Quantitation ofthe ingredients in-Gandhaka Parpam
A herbo Mineral Siddha Medicine

By

KASTHURI.S

(15PCH006)

Thesis 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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Signature of the

Supervisor

10.4.17
Signature of the

Head of the Department

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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	Black 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
IR SEM	High Resolution Scanning Electron Microscope
RESIMS	High-Resolution Electrospray Ionization Mass Spectrometry
CP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
CP-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

1. Introduction

1.1 Importance of herbal drug

Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries.**(Meena*etal.,* 2014)** Recently the entire population is showing interest in traditional medicine and it has increased the demand for Siddha drugs.

1.2 Siddha medicine

Siddha is one of the ancient medical system in India considered as the mother medicine of ancient Dravidians in South India and a unique system, have plenty of medicines which cures many disease without side effects. The word Siddha comes from the word Siddhi which means an object to be attained perfection or heavenly bliss. **(Manish *et al.,* 2015)**Siddha focused to "Ashtamahasiddhi," the eight supernatural powers. Those who attained or achieved the above said powers are known as Siddhars. There were 18 important Siddhars in olden days and they developed this system of medicine. Hence, it is called Siddha medicine.

According to siddha system human body is made up to of five elements (Fire, Water, Air, Earth, and Galaxy). The human body runs on the basis of vatha, pitha and kapha. Due to changes in the equilibrium of tri-dosha, mankind acquire diseases. **(Rajkumar*etal,* 2016)** The great Siddhars like Agasthiyar and Yugi muni classified the human's disease into 4448 types of diseases. Siddha medicines are in high demand as effective therapeutic retailers globally.

According to the Siddha medicine various psychological and physiological function of the body are attributed to the combination of seven elements:

- First is *saram* (plasma) responsible for growth, development and nourishment
- Second is *cheneer*(blood) responsible for nourishing muscles, imparting colour and improving intellect
- The third is *oun* (muscle) responsible for shape of the body
- Forth is *kollzuppu*(fatty tissue) responsible for oil balance and lubricating joints
- Fifth is *enbu*(bone) responsible for body structure and posture and movement

- Sixth is *moolai*(nerve) responsible for strength
- The last is *sukila* (semen) responsible for reproduction.

The development of this traditional system of medicines with perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in health care.**(Shukla*et al.,* 2010)** In Siddha system of medicine the drug sources are obtained from plant, mineral, metal and animals. According to their pharmaceutical preparations, **Siddha medicine could be categorized into:**

- ***Kudineerchuranam*** (decoction powder): It is a fine powder of drugs.
- ***Chendooram*****:** It is a red colour powder generally made of metallic compounds.
- ***Chunnam*****:** It is alkaline in nature.
- ***Kalangu*****:** It is based on mercury.
- ***Karpams*****:** It could be herbal or non-herbal in nature, made on a daily basis.
- ***Karruppu*****:** Mercury and sulphur are essentially present and its colour is dark black.
- ***Legiyams*** and ***rasayanams*****:** It contains ghee, honey and sugar, apart from herbal powder and juices.
- ***Mathirai*** and ***vadagam*****:** It is pills prepared from fine powdered paste.
- ***Maappagu*****:** It is flavoured medicinal syrup and contains generally aromatic herbs, honey and sugar.
- ***Mezhugu*****,** ***kuzhambu*****,** ***kalimbu*** **and** ***mai*****:** All of these categories have a waxy feel.
- ***Ney*****:** It is medicated ghee, which contains fat-soluble plant substances.
- ***Pakkuvam*** and ***theenooral*****:** It is herbal medicine with honey.
- ***Parpam*****:** It is prepared by the process of calcination.
- ***Patangam*****:** It contains mercury, camphor, etc.
- ***Thailam*****:** It is medicated oil; usually sesame seed oil, coconut oil, castor oil, etc are used in its preparation.
- ***Theeneer*****:** It is distilled essence, which contains volatile constituents of the drugs.

The unique formulations in Siddha include Parpam (mineral/metallic oxides),Chendhooram (mineral/metallic sulphides), Chunnam (caustic or major oxides) and Pathangam (sublimation) Parpam and Chendooram type of medicines are

highly used by the traditional medicine practitioners for its smaller dosage with higher therapeutic values. Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body. Treating the minerals with herbal juices may lead to reduction in particulate size even up to nano levels (less than 100 nm) enabling increased potency. These drugs are known to be effective even in low concentration.

In Siddha system of medicine there are 25 varieties of water-soluble inorganic compounds called 'MUPPU', different types of alkalies and salts, 64 varieties of mineral drugs that do not dissolve in water but emit, vapours when put in fire, 32 of these are natural and remaining are artificial, seven drugs that do not dissolve in water but emit vapour on heating.

The system has classified separately classes of metals and alloys, which melt when, heated and solidifies on cooling. These include items like gold, silver, copper, tine, lead and iron. These are incinerated by special processes and used in medicine. There is a group of drugs that exhibit sublimation on heating and includes mercury and its different forms like red sulphide of mercury, mercuric chloride and red oxide of mercury etc.

The treatment in Siddha system of medicine lays emphasis on paediatrics, toxicology and ophthalmology. Among plants, animals and minerals that constitute the Siddha drugs, preparations based on minerals and metals are used in majority in preference to preparation from plant. Though metals are used in Siddha medicines, they are subjected to rigorous treatments involving different steps before being converted to a therapeutic form. **(Nesapriya*etal.*, 2012)**

The preparation of majority of medicines involves tedious process which result in physicochemical transformation of the particles size, chemical composition which regulates the bio-mechanisms of the drug in the body, thus enhance the efficacy and reduce toxicity. **(R Shailaja*etal.*, 2016)**

1.3 THE ADVANTAGES OF SIDDHA MEDICINE

There are a number advantages associated with using herbal medicines as opposed to pharmaceutical products.

- **Reduced risk of side effects:** Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.
- **Effectives with chronic conditions:** Herbal medicines tend to be more effective for long-standing health complaints that don't respond well to traditional medicine.
- **Lower cost:** Herbs cost much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.
- **Widespread availability:** Herbs are available without a prescription. In some remote parts of the world, herbs may be the only treatment available to the majority of people.

1.4 WHY STANDARDIZATION OF HERBAL MEDICINE

Standardization means analysing the amount and potency of active ingredients believed to be present in each herb claimed in the formulation.

In recent years there is a spurt in the interest regarding survival of Ayurvedic forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the short coming of modern medicine have started getting more apparent and as majority of Ayurvedic formulations are prepared from herbs. It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get finished products in pharmacopoeias controlling the manufacturing through the use of formularies and the medication, with guaranteed purity, safety, potency and efficacy. **(Pravin H. Nikam*etal.*, 2012)**

The quality control of crude drugs and herbal formulations is of paramount importance in justifying their acceptability in modern system of medicine. But one of the major problems faced by herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations. The task of lying down

standard for quality control of herbal drugs and their formulations involves biological evaluation for particular disease area, chemical profiling of the material and lying down specification for the finished product. Therefore, in case of herbal drugs and product, the word "standardization" should encompass entire field of study from cultivation of medicinal plant to its clinical applications. Plant material and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality control are necessary.

1.5 Nano medicine

The preparation of majority of medicines involves tedious processes which result in physico-chemical transformation of the particles size, chemical composition which regulates the bio-mechanism of the drug in the body. Unique preparations of **Siddha system of medicine like *Parpam, Chenduram, Chunnam, Kattu*and *Padhangam*are "lifesaving" and "miracle" nanomedicines.** Most of the medicine of above category was found to contain nano particles and it seems Siddha has used special techniques to prepare each and every medicine. **(R.Shailaja and S Sugunthan.,2016).** Recent development in Nanotechnology are helpful in the diagnosis and treatment aspects of certain life threatening diseases like cancer and other diseases in cellular level.**(R.Manickavasagam and senthamil Rajam.,2016)**.

1.6. Importance of sulphur

Sulphur is a naturally occurring element that has several benefits on our health. It is a non-metallic solid element found in nature. It is required in human body for proper functioning. Sulphur is found in protein-rich animal foods, such as dairy, eggs, beef, poultry and seafood. In particular, the yolks of eggs are one of the highest sources of sulphur. Primary sources of sulphur include onions, garlic, turnips, kale, seaweed and raspberries. Nuts are an additional plant source of sulphur. **(Rajalakshmi P *etal.,* 2010)**It is the third most abundant based on percentage of total body weight. Traditionally sulphur is known with other names like **Ghandagam, KaarizhaiNatham, Parainatham, Paraiveerayam, Atheethaprakasam, Beejam, Selvivindhu, Sakthi, Sakthipeesam, Chenduraathrrathi, Natham, Narram,Parainatham, Ponnvarni, Rasa sronitham**. **(P Rajalakshmi*etal.,* 2012)**

It is the sixth most abundant macro mineral in breast milk. It is present in saliva, bile, protein, amino acids, and insulin. It is mainly present in sulphur

containing amino acids like cystine in our body and involved in protein synthesis. Sulphur in the body purifies blood, aids healthy digestion, and prevents toxic build-up synthesis, as well as several enzyme reactions. It helps with the production of collagen, which is a substance that forms connective tissues, cell structure and artery walls. It is a part of keratin, giving strength to hair, skin and nails, amino acids, and insulin.

The photosynthetic green sulphur bacteria and purple sulphur bacteria and some lithotrophs use elemental oxygen to carry out such oxidization of hydrogen sulphide to produce elemental sulphur. Sulphuric acid (H_2SO_4) is present in the digestive fluids of sea squirts. Hydrogen sulphide(H_2S) replaces H_2O in the photosynthesis of some bacteria. It very small concentrations can be metabolized, but in higher concentrations it kills quickly by preventing respiration.Carbon disulphide (CS_2) causes problems to the central nervous system.Sulphur is a component of gunpowder. The sulphur dioxide gas addition to fermented wine to produce traces of sulphurous acid its sulphite salts in the mixture has been used in winemaking.

1.7 Types of Gandhangam:

Based on the colour sulphur is of four types

- **White sulphur** is used to cure all diseases.
- **Parrot nose red**colourGandhgam
- **Yellow colour**Gandhagam resembles the colour of gooseberry, easily reacts with mercury to form kajjali, and is used in therapeutics.
- **Black Gandhagam** similar with the crow black colour is a rare variety used as a rejuvenator. In nature, both plant and animal origin substance contain sulphur in a permissible quantity such as leafy vegetable, egg, meat, garlic etc. **(P Rajalakshmi*etal.*,2012)**

1.8 Medicinal Uses of Gandhakam

Sulphur is useful in cough, asthma, consumption, general debility, enlargement of liver and spleen, chronic fevers etc. It is well known medicine for diseases of skin.Sulphur detoxifies at the cellular level and relieves pain. It has been called nature's **"beauty mineral"** because it keeps our complexion clear and youthful and hair glossy and smooth**. (P Rajalakshmi*etal.*, 2010)**

Sulphur has been shown to be effective for osteoarthritis, rheumatoid and psoriatic arthritis. Sulphur baths or mud-soaks can help alleviate the painful swelling caused by arthritis. By taking sulphur bath at night it can reduce stiffness. It also can improve walking ability and overall strength. Applying a cream containing DMSO (Dimethylsulfone) may reduce pain in some types of arthritis. Lastly, taking a supplement with 6,000 mg of MSM (Methylsulfonylmethane) sulphur can reduce pain associated with arthritis, but it may have more beneficial effects when paired with glucosamine. Sulphur is used in pharmaceutical skin preparations for the treatment of acne. It acts as a keratolytic agent and also kills bacteria, fungi, scabies mites and other parasites.

Precipitated sulphur and colloidal sulphur are used, in form of lotions, creams, powders, soaps, and bath additives, for the treatment of acne vulgaris, acne rosacea, seborrhoeic dermatitis, swelling and redness related to acne. Gandhaka has antibacterial and antifungal activity. It is effective both internally and topically as a microbicide agent. Sulphur can help ease the effects of several skin disorders, including psoriasis, warts, dandruff, eczema and folliculitis, which cause inflamed hair follicles. Dermatitis and scabies can be treated with a specialized sulphur ointment. Some sulphur treatments are available over the counter, but in severe cases. **(paechiyammal*etal.,* 2016).** Sulphur, which is insoluble in water, finds a crucial place in Siddha materiamedica along with mercury for use in therapeutics and in maintenance of health.

GandhagaParpam is mainly used therapeutically in eighteen types of Leucoderma, Flatulene, Hepatomegaly, Ascites, Gastric, Ulcer, Eye disease, Poisonous, Bites, Chronic veneral disease, Rheumatic fever, Diarrohea and respiratory symptoms. **(Nesapriya J *etal.,* 2012)**

1.9 Objective

Siddha system is the ancient and unique among the Indian system of medicines. The traditional systems of medicine have become significantly more popular all over the globe because of the curative property, less toxic and minimal side effects. Active research in the mechanism of curative actions of Siddha system of medicine is very much essential, to have widespread acceptance of the ancient practice. International bodies like world health organization, provides guidelines for prevention, control, safety, efficacy as well as evaluation and standardisation of herbomineral preparations.

Standardization of Siddha preparations is highly essential and it becomes mandatory with respect to regulatory concern. Standardization not only to mention its quality but also to maintain the standard of the finished product. As a consequence of focusing upon the current need of drug standardization the present work has been undertaken to standardize the traditional herbomineral Siddha formulation **GandhagaParpam (GP).** The objective of the work include,

To study the

i. **Organoleptic properties**

ii. **Physical properties**

- ✓ Solubility
- ✓ 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

2. REVIEW OF LITERATURE

The reviews of literature related to physiochemical analysis phytochemical constituents of the Siddha drug, biological activity of the Siddha drug **(GP)** 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 properties of GandhagaParpam as part of standardisation as per AYUSH Guidelines was done by **Geetha *et al.*, (2017).** The physico-chemical analysis of the drug showed the quality of the drug. From FTIR molecular structure of the sample was interpreted.SEM analysis 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.

- **Shailaja*et al.*,(2016)** confirmed the presence of nanoparticles like **nano mercury, nanogold, nanosilver,nanozinc** in Siddha medicines like LingaChenduram, PoornaChandrodayam, VelliParpam, KshayaKulandhagaChenduram, MuppooraChenduram, ThangaParpam, Naga Parpam, Naga Chenduram, CharaParpametc., with the aid of modern technology like SEM with EDAX, FTIR, ICP-OES, XRD, DLS etc.,

- **Madhavan*et al.*, (2016)** carried out the purification and standardization of sangu. Sangu (conch) is one of the animal products which were 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.

- **Madhavan*et al.*,(2016)** prepared and analysed SanguParpam according to the standard procedures. This is used frequently for management of peptic Ulcer disease. Physico chemical characters of SanguParpam were analysed 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. Organoleptic characters 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 *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 nano particles 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 typhiand 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 Anaemia. The result revealed that the metals in this preparation were in sulphide form which favours the therapeutic efficacy of the drug. Some particles in nano range were also identified.**Rajamaheswari *et al.*,(2014).**

¶ The processing and chemical characterization of *Kajjali* drug using various techniques, viz. XRD, SEM, XPS, Particle size analyser, TGA, and EDXRF have been reported. *In vitro* bovine shrimp assay and Osmotic fragility test have also been performed. XRD pattern of preparation delineate 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 characterisation of the study, clearly delineates in crystal view manner of its safety concern.**Thakur *et al.,* (2014).**

¶ The Mega SanjeeviMathirai a Siddha herbometallic drug was prepared by **Sathish *et al.,* (March – April 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**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 usingmodern 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 themedicines contained metal particles were also in the nano range.**ArunSudha*et al.,* (2009).**

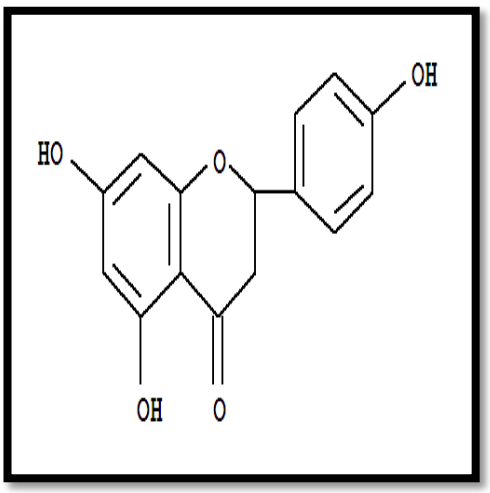
¶ **Elango*et al.,* (2006)** carried out the standardization of Kanthachendooram using pharmacognostical standardization method. Chendooram was prepared by using 8 ingredients, viz. 1. Purified Lode Stone, 2.Purified Sulphur, 3. Lead wort root powder, 4. Eclipta juice, 5. Lime juice, 6. Milk, 7.Egg albumin,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

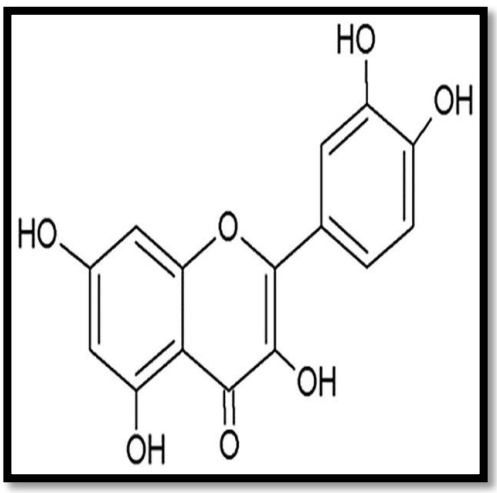
¶ **Anoop Austin *et al.,* (1999)** carried out the standardization of Linghachendooram' number 1 (i.e. Number 1 is prepared by titration process 'Churukku' process) using Cinnabar, the chief ore of mercury .which is a single drug useful in Siddha system of medicine. The standardization was carried out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

2.2 Biological activity:

- **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, Costusspeciosus, Lipidiumsativum,, Cynodandactylon, MerremiaemarginataBurm. F., PortulacaoleraceaLinn, Hemidesmusindicus R.Br. Anantmul,.Tribulusterrestris. Jatrophacurcas. CyperusrotundusL*****, Vitexnegundo,Withaniasomnifera*****(L) and*** ***Zingiberofficinale*****Rosc.**

- 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 ofThraatchathichooranam 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. This result revealed the active ingredients responsible for the beneficial effect of TC and thereby evidences the traditional claim.**Ramakrishnan*et al.*,(2015).**

¶ **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 andidentify 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 BG 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 reached 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. **Merish *et al.,* (2014 April).**

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

¶ **Akila*et al.,*(2014)** reported Physico chemical analysis of GomutrasilasathuParpam by ICP-OES elemental analyser and CHN analyser. 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.

¶ The antibacterial properties of three herbo- mineral siddha drugs (**Palakaraiparpam, Padikaraparpam, Uppuchenduram**) on clinically isolated *Enterococcus strains* were evaluated fifteen bacterial strains isolated from clinical samples were identified as *Enterococcus faecal* is*, 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 thatPadikaraParpam was considered sensitive against the tested *Enterococcus faecal* is 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 thatthe drug PadikaraParpam contains essential elements which are considered to be good anti-microbial activity against *Enterococcus spp*. **Shobha*et al*., (2014).**

¶ Characterization of physico– chemical traits of vaalai Rasa Chendooram was done by **Shajahan*et al*., (2013)** was prepared as per Agathiyarsiddhar method using the ingredients like sulphur, mercury, gold, alum, potassium nitrate and Aloe Vera. The prepared drug was analyzed for chemical properties using FTIR andDSC-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.

¶ Herbo mineral GandhagaMezhugu, especially used in skin diseases was prepared and evaluated for its antimicrobial potential. Antimicrobial efficacy of gandhagam (Raw sulphur), purifiedgandhagam and gandhagamezhugu were evaluated against six pathogens*Escherichia coli*, *Proteus vulgaris*, *Klebsiellapneumoniae*, *Staphylococcusaureus, Streptococcusmutans, Candida albicans* which was associated withvarious disease conditions. The agar check diffusion was used to determine the sensitivityof the samples, whilst the micro-dilution method was used for the determination of the MIC. Of the samples assayed, the samples of GandhagaMezhugu were observed to be the more effective against all the tested pathogens. Theresults provided evidence that the studied samples might be potential sources of newantimicrobial drug.**Shanmugapriya*et al.*, (2013).**

¶ Herbomineral drug **'ThamiraParpam'**by calcining the purified copper foils with earthworm, Clinuslotoidesjuice according to Siddha medicine was prepared by **Krishnaveni (2013).**Antimicrobial activity of ThamiraParpam was investigated

against both gram positive and gramnegative 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.

¶ The anti-ulcer properties of **TamiraParpam**(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**Krishnaveni (2012).** A significant antiulcer activity of TamiraParpamwas 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 TamiraParpamshowed 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 TamiraParpampossessed potential anti-ulcer activity in the three models that was reported.

¶ **ShipraBhargava*et al.*,(2012)** studied the antimicrobial activity of ZingiberOfficinaleextract and their phytochemical composition. Phytochemical screening revealed the presence of **alkaloids, saponins, tannins, flavonoids, terpenoid and phlobotannins**in the extracts. The ZingiberOfficinaleextracts 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, Willi*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.

¶ **Savarimuthu Michael *et al.,* (2011)** studied the antibacterial potential against Escherichia coli, Salmonella typhi, Vibrio cholerae, Klebsiellapneumoniae, and Staphylococcus aureus 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.

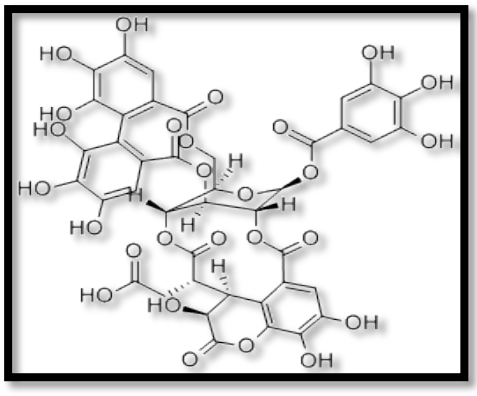
¶ **ThangaThirupathi*et al.,* (2002)**studied pharmacological validation of two siddha drugs SanguParpam and SilasathuParpam. These were evaluated for its antiulcer effect in albino rats. The result revealed that both siddha formulations are indeed good antiulcer agents.

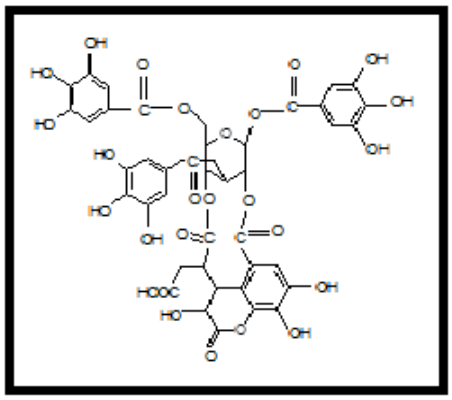
2.3 Phytochemical constituents of the plant

The drug contains the exracts of the following plants

- *Terminaliachebula* Retz ,
- *Zingiberofficinale,*
- *Allium cepa*

¶ The aqueous extract of galls from *Terminaliachebula* Retz. (Combretaceae) was fractionated on Diaion and refractionated on octadecyl silica column. Six phenolic compounds were isolated and identified as **Gallic acid , punicalagin ,isoterchebulin , 1,3,6-tri-*O*-galloyl-β-D-glucopyranose , chebulagic acid(3) and chebulinic acid (4) .** All of the compounds showed stronger DPPH radical scavenging and melanin inhibitory activities than **ascorbic acid, butylatedhydroxytoluene, α-tocopherol, arbutin and kojic acid,** the reference compounds.





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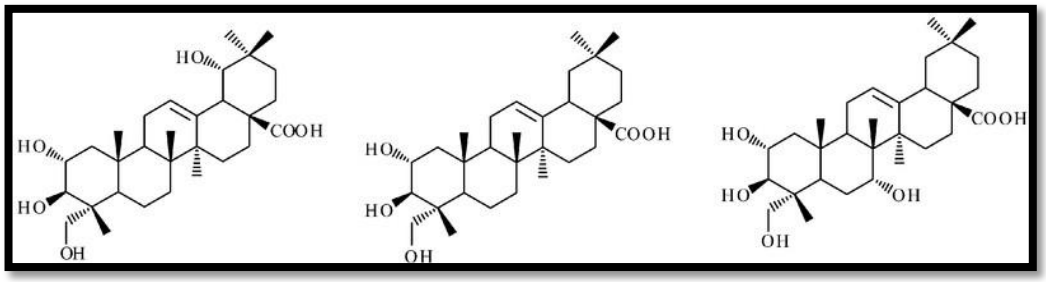
Gallic acid exhibited inhibitory activity against nitric oxide production in lipopolysaccharide-activated macrophages and all isolated compounds exhibited less activity than the reference compounds in mushroom tyrosinaseinhibition and human tumour cytotoxicity assays. This study had demonstrated that 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 (5).** From the plant their structures were determined by spectroscopic methods including NMR and HRESIMS techniques.**Ali*et al., (2009)***



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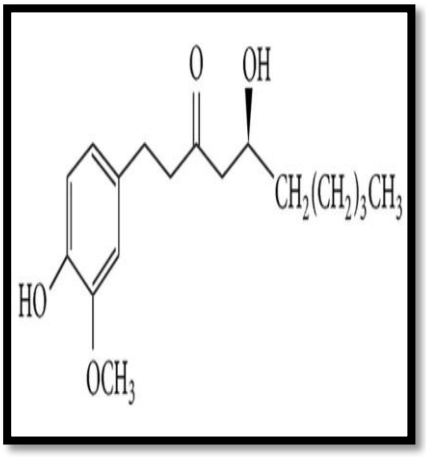
¶ Gas chromatography in conjunction with mass spectrometry, a technique employed to analyze non-volatile pungent components was applied to analyze unmodified partially purified fractions from the dichloromethane extracts of organically grown samples of fresh Chinese white and Japanese yellow varieties of ginger, *Zingiberofficinale* Roscoe (Zingiberaceae). This analysis resulted in the detection of 20 hitherto

unknown natural products and 31 compounds previously reported as ginger constituents. These include **paradols,dihydroparadols, gingerols (6), acetyl derivatives of gingerols, shogaols (7), 3-dihydroshogaols, gingerdiols, mono- and diacetyl derivatives of gingerdiols, 1-dehydrogingerdiones, diarylheptanoids, and methyl ether derivatives** were some of these compounds.



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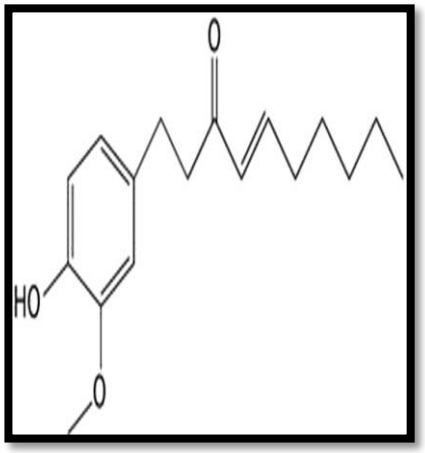

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The thermal degradation of gingerols to gingerone, shogaols, and related compounds was demonstrated. The major constituent in the two varieties was [6]-gingerol, a chemical marker for *Z. officinale*. Anti-inflammatory activities of silica gel chromatography fractions were tested using an in vitro PGE_2 assay. Most of the fractions containing gingerols and/or gingerol derivatives showed excellent inhibition of LPS-induced PGE_2 production. **Shivanand D. Jolad*et al.*,(2004).**

¶ The stability of the major flavonolglucosides, QDG and QMG, was studied in two varieties of onion (Red Baron and Crossbow) that were cured and stored for 6 months under normal commercial conditions and analysed at regular intervals. Onions *(Allium cepa)*were also cooked by boiling in water and by frying in oil under normal domestic conditions. Apart from a 50% loss of QMG during the initial drying process, little change in content and composition was observed over 6 months of storage. Neither boiling nor frying resulted in interconversion of the quercetin conjugates or production of free quercetin, although a 25% loss overall was recorded for each process.**Keith, Price*et al.*,(1997).**

Biological activity of the plant

- **ŽeljanaFredotovic*et al.*, (2017)** reported a comparative study of the phytochemical profile and the biological activity of two onion extracts, namely *Allium cepa L.* and *Allium _ cornutum* (Clementi ex Visiani 1842), members of the family Amaryllidaceae. The identification of flavonoids and anthocyanins, and their individual quantities, was determined by HPLC. The potency of both extracts to scavenge free radicals was determined by the DPPH radical-scavenging activity and ORAC methods. The DNA protective role was further tested by COMET assay and by Fenton's reagent causing double-strand breaks on the closed circular high copy pUC19 plasmid isolated from *Escherichia coli.* In the presence of both extracts, a significant decrease in DNA damage was observed, which indicated a protective role of *Allium cepa* and *Allium _ cornutum* on DNA strand breaks and additionally, cytotoxicity was tested on glioblastoma and breast cancer cell lines. The results revealed that both extracts had anti-proliferative effects, but the most prominent decrease in cellular growth was observed in glioblastoma cells**.**

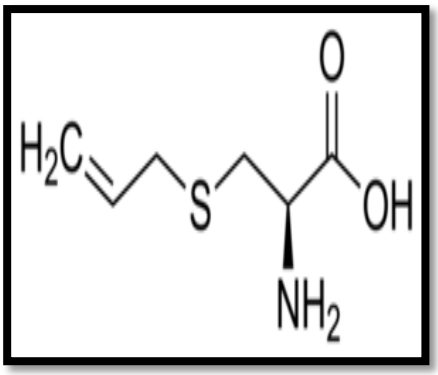
- The antibacterial activity of crude extract of *Terminaliachebula*Retz was studied against gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*and*Klebsiellapneumoniae*and gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcusaureus*. The antibacterial activity was studied by disc diffusion method. Extracts with the different solvent of *T. chebula*Retz exhibited the **antibacterial activity** 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 methanols, ethanol and acetone seems to be the best solvent when compared to other solvents.**TensinghBaliah*et al.*, (2014)**.

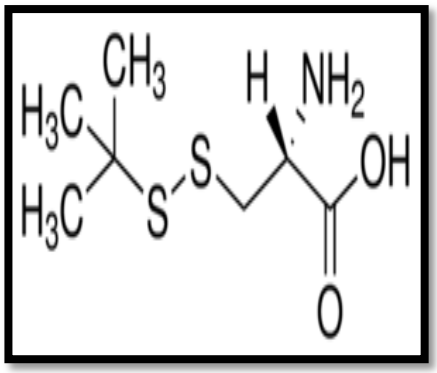
- An ethanol extract of *Terminaliachebula*fruit was studied for its antibacterial activity against clinically important standard reference bacterial strains was reported by **Kannan*et al.*,(2009).** The antimicrobial susceptibility was screened using the disc diffusion method and the MIC was determined using the broth micro dilution method.

The results showed that it was active against both gram-positive and gram-negative 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.**

¶ The hypoglycaemic potentials of ginger *(Zingiberofficinale)* were studied in rats by **Zainab, Al-Amin *et al.*, (2006)**. An aqueous extract of raw ginger was administered daily (500 mg/kg, intraperitoneally) for a period of 7 weeks to streptozotocin (STZ)-induced diabetic rats. Fasting blood serum was analysed for blood glucose, cholesterol and triacylglycerol levels. The streptozotocin -injected rats exhibited hyperglycaemia accompanied with weight loss, indicating their diabetic condition. At a dose of 500 mg/kg, raw ginger was significantly effective in lowering serum glucose, cholesterol and triacylglycerol levels in the ginger-treated diabetic rats compared with the control diabetic rats. The ginger treatment also resulted in a significant reduction in urine protein levels. In addition, the ginger-treated diabetic rats sustained their initial weights during the treatment period. Moreover, ginger decreased both water intake and urine output in the STZ-induced diabetic rats. The result indicated that raw ginger possesses hypoglycaemic, hypocholesterolaemic and hypolipidaemic potential as well as it effective in reversing the diabetic proteinuria observed in the diabetic rats. Thus it was proved that the ginger may be of great value in managing the effects of diabetic complications in human subjects.

¶ A number of studies have demonstrated the chemo preventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a number of organosulfur compounds derived from garlic. The chemo preventive activity has been attributed to the presence of organosulfur compounds in garlic. Several modes of action have been proposed these include its effect on drug metabolizing enzymes, antioxidant properties and tumour growth inhibition.





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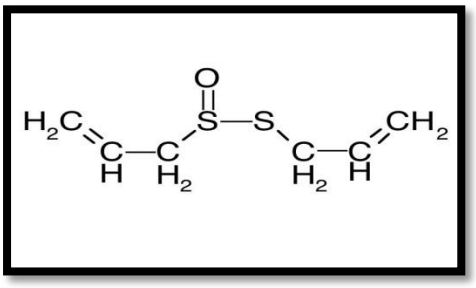
Most of these studies were carried out in the animal models. Also, recent research has focused on the anti-mutagenic activity of garlic. Recently, it has been observed that aged garlic extract, but not the fresh garlic extract, exhibited radical scavenging activity. The two major compounds in aged **garlic, S-allyl cysteine (8) and S-allylmercapto-L-cysteine (9),** had the highest radical scavenging activity. In addition, some organosulfur compounds derived from garlic, including S-allylcysteine, have been found to retard the growth of chemically induced and transplantable tumours in several animal models. Therefore, the consumption of garlic may provide some kind of protection from cancer development. **Martha Thomson *et al., (2003)***.

¶ **Stephen Parcell*et al.*, (2002)** emphasized the importance of elemental sulfur in humans and discuss the therapeutic applications of sulphur compounds in medicine. The sulphur containing amino acids are **methionine, cysteine, cystine, homocysteine, homocystine, and taurine**. Dietary sulphur containing amino acid analysis and protein supplementation may be indicated for vegan athletes, children, or patients with HIV, because of an increased risk for sulphur containing amino acid deficiency in these groups. Methylsulfonylmethane, a volatile component in the sulfur cycle, is another source of sulfur found in the human diet. Increases in serum sulfate may explain some of the therapeutic effects of Methylsulfonylmethane,, DMSO, and glucosamine sulfate. Organic sulfur, as SAAs, can be used to increase synthesis of S-adenosylmethionine, glutathione, taurine, and Nacetylcysteine .Methylsulfonylmethane,may be effective for the treatment of allergy, pain syndromes, athletic injuries, and bladder disorders. Other sulfur compounds such as

S-adenosylmethionine, DMSO, taurine, glucosamine or chondroitin sulfate, and reduced glutathione may also have clinical applications in the treatment of a number of conditions such as depression, fibromyalgia, arthritis, interstitial cystitis, athletic injuries, congestive heart failure, diabetes, cancer, and AIDS. The low toxicological profiles of these sulphur compounds, combined with promising therapeutic effects, warranted continued human clinical trials.

¶ Antioxidant properties of garlic compounds representing the four main chemical classes, **alliin, allyl cysteine, allyldisulfide, and allicin (10),** prepared by chemical synthesis was investigated by **Lip Yong Chung *et al.,* (2000)**. Alliin scavenged superoxide, while allyl cysteine and allyldisulfide did not react with superoxide. Allicin suppressed the formation of superoxide by the xanthine/xanthine oxidase system, probably *via* a thiol exchange mechanism. Alliin, allyl cysteine, and allyldisulfide all scavenged hydroxyl radicals; the rate constants calculated based on deoxyribose competitive assay were 1.4–1.7 × 10^{10}, 2.1–2.2 × 10^{9}, and 0.7–1.5 × $10^{10} M^{-1}$ second^{-1}, respectively. Contrary to previous reports, allicin did not exhibit hydroxyl radical scavenging activity .Alliin, allicin, and allyl cysteine did not prevent induced microsomal lipid peroxidation, but both alliin and allylcysteine were hydroxyl scavengers, and allyldisulfide was a lipid peroxidation terminator. The study indicated that allyldisulfide, alliin, allicin, and allyl cysteine exhibit different patterns of antioxidant activities as protective compounds against free radical damage.



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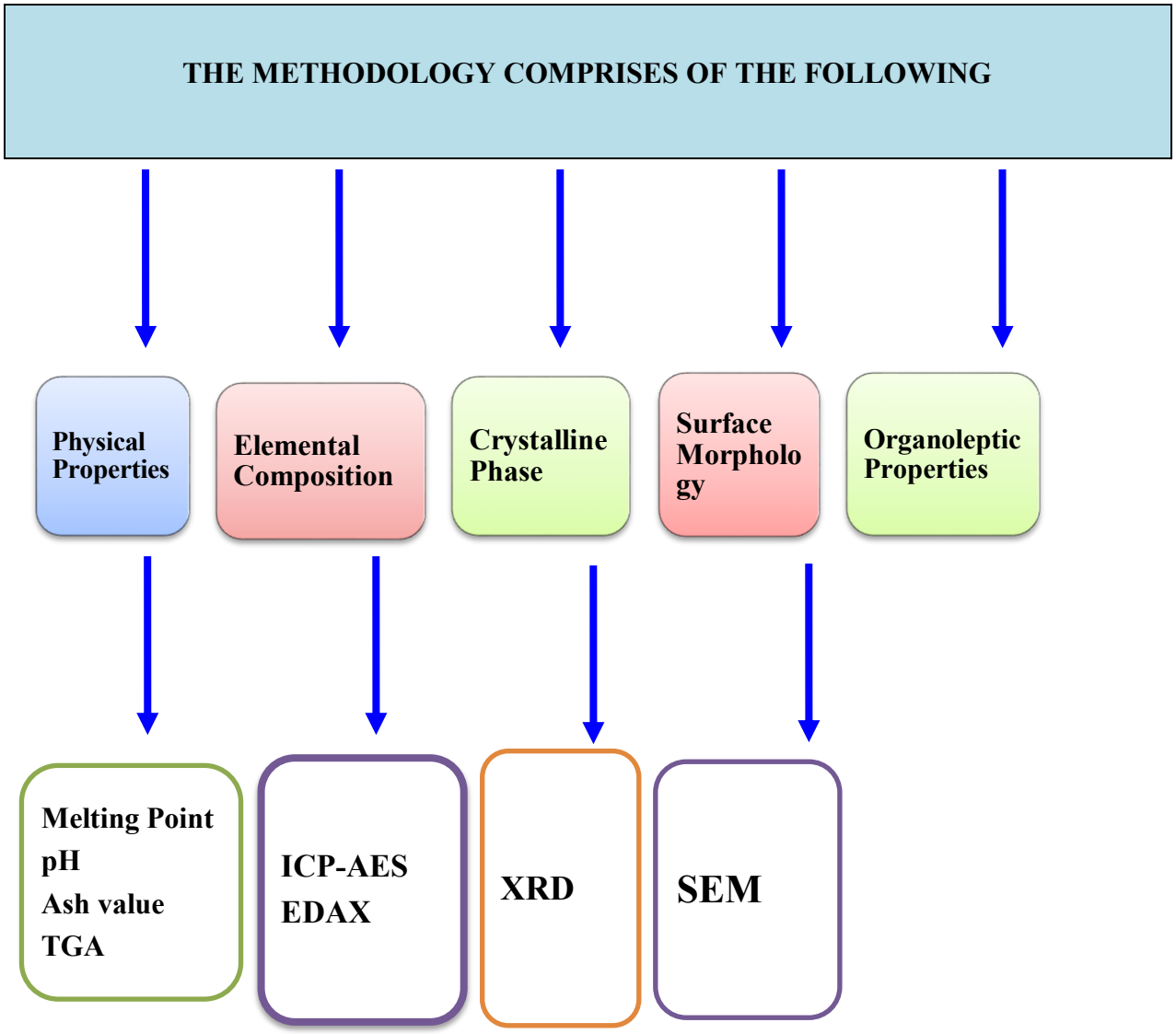
3. MATERIAL AND METHODS

In the present study evaluation of **Gandhaka Parpam(GP)**a Siddha drug 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.



3.1 ORGANOLEPTIC PROPERTIES

Organoleptic property of drug was examined according to conventional method given by Kokate. **(Kokate*etal.*, 2002).** The sample was evaluated for the organoleptic characters like colour, odour, appearance, taste and solubility. Solubility was tested in water, organic solvents, and concentrated acids including aqua regiathe characteristic changes were observed.

3.1.1 Test with concentrated hydrochloric acid

A small amount of the sample was treated with concentrated hydrochloric acid and the sample was found to float on the surface.

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 float on the surface.

3.1.3 Test with concentrated sulphuric acid

A small amount of thesample was treated with concentrated sulphuric acid and the sample was found to float on the surface.

3.1.4 Test with 5% aqueous sodium hydroxide

A small amount of the sample was treated with 5% aqueous sodium hydroxide. An oily appearance was observed.

3.1.5 Test with iodine solution

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

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 float on the surface.

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 float on the surface.

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

(i) Silica crucibles

(ii) Muffle Furnace - Furnace was fitted with an indicating pyrometer, tomaintain the temperature. **(Genuine)**

(iii) Analytical balance – with to 0.001 mg sensitivity. **(Shimaozu AY220)**

(iv) Desiccator

(v) 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 **GP**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.

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

W = Weight of air – dried drug.

3.2.3(B) Acid insoluble ash

The ash obtained by the procedure **(3.2.3.A)**was boiled with 2.5 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible, washed with hot water, ignited and cooled in desiccators. Then it was weighed. The percentage of acid insoluble ash was calculated with reference to the air- dried drug.

3.2.3(C)Water soluble ash

The ash obtained by the procedure **(3.2.3.A)** was boiled for 5minutes, with 25 ml of water. The insoluble matter was collected in a Gooch crucible, washed with hot water, and ignited for 15minutes not exceeding 450 ^{0} C. The insoluble matter was subtracted from the weight of the ash, the difference in weight represent the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the 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 washeated 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 grams

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 in 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.Afterthe 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

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.

3.5 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.

4. RESULTS AND DISCUSSION

In the present study quantitative analysis of the herbo mineral drug GP was carried out using modern analytical techniques. The results of the study were deliberated in the following folios.

4.1 ORGANOLEPTIC PROPERTIES

Organoleptic property includes study of morphology and other sensory characters like shape, size and fracture of drug .The results were summarised in Table 1

Table 1Organoleptic properties of GP

S.No	Parameters	Observation
1	Colour	Pale yellow
2	Odour	Mild rotten egg smell
3	Taste	Tasteless,
4	Sate of drug	Powder
5	Consistency	Soft

The behaviour of drug with acidic, basic and neutral reagent was observed. The results were tabulated in the **Table 2.** the treatment of GP with concentrated Hydrochloric acid, concentrated Nitric acidconcentrated Sulphuric acid, 5% aqueous potassium hydroxide showed no effect. The sample was insoluble in iodine solution and Glacial acetic acid. An oily appearance with sodium hydroxide.

Table 2 Behaviour of GP with different reagent

S.No	Chemical treatment	ervation
1	g powder treated with Concentrated Hydro Chloric Acid	Powder float on the surface.
2	g powder treated with Concentrated Nitric Acid	Powder float on the surface.
3	Drug powder treated with Concentrated Sulphuric Acid	Powder float on the surface.
4	g powder treated with 5% aqueous Sodium Hydroxide	Oily appearance
5	rug powder treated with Iodine Solution	sample was found to insoluble &if settled down slowly.
6	Drug powder treated with5% aqueous Potassium Hydroxide Solution	Powder float on the surface.
7	g powder treated withGlacial Acetic Acid	sample was found to insoluble &if settled down slowly.

4.2 PHYSICAL PROPERTIES

Physical properties of GP like solubility, ash value, and loss on drying, pH values and stability were determined as per standard procedures.

4.2.1 Solubility of a drug is an important biopharmaceutical parameter as it affects the rate of dissolution and thus affects the rate of absorption. Hence the solubility of GP was checked with solvents like DCM, HPLC Chloroform, Benzene, Cyclohexanone and water. The GP was found to be soluble in all the tested solvents except water.

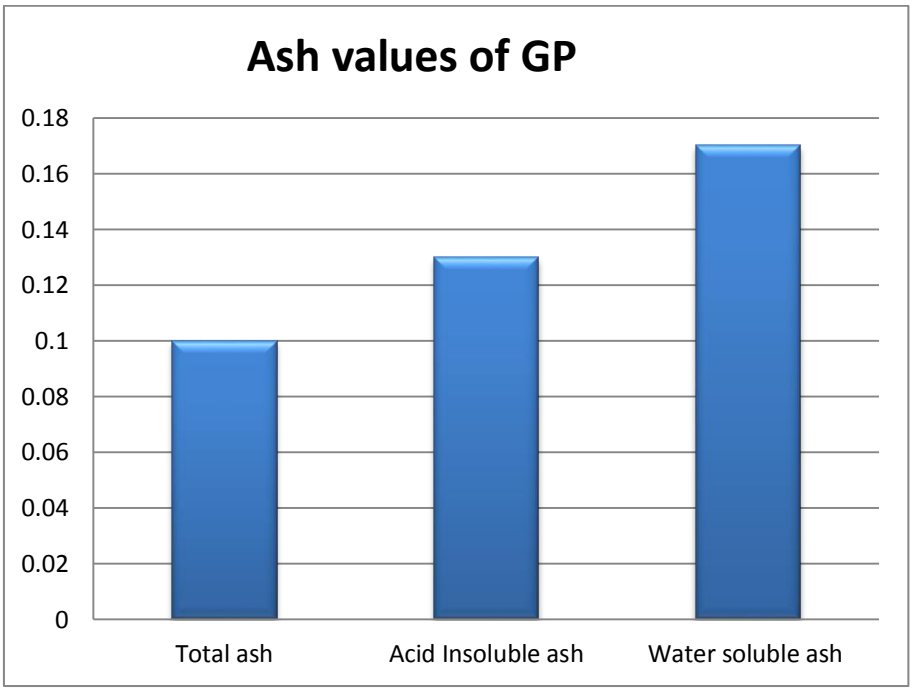
4.2.2 The melting point of the drug was determined by melting point apparatus**AjayR**. The melting point was found to be 320^0C.

4.2.3pH 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**4.7-5.0.**

4.2.4 Ash value:

Ash value is helpful in determining the quality of the crude drugs on powder. It usually represents the inorganic salts present in the drug. Water soluble ash is the measure of physiological inorganic components of the crude drug. **(Sharma *etal*.,2013)**Acid insoluble ash gives an idea about the non- physiological ash produced due to the adherence of inorganic dirt, dust to the crude drug. The ash value was determined using Muffle Furnace heated up to 700◦C. Hence the ash content of GP was found to be 0.1% .The result were shown in **Figure 11**

Figure 11 Ash value of GP



4.2.5 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 **0.16%** which indicated the low moisture content of the GP.

4.2.6 TGA

Figure 12, 13, 14 and 15 represents the TG curve of GP. The weight loss of GP started at 180^0C and completely decreased at 326^0C because of evaporation of sulphur. Above that there was no change in weight observed in the curve. The weight loss at 180^0C and 326^0C was 20% and 80%. DTG curve showed endothermic peak at 288^0C, 306^0C and 321^0C. DTA curve of GP was shown in the figure **(14&15).** It showed two decomposition peaks at 120^0C and 289^0C. TG-DTA results confirmed that the sulphur was present in its pure form viz pristine sulphur.

TG CURVE OF GP

Figure -12

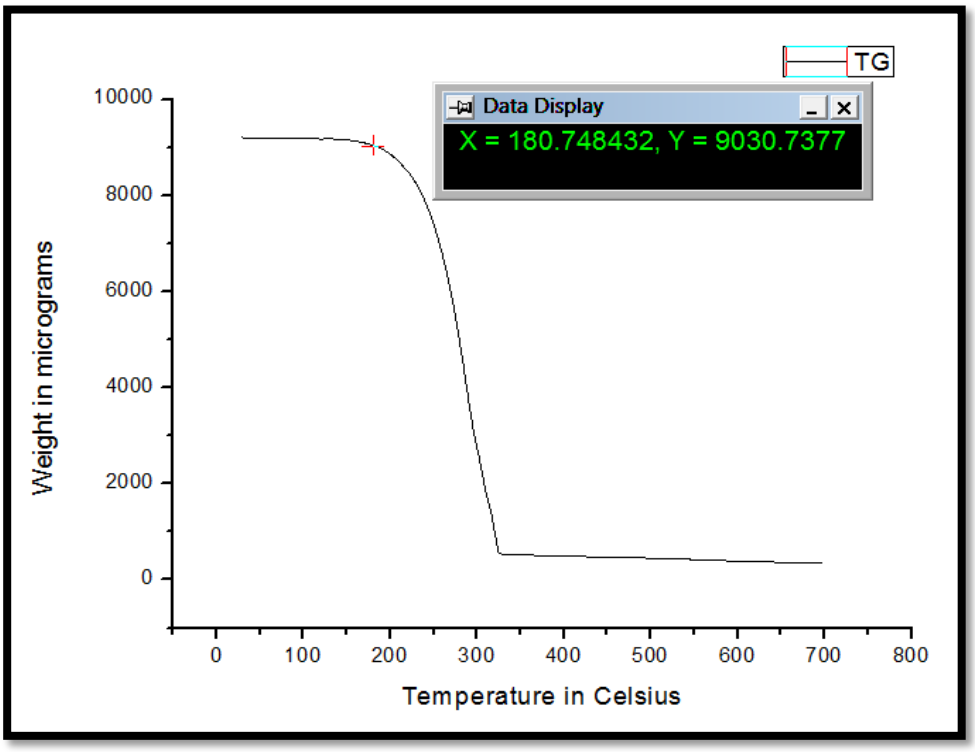
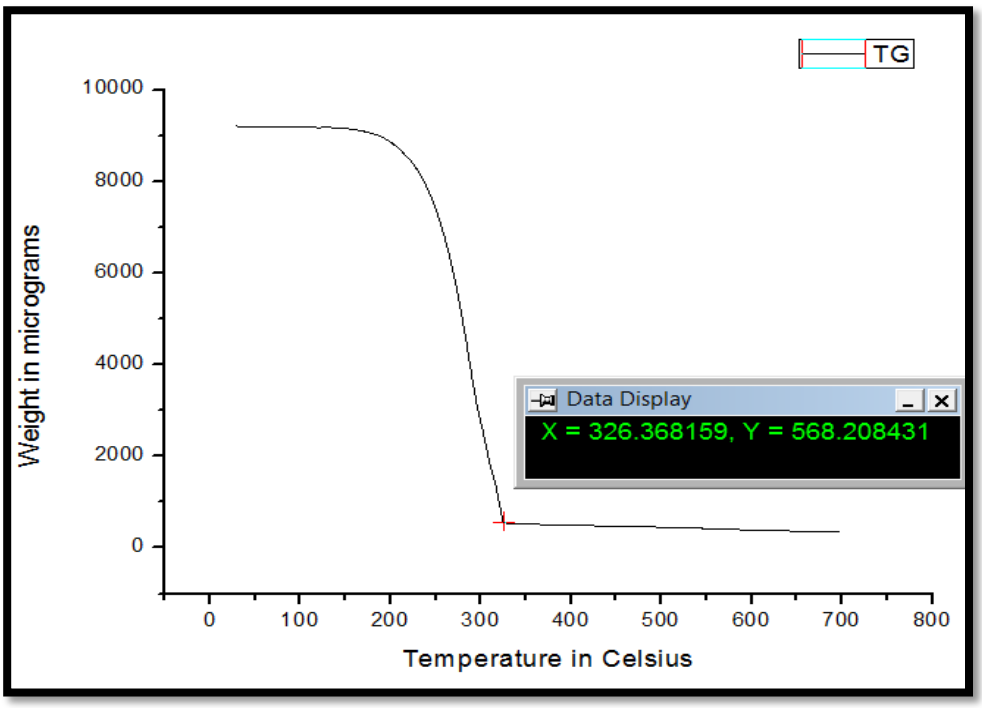


Figure -13



DTA CURVE OF GP

Figure -14

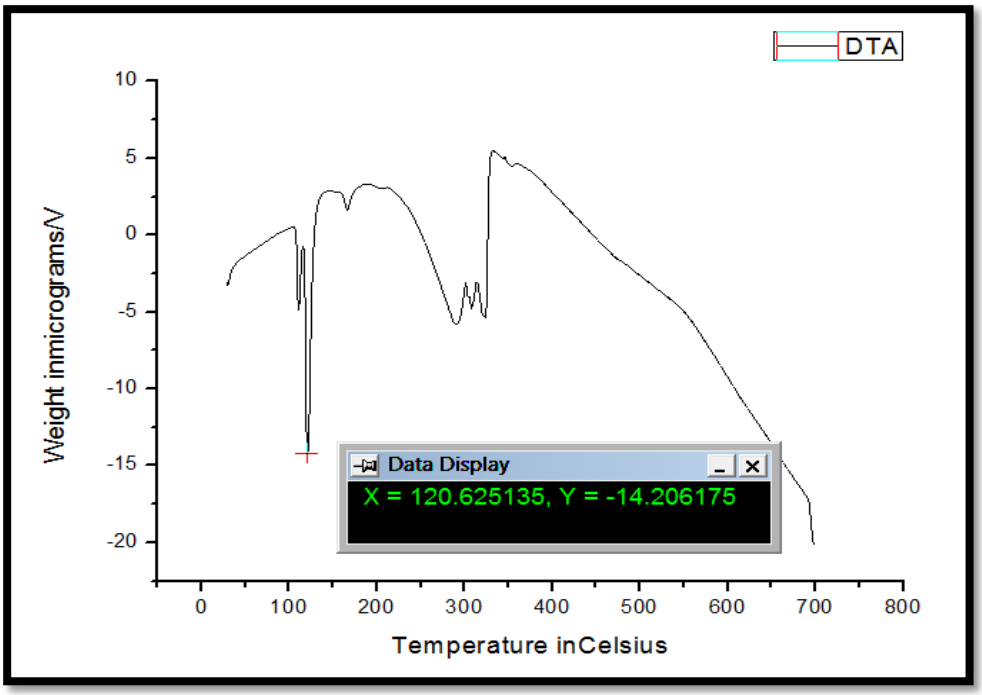
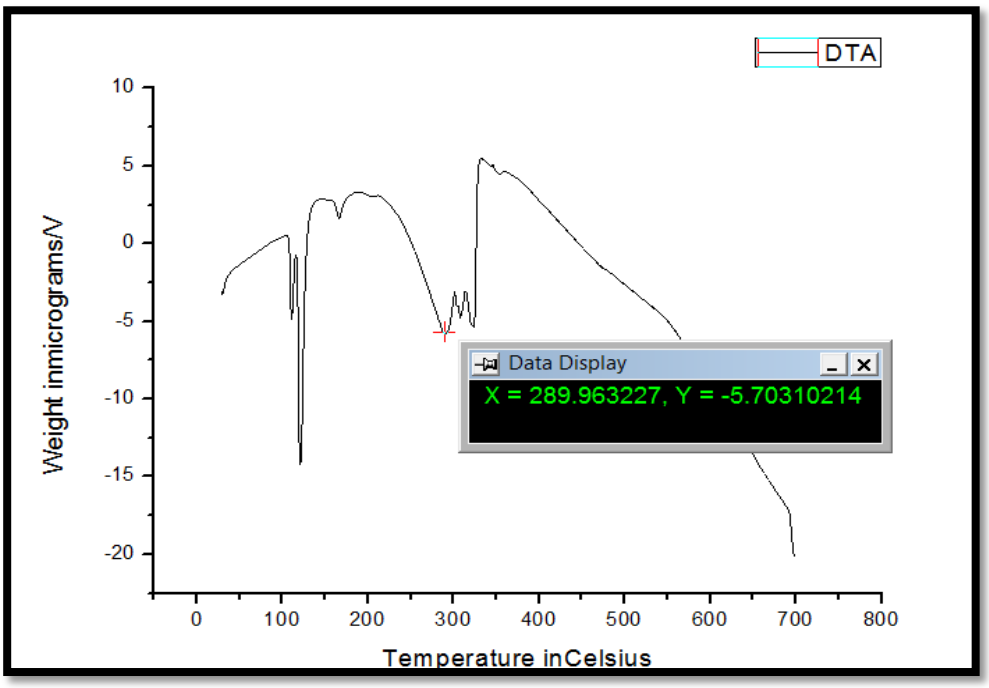
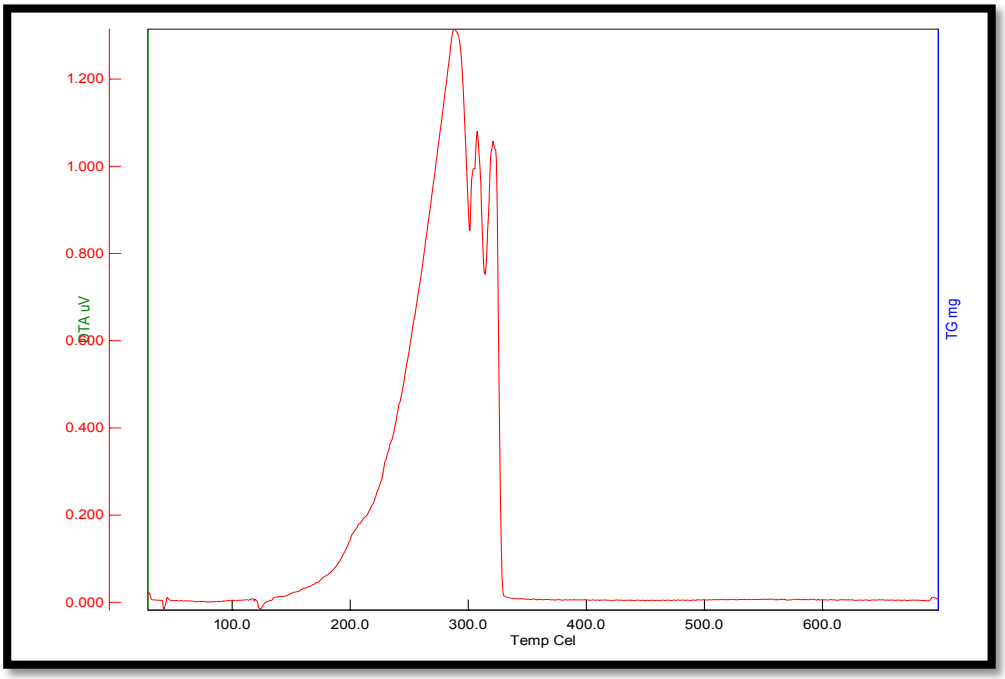


Figure -15



DTG curve of GP

Figure -16



4.3. ELEMENTAL COMPOSITION

4.3.1 ICP-AES

The drug GP sample was analysed by ICP-AES methods to detect the trace elements and other elements quantitatively. Average elemental content of ICP-AES analysis of GP was shown in **Table 3.** Results showed that GP contained seven elements namely Sulphur,Calcium,Zinc, Iron,Lead, And Mercury. The major element in GP was found to be Sulphur (1000 Ppm).

Table -3 Elemental compositions by ICP-AES Method

S.No	Elements	Vave length (ppm)	Detected limit
1	Arsenic (As1890)	BDL	0.10
2	Mercury (Hg1849)	36.8	0.10
3	Lead (Pb2203)	55.2	0.10
4	Zinc (Zn2025)	103.3	0.01
5	Calcium (Ca3158)	2700	0.05
6	Iron (Fe2599)	300	0.05
7	Sulphur (S1820)	1000	0.10

4.3.2 EDAX

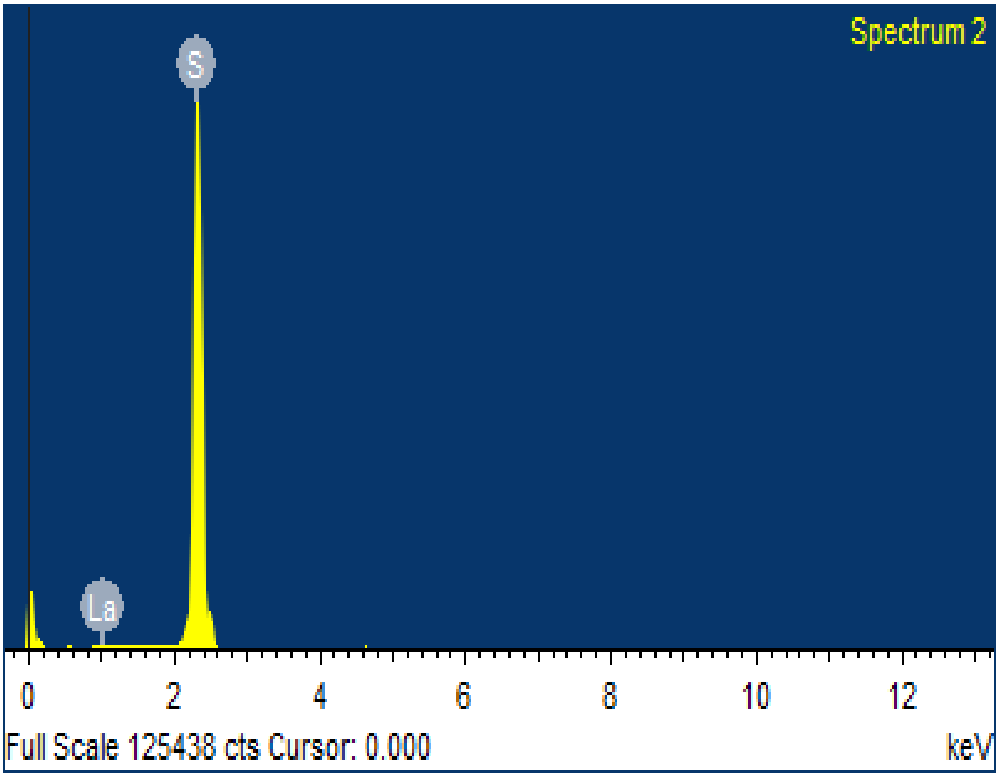
Elemental analysis of the GP showed the presence of Sulphur, Lanthanum, Oxygen **(Table 4 & 5)** were sulphur was found to be the major element in the oxide form (99.48 %). **(Figure 2 and 3)**

EDAX analysis of GP

Table- 4

Element	Veight%	Atomic%	ompound%	Formula
S	39.81	24.95	99.40	SO3
La	0.51	0.07	0.60	La2O3
O	59.68	74.97		
Totals	100.00			

Figure-17 EDAX spectrum of GP

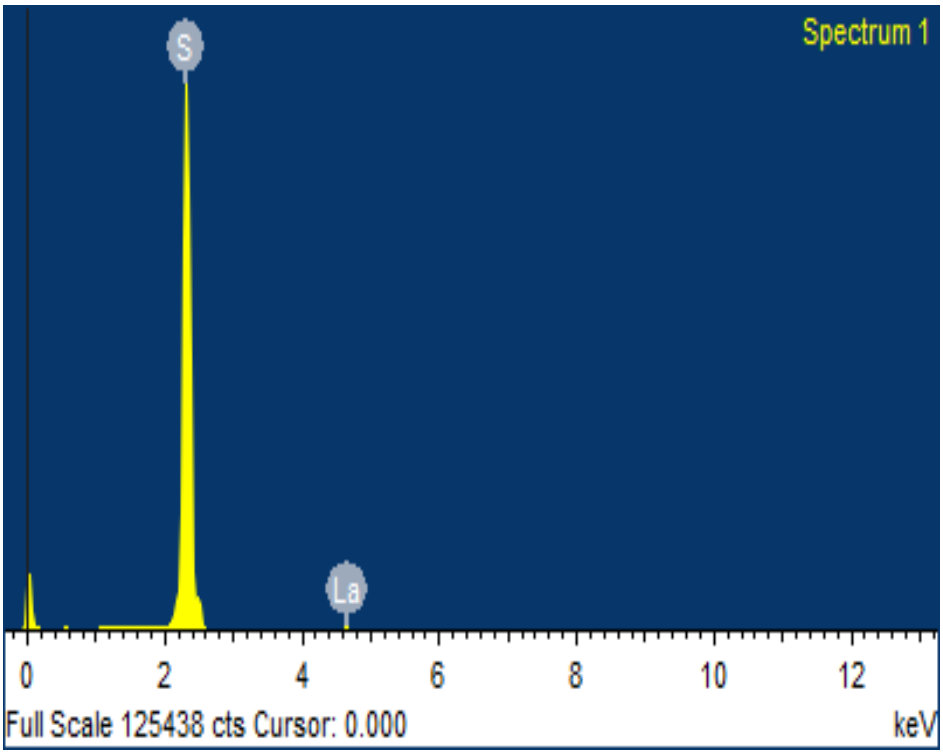


EDAX analysis of GP

Table - 5

Element	**Weight%**	**Atomic%**	**mpound%**	**Formula**
S	39.84	24.96	99.48	SO3
La	0.44	0.06	0.52	La2O3
O	59.71	74.98		
Totals	100.00			

Figure- 18 EDAX spectrum of GP



4.4 SURFACE MORPHOLOGY

4.4.1 SEM:

The particle size of GP was assessed by SEM.**(Figure 19, 20, 21, and 22)** Particle size, shape and surface area affects homogeneity, efficiency and granules and also the stability. SEM photograph of GP showed the presence of nanoparticles in the range from 10µ - 200 nm. The particles were found to have circular shape. Surface was found to be smooth so the flow ability was normal. GP showed difference in size and agglomeration of the particles. Agglomeration of particles may be due to repeated cycles of calcinations involved in the preparation of the drug**. (Arunsudha*et al*2009)**(The extremely small size of nanoparticles allows them to penetrate the cells and interact with cellular molecules). As the particle is in Nano size, a low dose of the drug is enough to treat disease.

SEM PHOTOGRAP'S OF GP

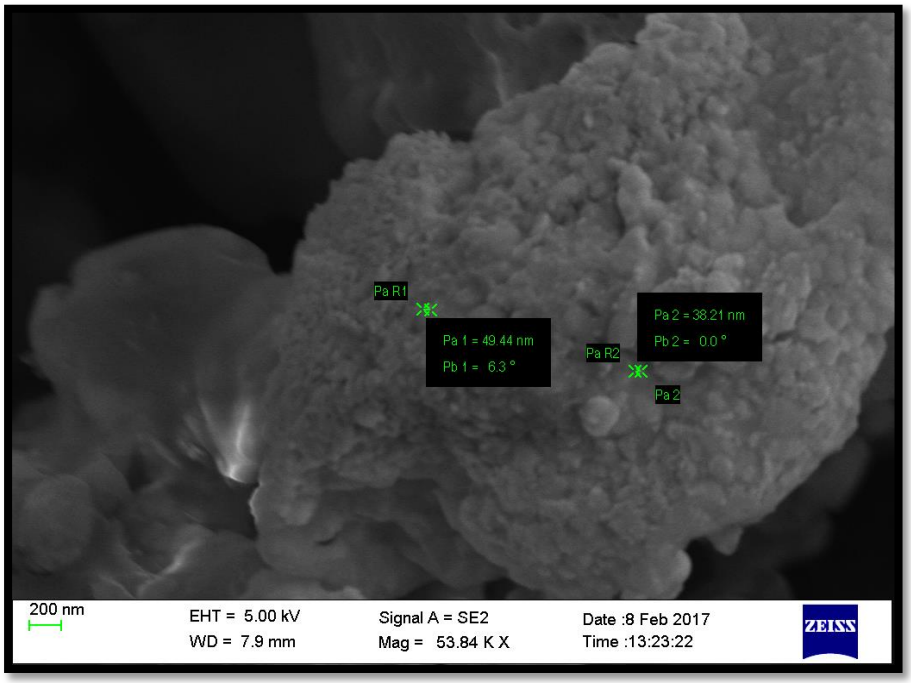


Figure – 19

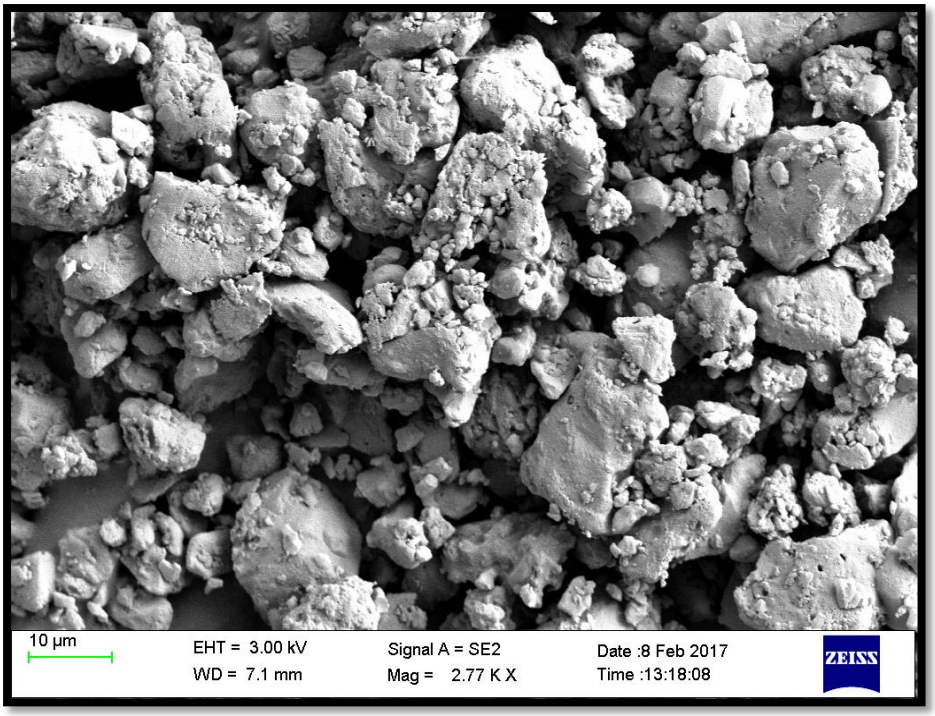


Figure -20

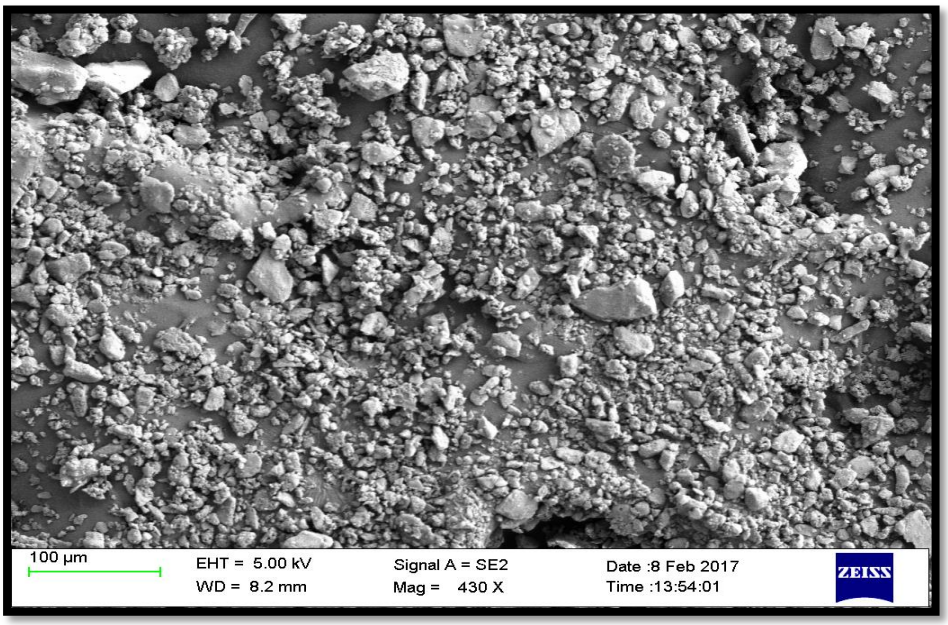


Figure -21

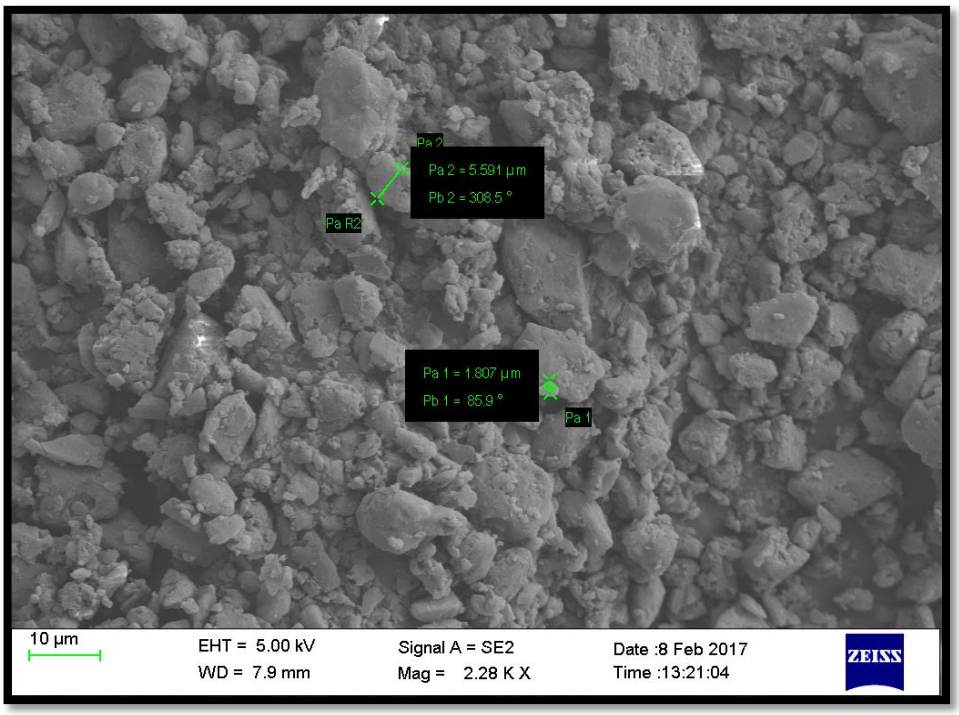


Figure -22

5. CRYSTALLINE PHASE

5.1 XRD

XRD technique was used for the characterization of compound through the crystalline phase identification. The phase identification of GP was done by matching the d-spacing with literature value. (**Thakur K.S *et al* 2014)** The d-spacing value of 3.277 at angle 2θ27.9 showed that the sulphur was present as Ortho-rhombic form in GP. From the XRD graph the particle size was determined at 37.2 nm.

XRD SPECTRUM OF GP

Figure -23

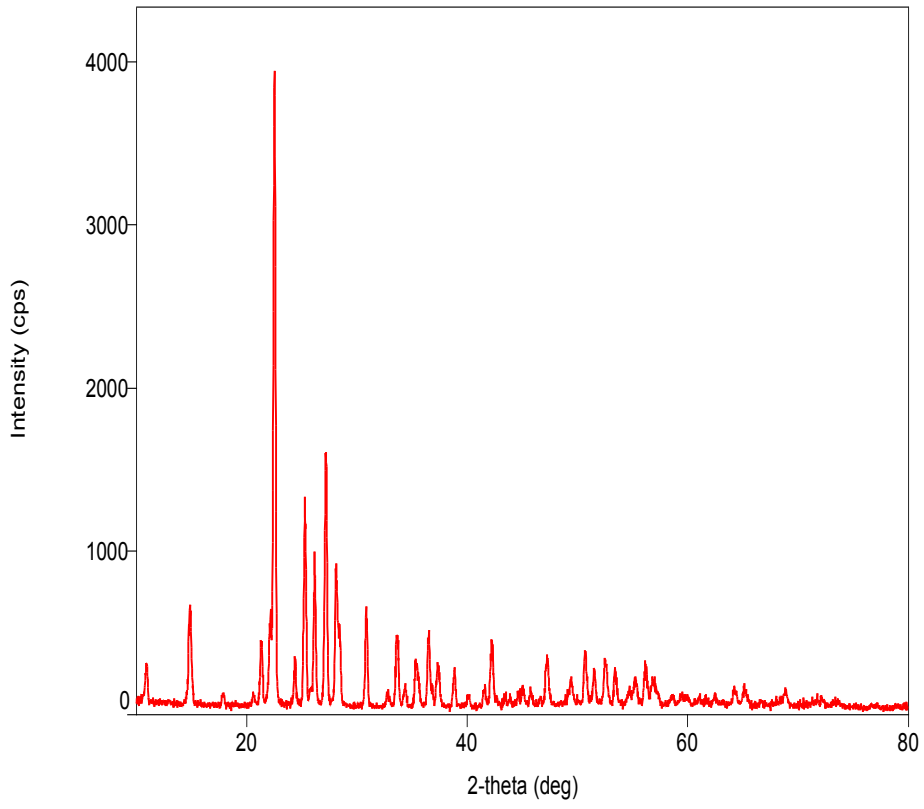
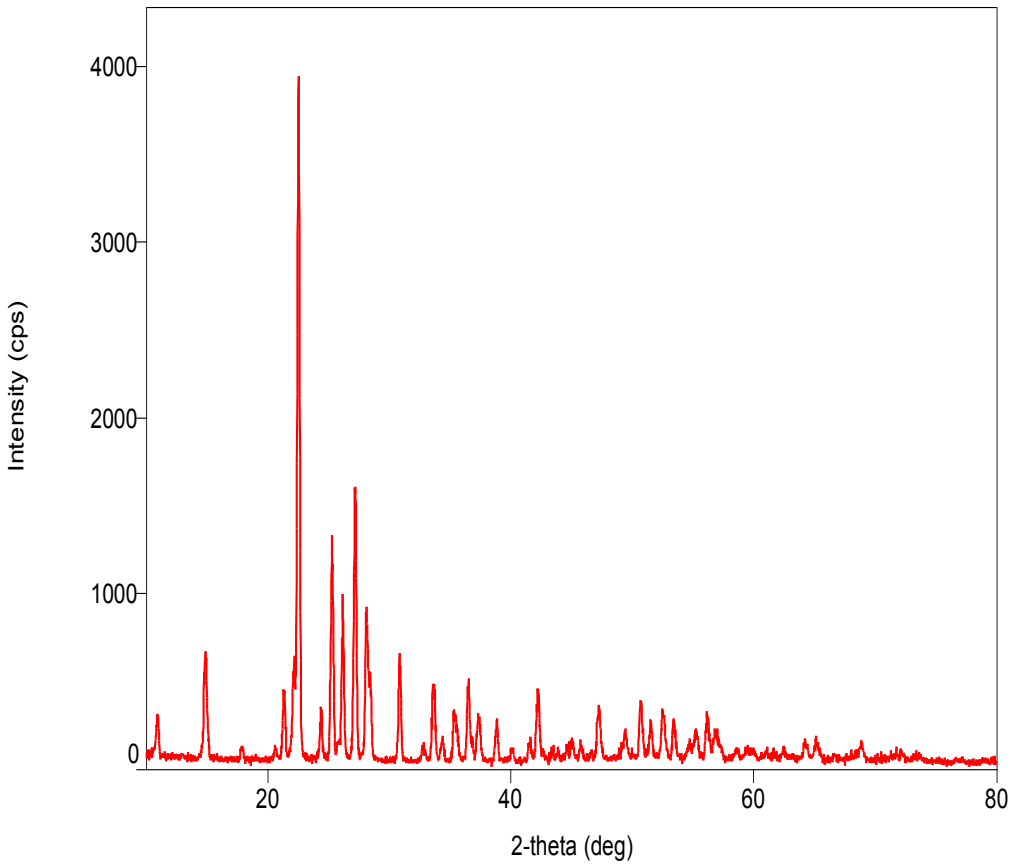


Figure – 24



SUMMARY AND CONCLUSION

In the present work physiochemical analysis of GP a Siddha formulation done to identify the chemical constituents present in the formulation. The findings of the results are summarized as follows; GP is preferably non-toxic to human in its therapeutic dose. Also hence, it ensured the efficacy of GP and established the fingerprint for standardization of the effective herbal formulation.

i. The following metals viz, Sulphur, Calcium, Zinc, Iron, Lead and Mercury were identified ICP-AES method. The major element in GP was found to be Sulphur (1000 Ppm)

ii. EDAX result delineated the presence Sulphur, Lanthanum, Oxygen and sulphur was found to be the major element in it's the oxide form (99.48 %)

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

iv. TG-DTA and XRD analysis results confirmed that the sulphur was present in its pure state viz pristine sulphur as Ortho-rhombic form.

The above investigation on GP using modern techniques was validating the bioavailability of this herbo-mineral formulation.

✓

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