constitute ultra small particles and are taken along with vechicles such as milk, honey, butter, ghee etc according to the diseases. This makes the drug s easily assimilable, eliminating their harmful effects and enhancing their biocompatibility. **Shibi.I.G *et al* (2012).**

Types of Chendooram

Table 1 Types of Chendooram

S.NO	Name of the Chedooram	Uses
1	***Abrachendooram***	Diabetes and urinary diseases
2	***Adikara Chendooram***	Menorrhagia, bleeding
3	***Annabhedi Chendooram***	Anaemia, Jaundice, oedema
4	***Arumugha Chendooram***	Anaemic conditions, scrofula, colic, headache and rheumatic diseases. Also in fissured soles, syphilis, sprue, uterine disorders, orchids and gastric ulcer.

5	***Ayachendooram***	sufficiency of seminal secretion, spermatorrhoea and premature ejaculation. Also in anaemia.
6	***ChandaMarutha Chendooram***	ritis, hemiplegia, paralysis, corea, delirium, fevers and syphilitic sores
7	***Kantha Chendooram***	crocytic anaemia, anaemia, obesity, oedema, scrotal swelling, rheumatic diseases, enlargement of liver and abdominal tumors.
8	***Linga Chendooram***	fevers and diseases due to vitiated "vatha" and "Kapha". Also in skin diseases, nervous debility
9	***Mandoora Chendooram***	Anaemia, jaundice, vomiting, indigestion, hiccup, tastelessness and loss of appetite.

(Dr.S.R.MuthusamyRimp 2010).

Quality of drugs like *parpam, chendhooram* shall be assessed by the analysis of physical characteristics. Properly finished *chendhooram* is tasteless, so fine and smooth, devoid of shining or glittering nature. Whilst analyzing, scrapping of finished *chendhooram* in between thumb and index finger results in lodging of them into the ridges of the fingers, placing of a little amount on water shall show floating i.e, it will not sink. If it is blowed in fire, it will not rebound to the base element. The preparations like *chooranam* should be devoid of moisture content and tackiness. The particles of *chooranam* should be minute and be in short of binding with each other.

As the effectiveness of drug depends upon the tiny or microscopic nature of the particles, the sieve of still finer mesh is mandatory in the processing of c*hooranam*. The *chooranam* prepared by the use of mechanical devices like pulverizer should be allowed to cool down by way of spreading and then mixed well for storage and packing. As there is a chance of plenty of microbes inhabiting on *Choornam*, it should be subjected to the process of purification (*Choorana thooimai*) also. That is baking of *Chooranam* in suitable instrument and then processed into fine powder. (**Dr. Shyamala Rajkumar *et al.*, 2016).**

The rigidity of the methods of preparation for a particular chendooram makes the drug, unique. However very few studies have been carried out to understand the physic-chemical nature of these type of traditional medicines. Though metal based Siddha medicines are time tested drugs. Extansive research works should be carried out to explore its effectiveness and to bring all India Traditional Systems into limelight. (**Shibi.I.G *et al.*, 2012).**

1.5 Standardization of Drug

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. **(Sheetal Verma et al., 2008).**

The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication whether included in modern system of medicine or one of the traditional systems.

Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implication in efficacy and safety. However, due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter though modern analytical technique are expected to help in circumventing this problem. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. Plant materials and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary. (**Kunle *et al.*, 2012).**

1.6 Objective

Siddha system of medicine has distinguished itself from the rest of the traditional medicines by its compassionate way of understanding the human disease and also by the unique way of treating the dreadful disease. As the practice of Siddha system of medicine hikes day by day and now people around the globe started realizing the importance of Siddha formulations and its applications towards various diseases.

Though Siddha system has its own value the concern on availability of physicochemical and standardization data of most of the Siddha formulation is highly doubtful. Hence standardization of Siddha drugs becomes highly essential in order to explore its potency and efficacy in the global market. Still now there is no proper documentary evidence available on standardization aspect of the formulation **Aya Chendooram (AC)**. This prompted us to peruse the systematic analysis of **Aya Chendooram (AC)**. The objectives include,

To study the

i. **Organoleptic properties**

ii. **Physical properties**

- ✓ Melting point of the drug
- ✓ pH values
- ✓ Determinations of ash values
- ✓ TG-DTA Analysis

iii. **Elemental composition**

- ✓ ICP-AES Analysis
- ✓ EDAX Analysis

iv. Crystalline phase

- ✓ XRD Analysis

v. Surface morphology

- ✓ SEM Analysis

Review of literature

2. REVIEW OF LITERATURE

The reviews of literature related to physiochemical analysis, phytochemical constituents and biological activity of the Siddha drug AC and phytochemical constituents of the plant that are used for the preparation of the drug are described below

2.1 Physio chemical analysis of the drug

- The physico-chemical analysis of Gandhagaparpam as part of standardization as per AYUSH Guidelines was done by **Geetha A *et al.*, (2017).** The physico-chemical analysis the drug showed the quality of the drug from FTIR molecular structure of the sample was Interpreted.SEM analysis of showed the size of the drug particle, which was in Nanometre which denoted that the trial drug could have potent drug delivery.XRF analysis disclosed the percentage of elements present in the drug.

- **R.Madhavan *et al.*,(2016)** carried out the purification and standardization of sangu. Sangu (conch) is one of the animal product which was classified under 'Uparasam' by saint Bogar. It is commonly used for Gunmam (peptic ulcer), gastric disturbances etc. Literature scanning revealed the study on standardization of SanguParpam using infrared spectrum and anti-inflammatory activity of sanguParpam in animal model and compared the anti ulcer effect of the same drug with SilasathuParpam in animal model and it proved the anti ulcer effect of the drug.

- **R Shailaja *et al.*,(2016)** confirmed the presence of nanoparticles like **nano mercury, nano gold, nanosilver, nano zinc** in Siddha medicines like LingaChendooram, PoornaChandrodayam, VelliParpam, KshayaKulandhagaChendooram, MuppooraChendooram, ThangaParpam, Naga Parpam, Naga Chendooram, CharaParpametc., with the aid of modern technology like SEM with EDAX, FTIR, ICP-OES, XRD, DLS etc.,

- **R.Madhavan *et al.,* (2016)** prepared and analyzed SanguParpam according to the standard procedures. Which was used very frequently for management of peptic Ulcer disease.Physico chemical characters of SanguParpam were analyzed by sophisticated instruments. Heavy metals like Mercury, Copper, and Lead were reduced after purification and preparation processes and powder property of the samples were good for absorption and flowability. An organoleptic character revealed that the purification and preparation processes were done in a hygienic condition and samples were alkaline in nature. XRD analysis confirmed the Calcium oxide as the major crystalline phase in samples. Findings revealed that more studies were needed to standardize the drug and to evaluate the importance of Siddha drug preparation technique, which may reveal the scope for chemical modulation of traditional methods.

- **Sathiyathilaga B *et al.,* (2014)** prepared a traditional Siddha herbo-mineral drug SanguChunnam. The chemical finger print was checked using techniques like FTIR and ICP-OES. The particle size and chemical elements of both quantitative and qualitative analysis of SanguChunnam were also assessed by SEM EDAX and XRF respectively. The FTIR results showed the presence of O-H stretching band, C-H stretching band, C=O stretching band indicating the presence of O-H and C=O functional groups. The SEM image of SanguChunnam had numerous nanoparticles which ranges between 56-172nm denoting a better bio-availability. The XRF results attributed the presence of 76% of CaO. The ICPOES analysis revealed that the heavy metals such as Arsenic, lead, Cadmium and Nickel were below detectable limits thereby proving SanguChunnam to be safe.

- Raw and purified drugs of Lingam (Cinnabar–Red sulphide of Mercury), Pooram (Mercury sub chloride), Rasa chendurum (Mercury sulphide) and the final product mupooraChendurum were characterized using the modern techniques FTIR, ICPOES, SEM and their anti microbial study were also carried out.FTIR analysis showed the broad peaks at 3584 cm^{-1}, 3524 $cm^{-1}$and 1612 cm^{-1}. Peak at ~2319 cm^{-1} and ~2395 cm^{-1} was assigned to C-H and C-H (methoxy compounds) stretching vibration correspondingly. Trace element analysis by ICP-OES indicated absence of heavy metals Lead, Copper,

Cadmium and Arsenic. Mercury concentration was under acceptable limit (0.97 ppm) at prescribed dose. Microbial load of the preparation had the highest antibacterial activity against Bacillus, Streptococcus and Vibrio species, medium activity against Salmonella typhi and Staphylococcus and considerably low activity against E. coli. SEM analysis indicated agglomeration of the particles and aggregated particle size of 83.3 nm. **MahalakshmiGopal *et al.*, (2014).**

¶ Modern techniques such as XRD, SEM with EDAX, FTIR were used to generate physico-chemical fingerprint of AKC, one of the herbo-mineral siddha medicine prepared as per the siddha classical text by the process of calcination. AKC is used in the treatment of various disorders especially Anemia. The result revealed that the metals in this preparation were in sulphide form which favors the therapeutic efficacy of the drug. Some particles in nano range were also identified.**K. Rajamaheswari *et al.*, (2014).**

¶ The processing and chemical characterization of *Kajjali* drug using various techniques, viz. XRD, SEM, XPS, Particle size analyzer, TGA, and EDXRF have been reported. *In vitro* bovine shrimp assay and Osmotic fragility test have also been performed. XRD pattern of preparation delineates its cubic and hexagonal form with 2θ position at 23.08, 26.42, 27.76, 28.80, 30.46, 31.25, 43.78, 51.86, and 54.30 with d-spacing of 3.83, 3.37, 3.21, 3.09, 2.93, 2.86, 2.06, 1.76 and 1.68 Aº respectively. SEM photomicrograph of *SamagandhakKajjali* particles showed the appearance of particles of 10 µm and less than 5 µm size particles i.e. up to 0.237 µm in size. The drug contains Mercury in the Mercury Sulphide form with free Sulphur and associated with organic contents, whereas EDAX showed the presence of 'Hg'(49.4 %), 'S' (48.70 %), 'Zn' (12.39 ppm), 'P' (0.67%) and 'Se' (0.04 ppm) in the final preparation of *Kajjali*. There is no significant ($p< 0.05$) difference in the *In vitro* toxicity and osmotic fragility of control group with treated ones. These findings help in understanding the therapeutic value, safety aspect and standardization of Ayurvedic drug- *Kajjali*. Though the metallic Mercury is known to be toxic to the biological system, no compelling evidence has been put forth to suggest any toxic effects of *Kajjali.* The results

of structural and chemical characterization of the study, clearly delineates in crystal view manner of its safety concern. **K.S. Thakur *et al.*, (2014).**

¶ Characterization of physico– chemical traits of vaalai rasa chendooram was done by **S. Shajahan*et al.*, (2013).** It **was** prepared as per Agathiyar siddhar method using the ingredients like sulphur, mercury, gold, alum, potassium nitrate and Aloe vera. The prepared drug was analyzed for chemical properties using Fourier Transform Infra-Red (FTIR), Zeta potential and Differential Scanning Calorimetry and Thermogravimetric Analysis (DSC-TGA). The result revealed that the heavy metals were absent except mercury (0.05ppm), which is below the acceptable limit. Gold (12.91%) is the major inorganic constituent presented with other heat stable organic compounds such as flavonoids, alkaloids, glycosides, serpentines, tannin and lignin. The particle size was found to be 846.5nm.

¶ The Mega sanjeevimathiraia Siddha herbometallic drug was prepared by **R. Sathish *et a., l*(2012)** and the chemical finger print , using modern analytical techniques like ICP-OES and FTIR were recorded for the drug. In addition, the particle size of mega sanjeevimathirai was ranged between 3 -10 μ also assessed by HR SEM. The results confirmed the absence of lead, arsenic and cadmium and also the presence of inorganic elements such as mercury (2.96ppm), sodium (6.26ppm), potassium (4.22ppm), calcium (15.10ppm), phosphorous (3.63ppm) and sulphur (6.25ppm). FTIR confirmed the presence of organic moieties such as IR (KBr, cm^{-1}), 3399 cm^{-1} (-OH structure of –COOH group), 1716 cm^{-1} (C=O structure of carboxylic acid), 1618 cm^{-1} (C=N structure), 1214 cm^{-1} (Asymmetric C-O-C structure), 1047 cm^{-1} (Symmetric C-O-C structure).

¶ Standardization of Sanguparpam by using IR spectrum was done by **V.N. Meenadevi *et al.*, (2010)**. It is used for the treatment of indigestion, acidity, hyperacidity, ulcer, carminative and piles. It is also used externally for various skin diseases, pimples and skin crack. Infrared spectroscopic studies of different samples of SanguParpam clearly indicated that the final product was identical in all the cases irrespective of mixing the different plant juices

available in the different regions used for the preparations. Moreover the spectral analysis helped to speculate the functional groups present in the drugs. From the nature of the functional groups, the curative property of the drug can be easily determined scientifically.

¶ The characterization of some of the metal based herbal medicines which were in traditional use for treating infectious diseases were done by using modern techniques such as XRD, SEM, EDAX, IR, TGA, ICP-OES and TEM to generate physico-chemical fingerprint.The results revealed that the metals in most of the herbal medicines were in the oxide or sulfide form. Some of the medicines contained metal particles were also in the nano range. **ArunSudha*et al.,* (2009).**

¶ K. **Elango *et al.,* (2006)** carried out the standardization of KanthaChendooram using pharmacognostical standardization method. Chendooram was prepared by using 8 ingredients, viz. 1. Purified Lode Stone, 2. Purified Sulphur, 3. Lead wort root powder, 4. Eclipta juice, 5. Lime juice, 6. Milk, 7. Egg albumin, 8. Madar Latex.

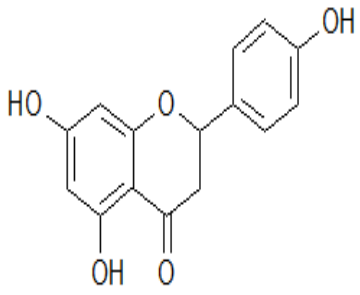
¶ **Anoop Austin *et al.,* (1999)** carried out the standardization of LinghaChendooram' number 1 (i.e., number 1 is prepared by titration process 'Churukku' process) using Cinnabar, the chief ore of mercury .which is a single drug useful in Siddha system of medicine. The standardization was carried out with respect to the presence of Mercury and Sulphur in the drug.

2.2 Biological activity

¶ **P. N. Sushilkumar *et al.,* (2016)** found out the action of different herbs used in the arthritic conditions. They also explain the recent studies on anti-arthritis and/or anti-inflammatory plants, ***Such as Asparagus racemosus, Boerhaaviadiffusa, Cinnamomumzeyllanicum, Boswelliaserrata*Roxb. exColeb.,*Cissusquadrangularis*and*Justiciatranquebariensis, , Lipidiumsativum,, Cynodandactylon, MerremiaemarginataBurm. F., PortulacaoleraceaLinn ,Hemidesmusindicus R.Br. (Anantmul,.***

Tribulusterrestris. Jatrophacurcas. CyperusrotundusL*, *Vitexnegundo, Withaniasomnifera* (L) *ZingiberofficinaleRosc, Costusspeciosus.

¶ ThraatchathiChooranam, a Siddha polyherbal formulation which comprises 32 medicinal plants, has the traditional claim for the management and treatment of cardio vascular diseases, diabetes mellitus, cough, asthma, ulcer etc., Standardization of ThraatchathiChooranam was done by using standard physio-chemical and phytochemical protocols such as Ash values, Extractive values, chemical profiling and marker quantification such as **Gallic acid, Ellagic acid, Naringenin (1), Quercetin (2) and Galangin** using HPTLC fingerprinting. In addition, residue analyses such as heavy metal content, microbial load, pesticide analysis were also examined to strength the standardization process. Qualitative phytochemical screening revealed the presence of total phenols, tannins, flavones, saponins and glycosides. Microbial load and heavy metals were found to be within the AYUSH permissible limits. Pesticide residues and aflatoxins were absent. Quantification of Gallic acid (1.8 mg/g), Ellagic acid (1.9mg/g), Naringenin (6.3 mg/g), Quercetin (21.4 mg/g) and Galangin (3.4 mg/g) confirms the presence of lead molecules. These results revealed the active ingredients responsible for the beneficial effect of Thraatchathi Chooranam and thereby evidence the traditional claim. **G. Ramakrishnan *et al.*, (2015).**



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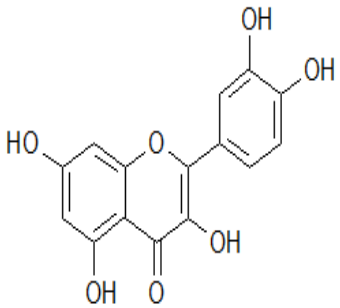

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¶ **HumayunRiaz *et al.,* (2015)** studied the antimicrobial property of ginger. Phytochemical screening of chloroform plant extract showed presence of different chemicals. Cultures of *E. Coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis* were used for the study and identify the antimicrobial strength. Effectiveness of ginger against different conditions attributed to its different constituents (**volatile oils, shogaols, Gingerols and diarylheptanoids**). phytochemical evaluation and antimicrobial assay of ginger root extract was also performed. Ginger possessed a noticeable antimicrobial activity which was confirmed by checking the susceptibility of different strains of bacteria and fungus by measuring the zone of inhibition.

¶ BG was prepared by heat treatment of whole garlic bulbs (Garlic-*Allium sativumL*) at high temperature under high humidity for several days, resulting in black cloves with a sweet taste **Sook Choi *et al.,* (2014).** To clarify how Black garlic changes during the 35 day aging period, the physicochemical characteristics, antioxidant contents, and antioxidant activities were evaluated under controlled conditions of 70 °C and 90% relative humidity. Reducing sugar and total acidity of Black garlic increased during the aging period, whereas pH decreased from pH 6.33 to 3.74. Lightness and yellowness values of Black garlic radically decreased during the aging period, whereas redness values increased significantly. Antioxidant components, including the **total polyphenol and total flavonoids contents of Black garlic**, increased significantly until the 21st day of aging ($p < 0.05$) and correspondingly, the antioxidant activities of Black garlic, measured by DPPH, ABTS, FRAP, and reducing power assays, were highest on the 21st day of aging. These results indicated that Black garlic possessed antioxidant properties during the aging period, and also to reach its optimal antioxidant properties at the 21st day of aging.

¶ Commonly identifiable, easily available, cost effective herbs such as Banyan tree (*Aalamaram*),Common Wireweed*(Arrivaalmookkupachilai*), Chay root (*Impural*),Barmuda grass (*Aruganpul*),Country Fig (*Atthi*),Purging nut (*Kaatamanakku*),Magic nuts (*Maasikkai*), Pomegranate (*Maathulai*),Red silk

cotton (*Mulelavu*),Rhusolina (*Othimaram*), Plantain tree (*Vaalai*),Kino tree (*Vengai*) were reported to have styptic action and wound healing properties as per the Siddha traditional literatures. This review listed the single herbs and poly herbal/metal/mineral drugs having potent styptic activity and documented as well as the potency of the herbs mentioned. **S. Merish *et al.*, (2014).**

¶ **Hazeena Begum V *et al.*, (2014)** reported the in vitro antioxidant activity of Poorna Chandrodayam Chendooram drug. The antioxidant activities of different concentrations of drugs were determined by t**otal polyphenolic content, ascorbic acid, total flavonoids**, DPPH radical scavenging activity, Hydroxyl radical scavenging activity and Nitric Oxide Scavenging activity. Then the results revealed that the effective antioxidant activity of Poorna Chandrodayam Chendooram was found to be increased with increasing concentration.

¶ The antibacterial properties of three herbo-mineral siddha drugs (PalakaraiParpam, PadikaraParpam, Uppu C henduram) on clinically isolated *Enterococcus* strains were evaluated Fifteen bacterial strains isolated from clinical samples were identified as *Enterococcus* faecalis, *E. faecieum*.The susceptibility of the microorganisms to the siddha drugs (PalakaraiParpam, PadikaraParpam, UppuChendooram) were screened by disc diffusion method and the effective drug's MIC value was calculated by agar dilution method. The results revealed that PadikaraParpam was considered sensitive against the tested *Enterococcus faecalis* and *Enterococcus faecium* .Agar dilution was performed with 1%, 0.5%, 0.25% and 0.12% of the drug dilution. MIC was found to be 0.5%. Finally it was concluded that the drug PadikaraParpam contains essential elements which are considered to be good anti-microbial activity against En-terococcus spp. **Shobha K.L *et al.*, (2014).**

¶ **B. Akila, *et al.*, (2014)** reported Physico chemical analysis of GomutrasilasathuParpam by ICP-OES elemental analyzer and CHN analyzer. ICP-OES elemental analysis to support the spermatogenic activity and the

presence of carbon through CHN analysis which increases the therapeutic potential of the medicine.

¶ Herbomineral drug 'ThamiraParpam' by calcining the purified copper foils with earthworm, Clinuslotoidesjuice according to Siddha medicine was prepared by **M.Krishnaveni(2013).**Anti-microbial activity of ThamiraParpam was investigated against both gram positive and gram-negative bacteria and also fungal stains of *Aspersillus fumigates, Aspersillusniger, Fusariumoxysporum, Mentographytessp* in invitro disc and minimum inhibitory concentration methods. The result evidenced the antimicrobial potential of ThamiraParpam and also efficiency of the drug was greater towards gram positive bacteria than gram negative bacteria.

¶ Herbo mineral Gandhaga Mezhugu, especially used in skin diseases was prepared and evaluated for its antimicrobial potential. Antimicrobial efficacy of gandhagam (Raw sulphur), purified Gandhagam and gandhaga Mezhugu were evaluated against six pathogens *Escherichia coli*, *Proteus vulgaris*, *Klebsiellapneumoniae*, *Staphylococcusaureus, Streptococcusmutans, Candida albicans* which was associated with various disease conditions. The agar well diffusion was used to determine the sensitivity of the samples, whilst the micro-dilution method was used for the determination of the MIC. of the samples assayed, the samples of gandhaga Mezhugu were observed to be the more effective against all the tested pathogens. The results provided evidence that the studied samples might be potential sources of new antimicrobial drug. **P. Shanmugapriya *et al.*, (2013).**

¶ The anti-ulcer properties of Tamira Parpam(bio-copper) from earthworm against Aspirin plus pylorus ligation induced gastric ulcer in rats, HCl-Ethanol induced ulcer in mice and water immersion stress induced ulcer in rats were investigated by **M.Krishnaveni (2012).** A significant antiulcer activity of Tamira Parpam was observed in all the models. Pylorus ligation model showed significant reduction in gastric volume, free acidity and ulcer index as compared to control. Results revealed that the TamiraParpam showed

significant (P<0.01) ulcer inhibition in HCl-Ethanol induced ulcer and ulcer protection index (P<0.01) in stress induced ulcer. This study indicated that Tamira Parpam possessed potential anti-ulcer activities in the three models tested were reported.

¶ **Shipra Bhargava *et al.,*** (2012) studied the antimicrobial activity of Zingiberofficinale Extract and their phytochemical composition. Phytochemical screening revealed the presence of **alkaloids, saponins, tannins, flavonoids, terpenoid and phlobotannins** in the extracts. The *Zingiber officinale* extracts were obtained by soxhlet apparatus and their chemical profile was determined through GC and GC-MS analysis resulted in the identification of 40 compounds in methanolic and 32 compounds in ethanolic extract. Their antimicrobial activity was tested against nine microorganisms that cause various diseases in human.

¶ **Paul *et al.,* (2011)** evaluated the protein adsorption, blood compatibility and complement activation potential of rasa chenduram and NagaParpam preparation, along with its physicochemical characterization. The particle size, morphology, elemental analysis, and in vitro cytotoxicity were evaluated initially. Red blood cell hemolysis, aggregation studies with blood cells, protein adsorption, complement C3 adsorption, platelet activation and tight junction permeability in Caco-2 cell line were investigated. The preparations with a crystallite size of 28-34nm did not induce any complement activation or protein adsorption. However, rasa chenduram induced hemolysis, platelet adhesion and activation. It was also highly cytotoxic. Naga parpam did not induce any hemolysis or platelet activation. However, was slightly cytotoxic. These preparations opened the tight junctions in Caco-2 cell experiments. The results indicated the importance of in vitro biological screening tests before application in clinical medicine from the biological safety point of view.

¶ **J. Savarimuthu Michael *et al*., (2011)** studied the antibacterial potential against Escherichia coli, Salmonella typhi, Vibrio cholerae, Klebsiellapneumoniae, and Staphylococcus aurous using herbo - mineral

siddha drugs such as Linga chendooram-1, Lingachendooram -2, Vajerakandi, Kantharasavillai, Sandamarutham and Rasa chunnam. The study suggested that these herbo – mineral Siddha preparations may be useful as an alternative medicine in the treatment of enteric bacterial pathogen.

¶ **ThangaThirupathi R.*et al.*,(2002)**studied pharmacological validation of two Siddha drugs SanguParpam and silasathu Parpam. These were evaluated for its antiulcer effect in albino rats. The result revealed that both Siddha formulations are indeed good antiulcer agents.

2.3 Phytochemical constituents of the plant

The drug contains the extracts of the following plants

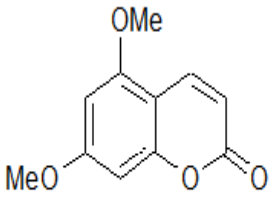
- ✓ *Terminaliachebula*
- ✓ *Terminaliabelerica*
- ✓ *Tinosporacordifolia*
- ✓ *Emblicaofficinalis*
- ✓ *Citrus Aurantifolia*
- ✓ *Aloe Barbadesis*
- ✓ *Syzygiumcummin*

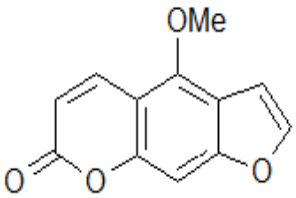
¶ **AzraKamal (2014)** investigated the preliminary phytochemical screening of the seeds of *Syzygiumcumini* belonging to family Myrtaceae. The results revealed the presence of medicinally important phytochemical constituents such as **alkaloids, glycosides, triterpenoids, steroids, saponins, flavonoids, tannins** except carbohydrates in the ethyl acetate and methanol extracts of *Syzygiumcumini* seeds and it justifies their use in the traditional medicines for the treatment of different diseases.

¶ The chemical composition and biochemical activity of *Aleovera leaves*. Proximate composition (moisture, ash, crude protein, crude lipid and crude fibre), **ascorbic acid, superoxide dismutase, catalase, peroxidase, amylase, reducing sugars** and total soluble sugars were determined by **Muaz Ahmed *et al*., (2013)**. Moisture content of 97.42 ± 0.13% was observed, while average

percent ash, fiber, protein and fat contents were 16.88 ± 0.04%, 73.35 ± 0.30%, 6.86 ± 0.06% and 2.91 ± 0.09% respectively along with traces of ascorbic acid (0.004 ± 0.05%). Variable levels (IU/mg) of superoxide dismutase (802.14 ± 55.6-2830.19 ± 37.09), peroxidase (1.46 ± 0.06-3.72 ± 0.19), catalase (1.56 ± 0.14-2.8 ± 0.19) and amylase (0.97 ± 0.82-24.02 ± 1.5) were observed in the extracts. Total soluble and reducing sugars accounted for 120.68 ± 7.24-363.03 ± 9.25 mg/mL and 97.23 ± 0.05-123.33 ± 0.74 mg/mL.

¶ Isolation and characterization of the active compounds from the hexane extract of the fruit peels of *Citrus aurantiifolia*, was done by **Nallely E. Sandoval Montemayor *et al.*, (2012).** The extract showed activity against one sensitive and three monoresistant (isoniazid, streptomycin or ethambutol) strains of *Mycobacterium tuberculosis* H37Rv. The active extract was fractionated by column chromatography, yielding the following major compounds: **5-geranyloxypsoralen; 5-geranyloxy-7-methoxycoumarin; 5, 7-dimethoxycoumarin (3); 5-methoxypsoralen (4) and 5, 8-dimethoxypsoralen**. The structures of these compounds were elucidated by 1D and 2D NMR spectroscopy.





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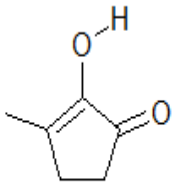
The GC-MS analysis of the hexane extract allowed the identification of 44 volatile compounds, were identified viz 5, 7-dimethoxycoumarin, 3-methyl-1,2-cyclopentanedione, 1-methoxy-ciclohexene, **corylone (5), palmitic acid (6) , 5,8-dimethoxypsoralen , β-terpineol(7) , and**

umbelliferone (8). Four isolated coumarins and 16 commercial compounds identified by GC-MS were tested against *M. tuberculosis* H37Rv and three multidrug-resistant *M. tuberculosis* strains using the Microplate Alamar Blue Assay. The constituents that showed activity against all strains were 5,8-dimethoxypsoralen(MICs = 25–50 mg/mL), 5-geranyloxypsoralen(MICs = 50–100 mg/mL), palmitic acid (MICs = 25–50 mg/mL), linoleic acid (MICs = 50–100 mg/mL), oleic acid (MICs = 100 mg/mL), 4-hexen-3-one (MICs = 50–100 mg/mL), and citral (MICs = 50–100 mg/mL). Compound 5,8-dimethoxypsoralenand palmitic acid were the most active ones. The ant mycobacterial activity of the hexane extract of *C. aurantifolia* could be attributed to these compounds.



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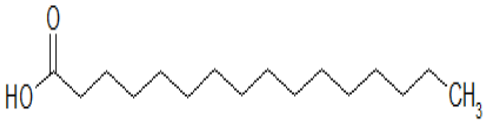

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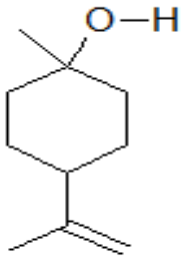

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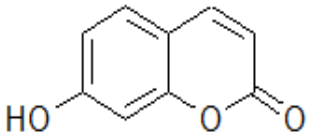

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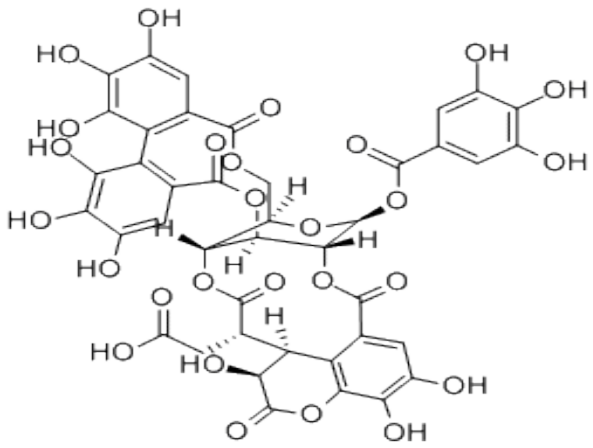
¶ The aqueous extract of galls from *Terminalia chebula* Retz. (Combretaceae) was fractionated on Diaion and refractionated on octadecyl silica column. Six phenolic compounds were isolated and identified as **gallic acid (10) ,**

punicalagin , isoterchebulin , 1,3,6-tri-*O*-galloyl-β-D-glucopyranose , chebulagic acid (9) and chebulinic acid (11). All of the compounds showed stronger 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and melanin inhibitory activities than ascorbic acid, butylatedhydroxytoluene, α-tocopherol, arbutin and kojic acid, the reference compounds.



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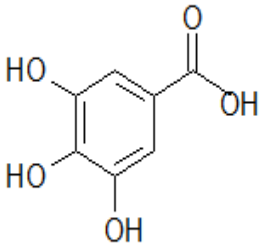

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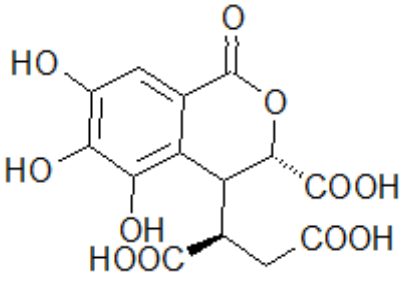

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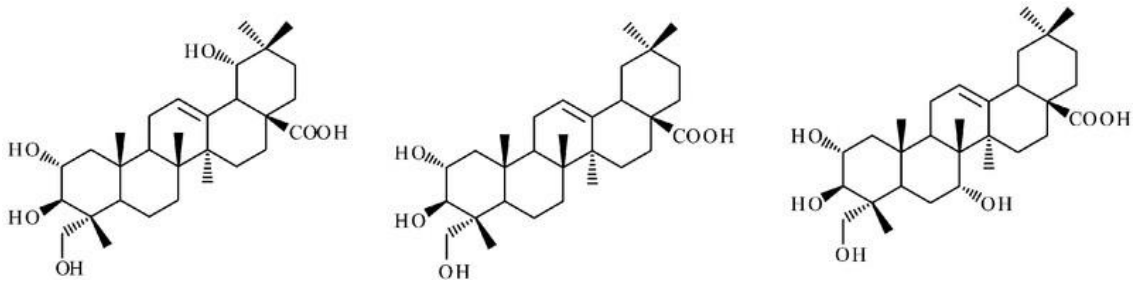
Gallic acid exhibited inhibitory activity against nitric oxide production in lipopolysaccharide-activated macrophages and all isolated compounds exhibited less activity than the reference compounds in mushroom tyrosinase inhibition and human tumour cytotoxicity assays. This study had demonstrated that the phenolic compounds isolated from galls of *T. chebula* might

contribute significantly due to their antioxidant and whitening activities. **AranyaManosroi *et al.*,(2010)**.

¶ *Terminaliachebula*Retz., tree belongs to the genus *Terminalia* of the Combretaceae family is a rich source of tannins and other phenolic compounds, **isolated olanolic acid-derived triterpenes (12)** from the plant and their structures were determined by spectroscopic methods including NMR and HRESIMS techniques. **Z Ali*et al., (2009).***



12

¶ Free amino acids, free monosaccharide's and total saccharides released upon hydrolysis, sterols, and triterpenoids of the leaves of *Aloe barbadensis* were determined by **G. R. Waller *et al* (1978)**. Some seventeen amino acids, D-glucose, and D-mannose were present in the water-soluble fraction. **Cholesterol, campesterol, *β*-sitosterol, and lupeol** were found in substantial amounts in the lipid fraction. An unknown(s) complex alkaloid was detected using Dragendorff's reagent.

Biological activity of the plant

¶ Phytochemical study of *Phyllanthusemblica* Linn *Amla*.disclosed major chemical constituents including tannins, alkaloids, polyphenols, vitamins and minerals. Gallic acid, ellagic acid, emblicanin A & B, phyllembein, quercetin and ascorbic acid are found to be biologically effective. Th results on amla revealed its analgesic, anti-tussive, antiatherogenic, adaptogenic; cardio, gastro, nephro and neuroprotective, chemopreventive, radio and chemo modulatory and anticancer properties and also reported to possess potent free radical scavenging, **antioxidant, anti-inflammatory, anti-mutagenic,**

immunomodulatory activities, which are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, liver and heart diseases. **Swetha Dasaroju *et al.*,(2014).**

¶ The **antibacterial activity** of crude extract of *Terminalia chebula*Retz was studied against gram negative bacteria such as *Escherichia coli*, *Pseudomonas*

¶ *aeruginosa* and *Klebsiella pneumonia* and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*. The antibacterial activity was studied by disc diffusion method. Extracts with the different solvent of *T. chebula*Retz. exhibited the antibacterial activity on bacterial strains. All extracts inhibited the growth of all test microorganisms and in disc method, with the range of concentration of 100µl, 150µl and 200µl the extract, the growth of all microorganisms was inhibited and also showed dose dependent activity. of the eleven solvent used methanol, ethanol and acetone seems to be the best solvent when compared to other solvents. **N.Tensingh Baliah *et al.*, (2014)**.

¶ **Karina *et al*., (2013)** evaluated the influence of high hydrostatic pressure applied for 5 minutes, on **antioxidant capacity, total phenolic content, colour, firmness, rehydration ratio, and water holding capacity** of Aloe Vera gel(*Aloe barbadensis Miller*) stored for 60 days at 4 °C. The analyzed properties of the pressurized gel showed significant changes after the storage period. The highest value of total phenolic content was found at 550 MPa and a decrease in the antioxidant capacity was observed for all pressurized gel samples when compared to the control sample (p <0.05). The smallest changes in product colour were observed at pressure levels between 150 and 250 MP. The application of high hydrostatic pressure resulted in lower gel firmness, and the lowest value was found at 150 MPa (p <0.05). The untreated sample showed a greater decrease in firmness, indicated that high pressure processing preserves this property. The application of high hydrostatic pressure exhibited modifications in the food matrix, which were evaluated in terms of rehydration ratio and water holding capacity.

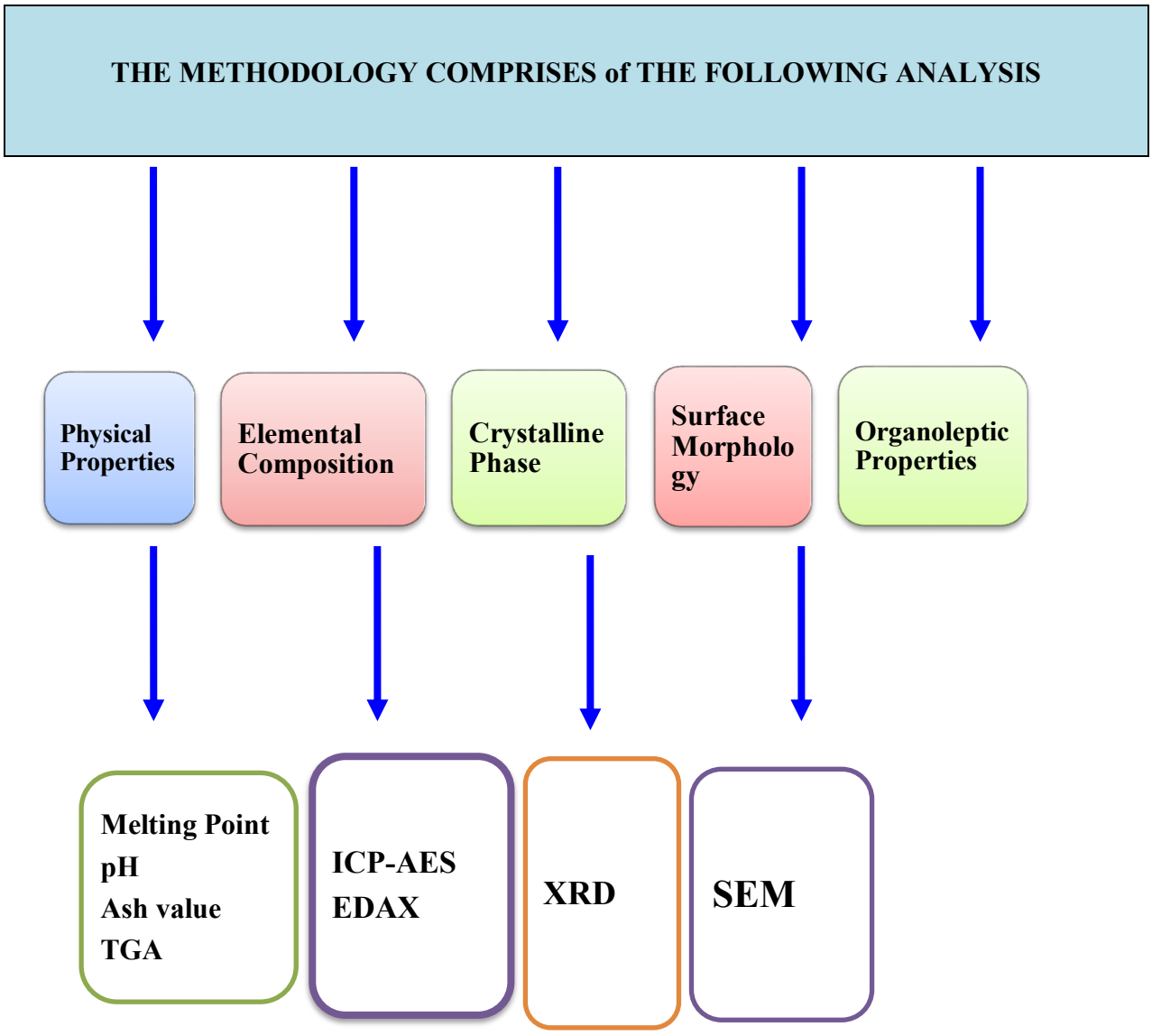
¶ The **cancer chemo preventive activity** of *Phyllanthus emblica* both in vivo and in vitro and pharmacological properties and relative safety of Amla (*Phyllanthus emblica*) was reported by **Ekta Singh *et al.*, (2011).**

¶ An ethanol extract of *Terminalia chebula* fruit was studied for its **antibacterial activity** against clinically important standard reference bacterial strains by **Kannan P *et al.*, (2009).** The antimicrobial susceptibility was screened using the disc diffusion method and the MIC was determined using the broth microdilution method. The results showed that it was active against both gram-positive and gramnegative bacteria. The *T. chebula*fruit extract was highly effective against *Salmonella typhi* SSFP 4S, *Staphylococcus epidermidis* MTCC 3615, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441 and *Pseudomonas aeruginosa* ATCC 27853. The MIC was determined as 1 mg/ml for *Salmonella typhi*. These results indicated that the *T. chebula* dry fruit possessed a potential broad spectrum of antimicrobial activity.

Materials & Methods

3. MATERIAL AND METHODS

In the present study evaluation of **Aya Chendooram (AC)** a Siddha formulation was carried out. The drug was collected from Siddha drug suppliers, Trippur. It is commercially available formulation that can be used for treating various diseases in traditional clinical practice in India and are usually prepared from purified mineral, triturated with decoction of herbal juices. The detailed methodology adopted is given below.



3.1ORGANOLEPTIC PROPERTIES

Organoleptic property of drug was examined according to conventional method given by Kokate. **(Kokateetal., 2002).** The sample was treated with some acidic, basic and neutral reagents and the characteristic changes were observed.

3.1.1Test with concentrated hydrochloric acid

A small amount of the sample was treated with concentrated hydrochloric acid and the sample was found to partly soluble and settled down slowly.

3.1.2 Test with concentrated nitric acid

A small amount of the sample was treated with concentrated nitric acid and the sample was found to insoluble and settled down slowly.

3.1.3 Test with concentrated sulphuric acid

A small amount of the sample was treated with concentrated sulphuric acid and the sample was found to settle down slowly.

3.1.4 Test with 5% aqueous sodium hydroxide

A small amount of the sample was treated with 5% aqueous sodium hydroxide. The sample was found to insoluble and settled down slowly.

3.1.5 Test with iodine solution

A small amount of the sample was treated with iodine solution. The sample was found to insoluble..

3.1.6 Test with 5% aqueous potassium hydroxide solution

A small amount of the sample was treated with 5% aqueous potassium hydroxide solution. The sample was found to insoluble.

3.1.7 Test with Glacial acetic acid

A small amount of the sample was treated with glacial acetic acid solution. The sample was found to insoluble and settled down slowly.

3.1.8 Test with 5% aqueous potassium hydroxide solution

A small amount of the sample was treated with 5% aqueous potassium hydroxide solution. The sample was found to insoluble and settled down slowly.

3.2 PHYSICAL PROPERTIES

Physical properties like ash value, water soluble extracts, loss on drying, pH values and stability were determined as per method described in Indian Pharmacopoeia.

3.2.1 Melting point of the drug

The melting point of the sample was determined using melting point apparatus. **(Ajay R)**

3.2.2 pH values

The pH value of the sample was determined by pH meter **(QC/Micro/pH – 101, Sr No. 1311605).**

3.2.3 Determinations of ash values

Apparatus

1. Silica crucibles
2. Muffle Furnace - Furnace was fitted with an indicating pyrometer, to maintain the temperature. **(Genuine)**
3. Analytical balance – with to 0.001 mg sensitivity. **(Shimaozu AY220)**
4. Desiccator
5. Drying oven - with temperature control of 105 ± 2°C. **(Sigma)**

3.2.3 (A) Total ash (TA) value

Accurately 2 to 3 g of air–dried samples of the **AC** was weighed in a silica dish and incinerated at a temperature not exceeding 700°C until ash free from carbon was obtained. Then it was cooled and weighed. The process was repeated until at least two consecutive constant weights were obtained. The results were expressed as range or mean value ± standard deviation. The percentage of ash was calculated with reference to the air – dried drug.

$$\text{Ash \%} = \frac{W}{\text{Loss in weight} \times 100}$$

W = Weight of air – dried drug.

3.2.4 Loss on drying (LOD)

Accurately 2 gram of the sample was taken in a tared crucible and initial weight was taken. The sample was heated in a Muffle Furnace maintained at 105-110°C, for 3 h, after which the sample was allowed to cool to room temperature for 30 minutes in desiccators, and subsequently weighed. This procedure was repeated until a Constant weight was obtained.

$$\text{Loss on drying (\%)} = \frac{\text{W}}{\text{Loss in weight x 100}}$$

Where W = weight of the sample powder in g.

The results are expressed as a range or as mean ± standard deviation.

3.2.5 Thermal gravimetric analysis

` Thermo gravimetric analysis was conducted using a TA instrument 951 thermo gravimetric analyser (TGA). The 951 model is a horizontal design TGA. Each test was conducted with a flow rate of 50cc/ min of nitrogen through the furnace and balance sides of the TGA. Approximately 25- 30 mg of sample was weighed onto a platinum pan for each sulphate decomposition test. The sample was then held at ambient conditions for 40 min before it was heated at a rate of 5^0C/ min to 200^0C where the temperature was held for 1 hour. The sample was then heated at the same heating rate to 300^0C and held for 15 min. this step was then repeated, raising the temperature to 100^0C increments and holding for 15 min until a temperature of 700^0C was reached (that is 100^0C, 200^0C, 300^0C, 400^0C, 500^0C, 600^0C and 700^0C). The final step in the decomposition program was to increase the temperature to 700^0C at a rate of 5^0C/ min where the temperature was held for 15 min.

3.3 ELEMENTAL COMPOSITION

3.3.1 ICP-AES Analysis

The ICP-AES analysis was done using Perkin Elmer Optima 5300 DV analyser. 0.2 gram of the sample, 5 ml of Nitric acid and 2 ml of Perchloric acid was added and digested. After the digestion was completed, the flask was allowed to cool and the contents were transferred to a beaker and heated to remove all the acid. The

resulting solution was diluted to 50 ml with deionised water and used for further analysis.

3.3.2 EDAX Analysis

The elemental composition of the sample was analyzed by EDAX **(51ADD0048 Model- Oxford Instrument)** analyser. EDAX provides a good estimate of the concentration of the main elements in the sample in a significantly faster way compared to ICP-AES method.

3.4 SURFACE MORPHOLOGY

3.4.1 SEM Analysis

To evaluate of surface topography, morphology (shape and size of the particles) of the sample SEM analysis were carried out by using **Fe-SEM analyser (51ADD0048 Model- Oxford Instrument).** A small quantity of the sample was sprinkled on a carbon tape mounted on a specimen stub and sputter coated with gold for best images and to avoid charging of instances, in order to get a higher quality secondary electron image for SEM examination.

1.5 CRYSTALLINE PHASE

3.5.1 XRD

To determine the different crystalline phase present in the sample, XRD patterns were obtained using anX-ray powder diffractometer**.** Powdered sample were studied by placing a thin layer in conventional cavity mounts. The sample were scanned from (10- 90^0) 2θ.

Results & Discussion

4. RESULT AND DISCUSSION

The present study dealt with the chemical characterisation of AC a Siddha formulation used for the treatment of anaemia. The characterization was done by systematic procedure that are used to standardize the drug formulations and the results of the analysis are discussed below

4.1 ORGANOLEPTIC PROPERTIES

- Organoleptic properties of the drug refers to the evolution of a drug by means of sensory organs like colour, odour ,taste, size, shape and texture. The properties were given in **Table -2.**

Table -2 Organoleptic properties of AC

S.No	Parameters	Observation
1	Colour	Red
2	Odour	Odourless
3	Taste	Tasteless
4	State of drug	Powder
5	Consistency	Soft

- The sample was treated with some acidic, basic and neutral reagent and characteristic changes were observed and the results were tabulated in **Table-3**. Of all the reagents tested the AC was found to be partially soluble in Concentrated Hydrochloric acid and was insoluble in other reagents.

Table -3 Behaviour of AC with different reagent

S.No	Chemical Treatment	Observation
1	Drug powder treated with concentrated Hydrochloric Acid	Partially dissolved and settled down slowly.
2	Drug powder treated with concentrated Nitric Acid	The sample was found to insoluble & settled down slowly.
3	Drug powder treated with concentrated Sulphuric Acid	The sample was found to insoluble & settled down slowly.
4	Drug powder treated with 5% aqueous Sodium Hydroxide	The sample was found to insoluble & settled down slowly.
5	Drug powder treated with Iodine Solution	The sample was found to insoluble & settled down slowly.
6	Drug powder treated with 5% aqueous Potassium Hydroxide Solution	The sample was found to insoluble & settled down slowly.
7	Drug powder treated with Glacial Acetic Acid	The sample was found to insoluble & settled down slowly
8	Drug powder treated with 5% aqueous Potassium Hydroxide Solution	The sample was found to insoluble & settled down slowly.

4.2 PHYSICAL PROPERTIES

Physical properties of AC like ash value, loss on drying, pH values and stability were determined.

4.2.1 The melting point of the drug was determined by melting point apparatus **Ajay R**. The substance did not melt till 500^0C.

4.2.2 pH of the formulation plays a significant role in the living biological system with respect to aid in absorption and distribution through systemic circulation .The pH value of drug was determined by pH meter **(QC/Micro/pH – 101, Sr No. 1311605)**. The pH value was found to be in the range of **4.0-5.0**.

4.2.3 Ash value

The ash content of the crude drug is generally taken to be the residue remaining after the incineration. Ash constitutes the inorganic residues remaining after the water and organic matter have been removed by heating. The residue will contain Carbonates, Phosphate and Silicates of Sodium, Potassium, Calcium and Magnesium.**(Sharma *et al.*, 2013)** Hence the total ash value of AC was determined using Muffle Furnace heated up to 700◦C.The ash content of AC was found to be **0.08%**. The lesser value of the ash content indicated that the formulation contained more of inorganic salts.

4.2.4 Determination of loss of ignition

Loss on drying of the sample was carried out using Muffle Furnace maintained at 105-110°C. Loss on drying was found to be **very less (below detectable level)** which indicated the absence of moisture content in AC.

4.2.5 TGA

The TGA curve of AC was shown in **Figure: 13, 14**and **15.** The TGA curve resembled the TG curves of α-Fe_2O_3. **(X.Cao et al., 1997).** The first weight loss appeared at 28◦C with 15% weight loss. After that there was no decomposition observed till 700^0C. This is because, AC mainly contained Fe_2O_3 and the melting point of Fe_2O_3 is 1545^0C. DTG curve showed the large exothermic peak at 321^0C which was attributed to α-Fe_2O_3 nanoparticles. **(Xin Zhang *et al.,* 2013**). All the above results confirmed the presence of α-Fe_2O_3 in AC.

Figure 13 TG of AC

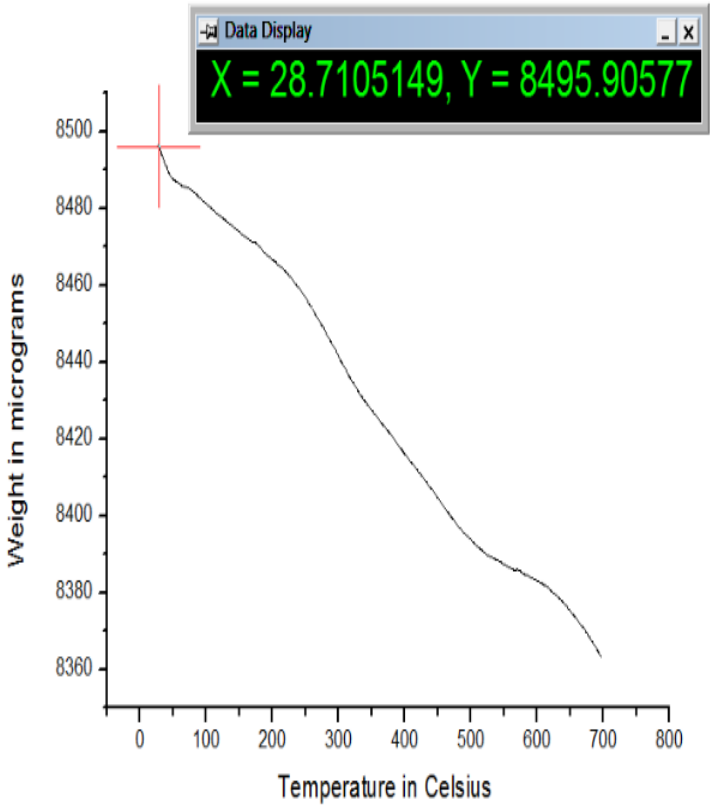


Figure 14 DTG of AC

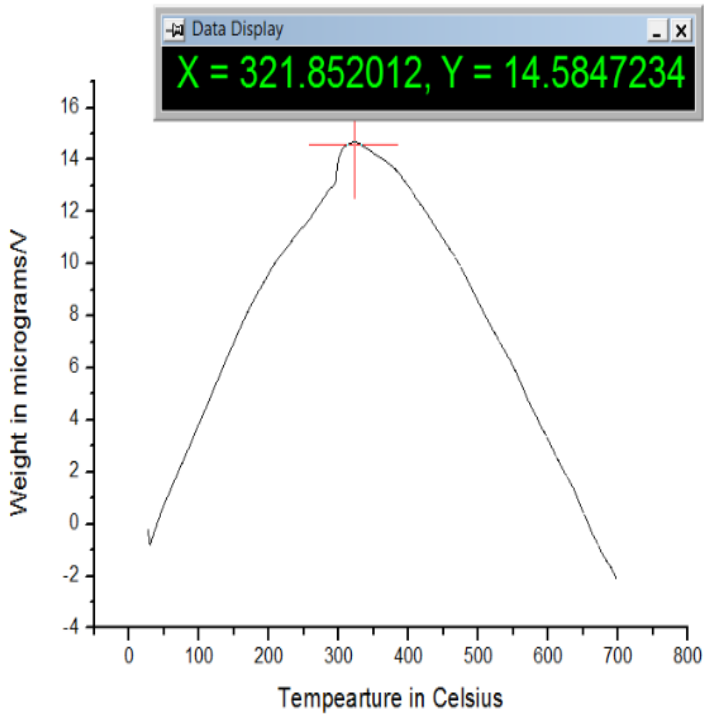
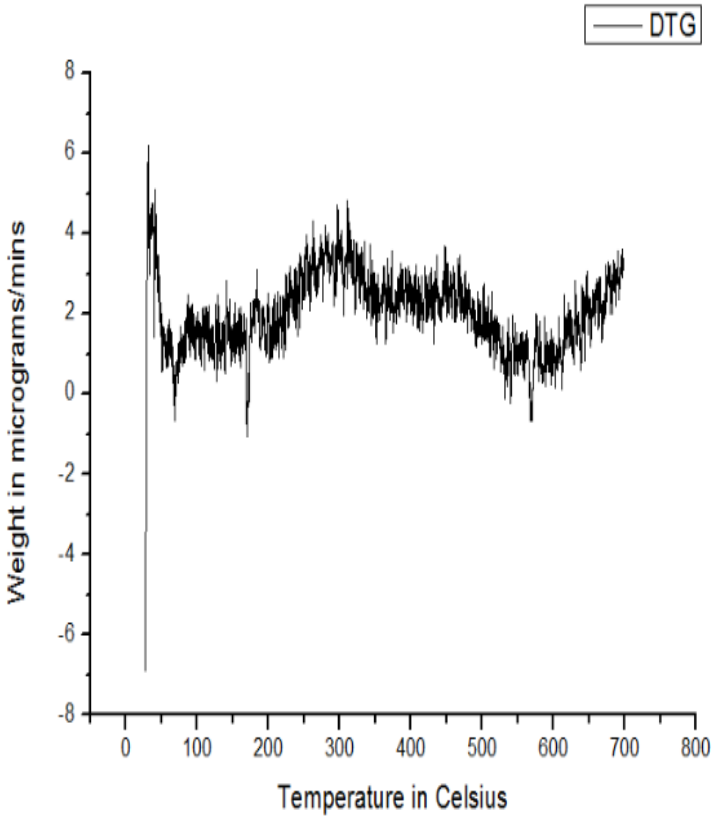


Figure 15 DTG of AC



4.3 ELEMENTAL COMPOSITION

4.3.1 ICP-AES

The drug AC was analysed by the ICP-AES (**Table -4)** to detect the trace elements and other elements present in it quantitatively. The results from quantitative analysis of AC by ICP-AES revealed the presence of Iron, Calcium, Zinc, Sulphur, Lead and Mercury. The major element was found to be Iron (127000 ppm). The arsenic was found to be below detectable level and other heavy metals like mercury and lead to lesser extent.

Table -4 Elemental Compositions by ICP-AES Analysis

S.No	Elements	Wave length (ppm)	Detected limit
1	Arsenic (As1890)	BDL	0.10
2	Mercury (Hg1849)	6.2	0.10
3	Lead (Pb2203)	8.5	0.10
4	Zinc (Zn2025)	8.1	0.01
5	Calcium (Ca3158)	900	0.05
6	Iron (Fe2599)	127000	0.05
7	Sulphur (S1820)	73200	0.10

4.3.2 EDAX

The elemental composition of AC was determined by EDAX (**Table 5, 6**) which confirmed the presence of metals like Magnesium, Aluminium, Silicon, Iron, and Potassium as their oxides. Iron oxide is the main component equivalent to 92.56 %. **(Figure 16 and 17)**

Table-5 EDAX Analysis of AC

Element	Weight%	Atomic%	Compd%	Formula
Mg K	0.38	0.54	0.63	MgO
Al K	0.27	0.35	0.52	Al2O3
Si K	2.27	2.76	4.85	SiO2
Cl K	0.42	0.40	0.00	
K K	0.85	0.74	1.02	K2O
Fe K	71.95	44.12	92.56	FeO
O	23.86	51.08		

Figure 16 EDAX Spectrum of AC

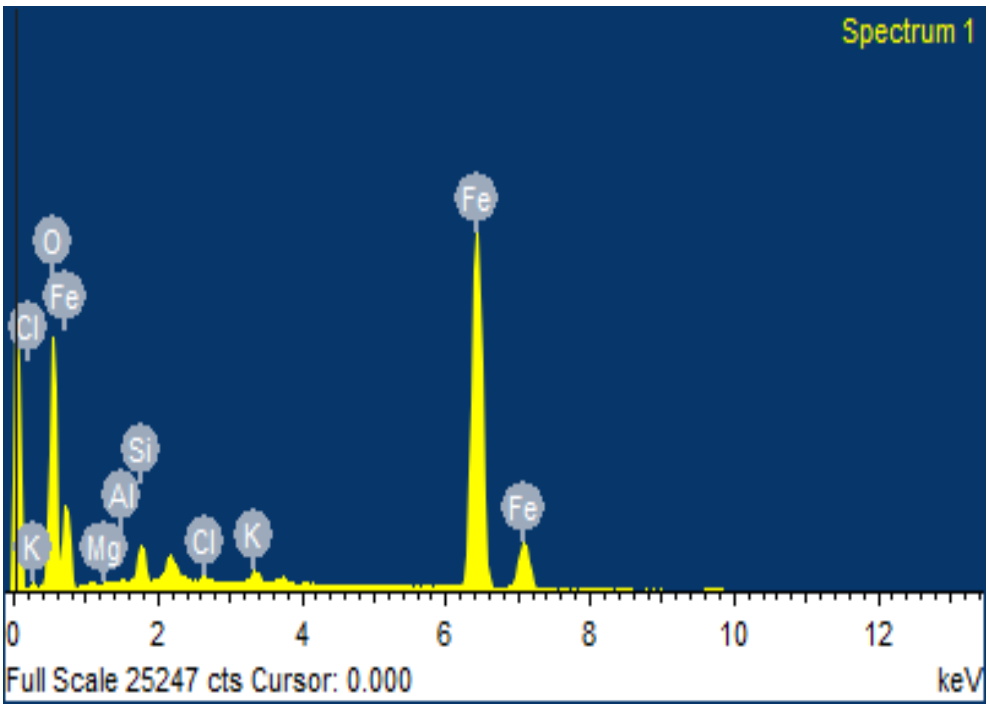
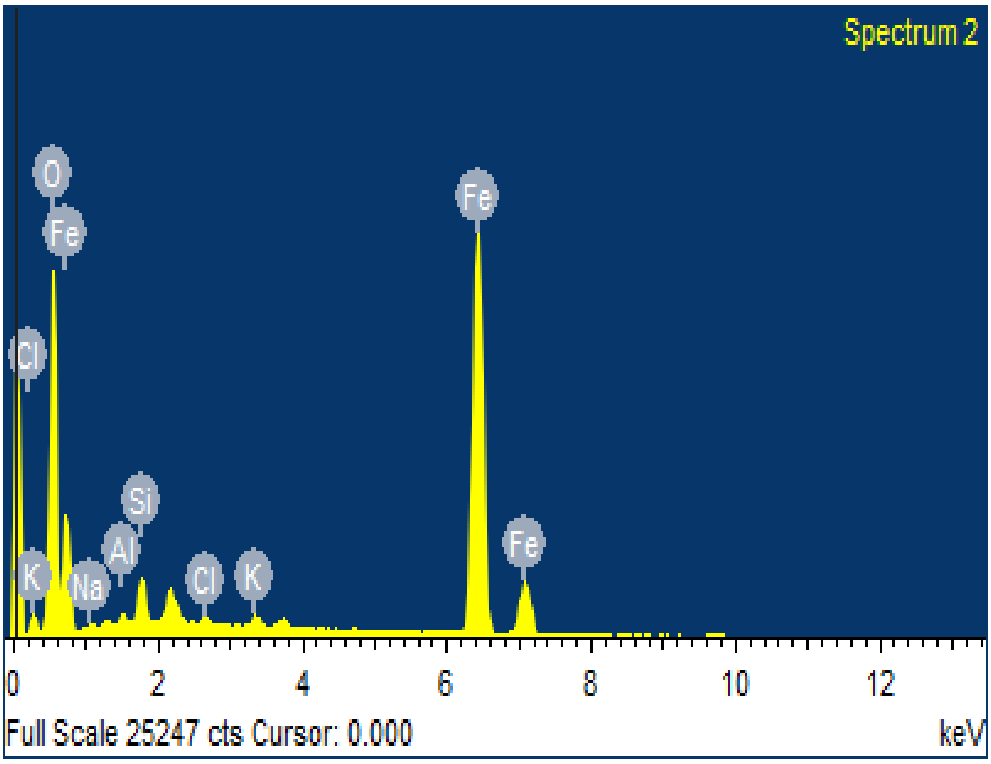


Table-6 EDAX Analysis of AC

Element	Weight%	Atomic%	Compd%	Formula
Na K	0.84	1.23	1.13	Na2O
Al K	0.63	0.79	1.19	Al2O3
Si K	2.58	3.10	5.51	SiO2
Cl K	0.45	0.43	0.00	
K K	0.55	0.48	0.67	K2O
Fe K	70.78	42.86	91.06	FeO
O	24.18	51.11		
Total	100.00			

Figure 17 EDAX Spectrum of AC



4.4 SURFACE MORPHOLOGY

4.4.1 SEM

The particle size of AC was assessed by SEM. Particle size distribution and particle shape affects various chemical and physical properties of the drug substance as well as the biopharmaceutical behaviour. SEM analysis of AC showed the presence of nanoparticles in the range from 10 - 200 nm. The particles were circular, triangle and needle in shape. Surface was found to be smooth so the flow ability was normal. **(Figure 18, 19, 20 and 21).** The extremely small sizes of nanoparticles allow them to penetrate the cells and interact with cellular molecules. As the particle was in nano size, a low dose of the drug is enough to treat disease.

SEM photograph of AC

Figure 18

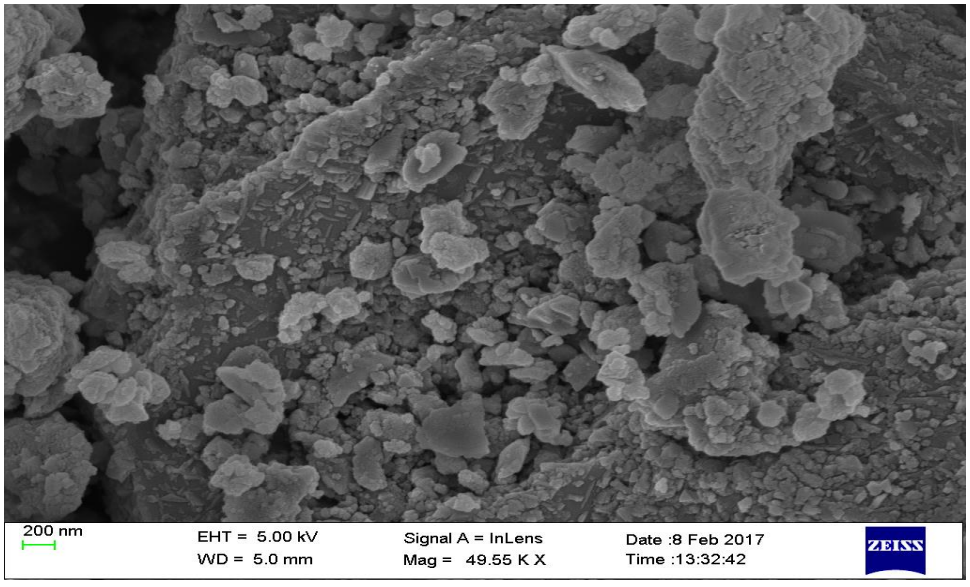


Figure 19

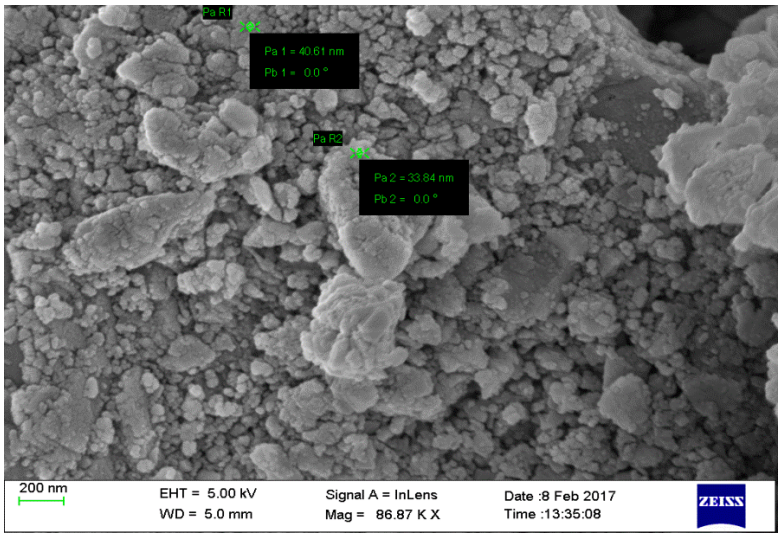


Figure 20

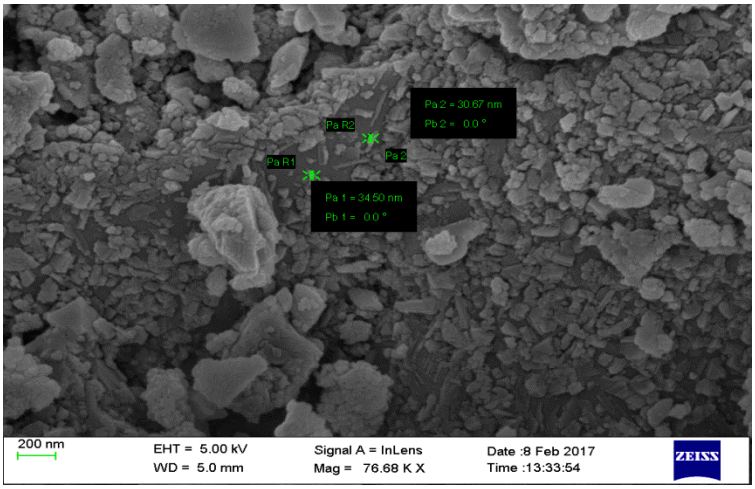
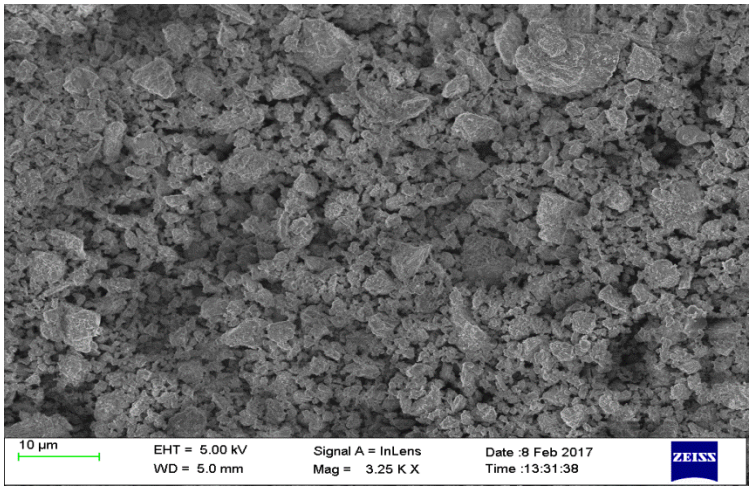


Figure 21



5. CRYSTALLINE PHASE

5.1 XRD

XRD studies were conducted to identify the bulk crystalline phases present in AC. The sharp peaks observed in the XRD pattern of AC confirmed that Fe_2O_3 is present in crystalline form. The comparison of XRD patterns with literature **(T. Radu.,*et al* 2017 fig ref.,23b)** showed the presence of α-Fe_2O_3 also XRD pattern revealed the existence of a pure inverse spinal crystalline structure. (**X.Cao *et al*., 1996). (Figure 22 & 23a).** From the XRD graph the particle size was determined at 27.72 nm.

XRD SECTRUM of AC

Figure 22

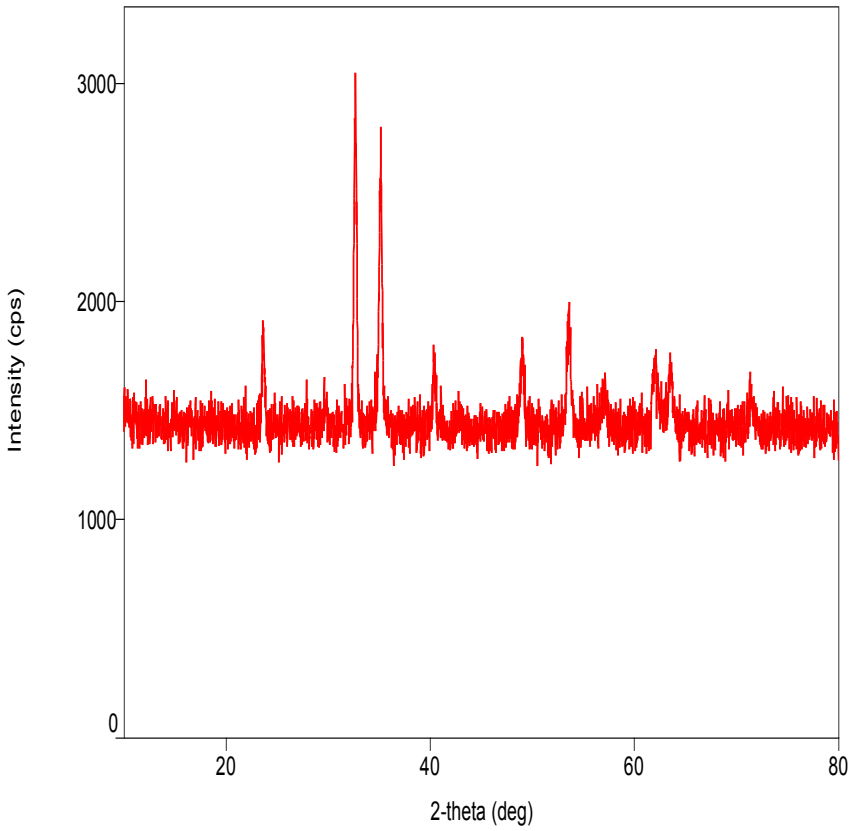


Figure 23b -XRD Spectrum of α-Fe_2O_3 ((T. Radu.,*et al* 2017)

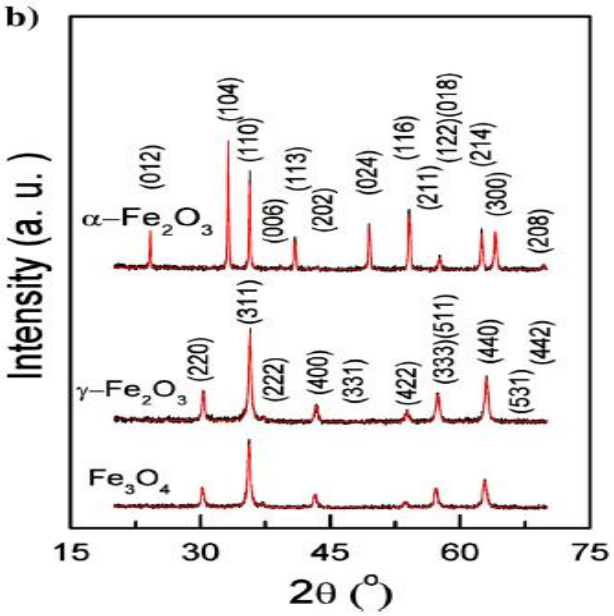
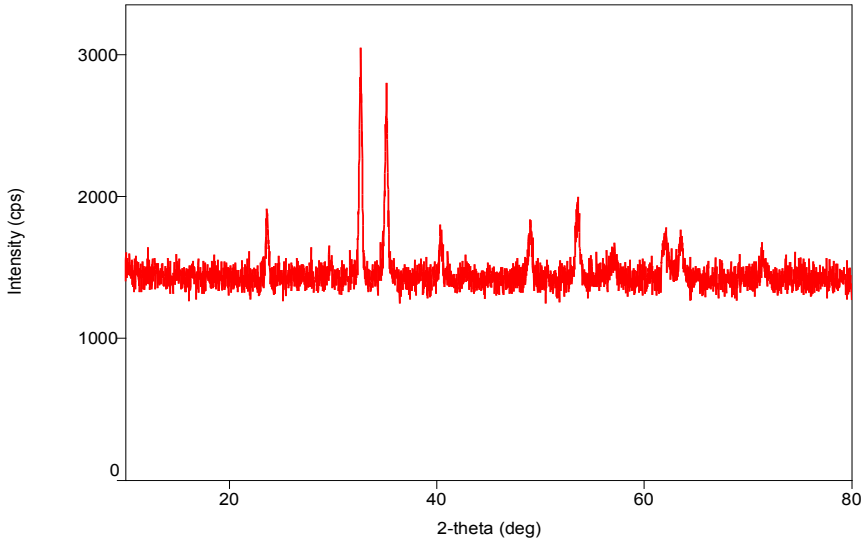


Figure 23 a



Summary & Conclusion

SUMMARY AND CONCLUSION

The present study aimed at the characterization of the physic-chemical traits of the traditional India Siddha medicine, and results of the findings are summarized as follows

i. The ICP-AES analysis revealed the presence of the following metals namely Iron, Calcium, Zinc, Sulphur, Lead and Mercury. The major element was found to be Iron 127000ppm.

ii. Magnesium, Aluminium, Silicon, Iron, Potassium was present in their oxide forms which were confirmed by EDAX. Iron oxide was the main component equivalent to 92.56%.

iii. SEM analysis showed the presence of nanoparticales in the range 10µ - 200 nm

iv. Powder XRD technique was used to identify the crystalline phase and from the XRD patterns the major chemical entity in AC was found to be α-Fe_2O_3.

v. TG-DTA technique results further confirmed the presence of α-Fe_2O_3 nano particles.

The results of the present investigation render some valuable information about the Siddha formulation AC to the future researchers and also provide evidence based information on standardization part of this noble formulation.

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