Physico-Chemical Properties of Vatari Chooranam

a Traditional Herbal Preparation

By

N.KEERTHIKA

(16PCH008)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher

Education for Women, University

(Estd.u/s of UGC Act 1956)

Coimbatore- 641043

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In Partial Fulfilment of the Requirements for the Degree of

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Signature of the

Supervisor

Signature of the

Head of the Department

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N.KEERTHIKA

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LIST OF ABBERIVATIONS

VC	Vatari chooranam
HPTLC	High performance liquid chromatography
TLC	Thin layer chromatography
FTIR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscope
GLC	Gas liquid chromatography
XRD	X ray diffraction
TGA	Thermo gravimetric analysis or thermal gravimetric analysis emission spectroscopy
EDAX	Energy dispersive spectroscopy
FOA	Field operating activity
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
DPPH	Diphenyl-1-pierylhydrazyl
pH	Potential hydrogen
AOAC	Association of official analytical chemist
SOD	Sphincter of oddidys function
BHA	Butylated hydroxyanisol
CODEX	Codex alimentaries
STZ	Streptozotocin
AYUSH	Ayurveda ,Yoga and naturopathy, unani
ANOVA	Analysis of varience
OPD	Outpatient department

GC	Gas chromatography
SBP	Spontaneous bacterial peritonitis
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
WHO	world health organisation
GC-MS	Gas chromatography-Mass spectrometry
LC-MS	Liquid chromatography-Mass spectrometry
1D NMR	Deuterium neutron magnetic resonance
HPLC	High performance liquid chromatography
LOD	Loss on drying
UV	Ultraviolet- visible spectroscopy
LC-NMR	Liquid chromatography-nuclear magnetic resonance
IR	Infrared spectroscopy
FIR	Far infrared spectroscopy
DTA	Differential thermal analysis
SFC	Supercritical fluid chromatography
SPE	Solid phase extraction
CE	Capillary electrophoresis
MAE	Microwave – assisted extraction
TEM	Transmission electron microscopy
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene

TBA	Thiobarbituric acid
PCA	Principal component analysis
DMSO	Dimethyl sulfoxide

INTRODUCTION

1. INTRODUCTION

1.1 HERBAL MEDICINES

Herbal medicine is the branch of science which is used from ancient period of time. In 20th century herbal medicine is the only source for medicinal system because on those days synthetic medicines were not improved. Herbal medicines are also known as **phytomedicines,** herbal formulations are used to alleviate the disease. The knowledge on **ethno medicine** is very much important for the preparation of herbal medicine.

Ethno medicine deals with herbs, animals, minerals etc and it is considered as the mother of herbal medicine like **Ayurveda, Siddha and Unani.** Synthetic medicines are avoided due to their adverse effect and dissatisfaction, hence herbal medicines were used as alternatives for synthetic medicine nowadays. Hence standardisation of herbal medicine is very much important to determine the efficacy, safety and quality of the medicine .WHO gives different quality control parameters for standardisation of the drug.

1.2 ADVANTAGES OF HERBAL MEDICINES

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 modern 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. One example is the herbs and alternative remedies used to treat arthritis. Vioxx, a well-known prescription drug used to treat arthritis, was recalled due to increased risk of cardiovascular complications. Alternative treatments for arthritis, on the other hand, have few side effects. Such treatments include dietary changes like adding simple herbs, eliminating vegetables from the nightshade family and reducing white sugar consumption.

Lower cost: Another advantage to herbal medicine is cost. Herbs cost much less than prescribed medications. Research, testing, and marketing add considerable cost to prescribed medicines. Herbs tend to be inexpensive compared to drugs.

Widespread availability: Yet another advantage of herbal medicines are their availability. Herbs are available at wide spread throughout. Hence preparation of herbal medicine is easier.

Disadvantages of herbal medicines

Herbs are not without disadvantages, and herbal medicine is not appropriate in all situations. These are a few of the disadvantages to consider:

Inappropriate for many conditions: Modern medicine treats sudden and serious illnesses and accidents much more effectively than herbal or

- alternative treatments. An herbalist would not be able to treat serious trauma, such as a broken leg, appendicitis etc.

- **Lack of dosage instructions:** Another disadvantage of herbal medicine is the very real risks of self-dosing which is harmful. Many herbs do not come with instructions.

- **Poison risk associated with wild herbs:** In the preparation of polyherbal drugs, if the herbs are not identified properly and if correct part of the plant is not used then the drug becomes poisonous.

- **Medication interactions:** Herbal treatments can interact with medications. Nearly all herbs come with some warning, for example like the herbs used for anxiety such as Valerian and St. John's Wort, can interact with prescription medication like antidepressants.

- **Lack of regulation:** Because herbal products are not strictly regulated, consumers also run the risk of buying inferior quality herbs. The quality of herbal products may vary among batches, brands or manufacturers. This can make it much more difficult to prescribe the proper dose of an herb.

1.3 SIDDHA MEDICINE

Siddha treatment is classified into three categories **1. Devamaruthuvam** which is commonly used as divine method, **2.Manudu maruthuvam** (rational method), **3. Asura maruthuvam** (surgical method). Divine method involves the consumption of medicine orally like parpam, chendooram, guru, kuligai made with mercury, sulphur and pashanams are also used. Rational method medicines are made of herbs like churanam, kudineer or vadagam. Surgical method involve the incision, excision, heat application, blood letting or leech application. Other than these three methods, siddha treatment method can also be classified into purgative therapy, emetic therapy, fasting therapy, steam therapy, oleation therapy, physical therapy, solar therapy, blood therapy, yoga therapy.

Siddha medicines are traditionally used system from ancient period of time especially in South India. Siddha medicines are prepared with the formulation of herbs, minerals, animals, mineral origin etc. These medicines are prepared in small quantity depending on their need for specific diseases. Heavy metal analyses are done for the siddha medicine as a result it is found the metals are present in some formulation like parpam and chendooram. The medicine which is mixed with heavy metals are called as herbo medicines.

1.4 IMPORTANCE OF STANDARDIZATION

In recent year there has been great demand for plant derived product in developed countries. Nowadays herbal medicines are prepared in bulk quantity depending upon their demand and usage hence standardization is very much important. Various herbs and plant materials are used for the preparation and the formulation also varies for each medicine. Environmental conditions such as sunlight, rainfall, altitude, temperature, soil, storage condition varies during the collection of plants, every plant has some chemical constituent which are responsible for the metabolic activity of the plant.These compounds either alone, or their combination are mainly responsible for the pharmacological activities or therapeutic action of the human body.

Adulteration is one of the reasons for adverse effect and inferiority of the medicine, some of the synthetic chemicals are used to enhance the natural character. Imperfect preparation, incorrect storage of the medicine and herbs of superior quality is mixed with cheaper quality products comes under adulteration. Hence to overcome such situations standardization is very much important. The chemical evaluation such as biological or pharmacological activity and qualitative chemical test are used to identify isolation, purification, identification and physical evaluation of the herbal medicine. Moisture content, specific gravity, optical rotation, refractive index, melting point, viscosity and solubility in different solvents were also done to know the purity. Specific biological and pharmacological activity can also be identified. Antibiotic activity such as salmonella typhi, styphyloceous aureus and E.coil are used to determine the antiseptic values of the herbal medicine.

Analytical evaluation and photometric analysis such as UV, IR, MS, NMR, TLC, HPTLC, GC can be used to identify the constant composition of herbal preparation. Organoleptic evaluations, pharmacognostic evaluation, phytochemical analysis, xenobiotics, microbial load testing, toxicity testing and biological activity were involved in standardization process. By these standardization techniques the quality, purity and safety of the herbal medicines can be ensured.

1.5 OBJECTIVE

Vatari Chooranam

Vatari chooranam is a traditional polyherbal formulation which is used for the treatment of rheumatoid arthritis. These medicines were prepared in marketed formulation and house preparation. Fenugreek seeds, ginger (a rhizomes rather than an root), ashwagnadha(root), kutki(rhizome), vidhara(root) were used for the preparation of vatari chooranam. The standardization technique used for the formulations such as organoleptic properties and physicochemical studies. Water soluble extract, alcohol soluble extract, ether soluble extract, hydroalcoholic soluble extract, total ash, water soluble ash, acid insoluble ash, water content, moisture content, bulk density, tap density, Hausners ratio, Carr's index pH of suspension were carried out under organoleptic and physiochemical studies as per the WHO guide line for this chooranam **(Sharma.V *et.al.,2012).*** Hence the present study aimed to identify the elemental composition present in the chooranam, by the following techniques

- **Energy dispersive spectroscopy (EDAX)**
- **Thermal gravimetric analysis (TG-DTA)**
- **X ray diffraction (XRD)**
- **Scanning electron microscopy (SEM)**

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review is based on the physicochemical analysis, phytochemical analysis, organoleptic properties of the herbal drug and the phytochemical constituents of the plant are used for the preparation of the drugs were also mentioned.

2.1 STANDARDIZATION OF HERBAL DRUG

- ✓ Aavaraivithaadhi chooranam was used for the treatment of diabetes mellitus. This chooranam was tested in animal model wistar albino rats, streptozotocin was injected which induced diabetes mellitus in animal model. These rats were separated into four groups and each group contain six animals. Group I contained normal animal, Group II contained diabetic control, Group III contained standard and the Group IV animal aavaraivithaadhi chooranam was tested . The blood glucose level was found to be reduced which was equal to standard glucose level and the body weight was also maintained. By taking acute toxicity study they concluded that the formulation of aavaraivithaahi chooranam was found to be effective for diabetes mellitus **(Thenmozhi.p *et.al.*, (2014)** .

- ✓ Amukkirai chooranam was a light whitist brown coloured fine powdered substance which was prepared from the root of *withania somnifira dunal(solanacae)* and it is used as an remedy for rheumatism, weakness, stress control,sleeping disorder, gastric ulcer, anaemia etc. Six root powdered sample was standardized. The standardization involved macroscopic, organoleptic characters, physicochemical properties, phytochemical screening, fluorescence analysis, elemental analysis, aqueous and ethanol extractive values, TLC and HPTLC fingerprint analysis were done. Heavy metal analysis Zn, Fe, Ni, Cu, Cr,Cd reveled that the concentration of the heavy metal was below WHO/FOA permissible limits, on the other hand the element such as Ca,Na and Mg where detected in lesser amount **(Vinotha samugarajah *et.al.*, (2014)**.

- ✓ **Anitha John *et.al.*,(2015),** standardized Chuntaivatral chooranam using the analytical parameters such as organoleptic analysis, TLC photodocumentation

and HPTLC finger print profile. The quality and purity of the sample was identified using the pharmacopeal standards , loss of drying, total ash, water soluble ash, acid insoluble ash in water and alcohol and pH. In preliminary phyto chemical analysis terpenoids, flavinoids, tannis, quinines, acids, glycosides, sugar, saponins, and coumarins were found to be present.

- ✓ **Nandhagopal.k *et.al.*, (2013),** analysed Karchure chooranam which was prepared from *phoenix dactylifera linn (arecaceae)* commonly known as date palm. This chooranam is used for the treatment of diabetes. Albino rats of 210-230g weight was used for the analysis and they were maintained under standard condition of humidity, temperature and light. The significant inhibitory effect was screened at 500mg/kg for then invivo anti diabetic activity on alloxan induced diabetic rats. For standard reference Glibenclamide was used, the anti diabetic activity was compared with that standard . From these analysis it was concluded that after 28 days daily treatment with Karchure chooranam it was found that the blood glucose level of diabetic rats were reduced.

- ✓ **Juliet.L *et.al.*,(2015),** standardised elachi chooranam in order to predit the quality of the drug, based on the physical and chemical standards. Elachi chooranam is effective on anti ulcer property. Physiochemical investigation, organoleptic properties, preliminary phyto chemical analysis, heavy metal analysis, microbial evaluation and analysis of aflatoxins , extractive values and ash values were done. From the above analysis purity and quality of the drug was confirmed.

- ✓ Kabasurakudineer chooranam is a formulation of 15 ingredients which is used for the treatment of fever with or without respiratory infection. The analytical methods such as chromatographic studies, physicochemical parameters such as total ash value, acid insoluble ash value, loss of drying and pH were carried out. Preliminary phytochemical analysis of the chooranam revealed the presence of phenols, terpenoids , steroids, flavinoids, quinines, coumarins, alkaloids, tannin, acid, glycoside **(Anitha john *et.al.*,*(2015)***.

- ✓ **Samraj.K *et.al.*, (2014),** standardized Magizham pattai chooranam is used as aphrodisiac drug. The standardisation was based on the physicochemical analysis, ash value, moisture content behaviour, florescence analysis. Preliminary phytochemical screening of the extract reveled the presence of alkaloids, carbohydrates, glycoside, saponins, triterpenoids, flavinoids and fatty acids. The heavy metal analysis and volatile oil analysis were done, and these analysis showed that chooranam contained below detection limit of toxic heavy metal.

- ✓ Milagathi chhoranam is a polyherbal formulation which is used for the treatment of ulcer. All the herbs for the preparation are produced and authenticated. The analytical methods such as physio chemical parameters, TLC profiling, HPTLC fingerprint were done. Physicochemical analysis like partical size, loss of drying, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractive, and pH were identified. The phytochemicals such as steroid, triterpene, flavonoids, coumarin, alkaloid, phenol, tannins, acid, glycoside, and saponins were also identified. These analysis revealed the standardisation profile and quality control assement of the polyherbal formulation **(Priya.F *et.al.*, 2014)** .

- ✓ **Mary suja. R *et.al.*, (2017),** Prepared paavu chooranam from 14 traditionally used herbs and explored for the treatment of breast cancer. The antioxidant activities of the chooranam was analysed by hydroxyl radical scavenging, DPPH, nitric oxide radical screening, hydrogen peroxide radical scavenging and reducing power activity. This polyherbal formulation was evaluated with standard antioxidant compounds like L ascorbic acid, gallic acid and vitamin C. from these analysis they concluded that Paavu chooranam has antioxidant potential.
- ✓ Seeraga chooranam is a polyherb composed of a variety of herbs. The phytochemical screening performed with solvent and aqueous extract, as an result it is found that saponins, flavonids, terpenoids were present and

cardiac glycosides were absent. Due to the presence of phytocompound it is found that the chooranam has medicinal value and used for the treatment of diabetes, pneumonia, cardiac disease etc. They conclude that phytochemical compound of seeraga chooranam has strong role on antitumor, antioxidant, anti inflammatory, anticancer, antiviral, antibacterial, and analgesic properties **(Mahalakshmi. K *et.al.,(2015)***.

- ✓ The clinical study of thelkodukku chooranam were conducted on the 50 patients of either sex between 13 to 60 years of age group who were affected by scabies lesions. This chooranam was given orally to the patients by mixing it with milk and the paste of the fresh leaf was applied externally in the affected area for a period of 10 months (august 2011- may 2012) in siddha medical college. The patients were instructed to avoid any topical and oral treatment during the study period without consulting the doctor. The result showed that chooranam was effective and the disappearance of itching were observed, from this it was concluded that thelkodukku chooranam has scabical activity and no harmful or side effectes were observed on the patients **(Siva saravanan KS *et.al.,(2013)***.

- ✓ Thraachathi chooranam of siddha formulation composed of 32 medicinal plants, used for the treatment of cardio vascular disease, diabetes mellitus, cough, astma, ulcer etc. The standardisation of this drug was carried out by Physiochemical and Phytochemical protocol such as ash values, extractive values, chemical profiling and marker quantification such as gallic acid, ellagic acid, naringenin, quercetin and galagin using HPTLC finger print were identified, phytochemical screening showed the presence of phenols, tannins, flavones, saponins, glycosides. From the heavy metal analysis it was confirmed s that the metal where in AYUSH permissible limit **(Ramakrishnan.G *et.al.,(2015)***.
- ✓ Vellarugu chooranam is a reputed drug which is used for the treatment of vata diseases, arthritis, constipation and diabetes mellitus. This chooranam is prepared from the whole plant of *Enicostemma littorale blume*. The standardisation involved organoleptic properties, phytochemical analysis,

fluorescence analysis, elemental analysis, physicochemical analysis parameters like ash values, moisture content, TLC and HPTLC fingerprint was also done. From these analysis the quality and purity of the Vellarugu chooranam was identified **(Vinotha sanmugarajah *et.al.,(2014).***

- ✓ **Periysami.D *et.al.,(2016),*** Vidathri chooranam is a brown coloured powder which is prepared as per classical siddha literature. This chooranam is used for the treatment of skin disease especially for kaanakadi(urticaria). The standardisation analysis was done using physicochemical analysis such as total ash, loss on drying, microbial coad, heavy metal analysis, pesticides, residues, aflatoxins, TLC and HPTLC. The preliminary phytochemical test for protein, flavonoids, quinine, phenols, tannins, alkaloids, glycosides, cardiac glycosides, reducing sugar, coumarin, anthroquinone, saponins were identified. On heavy metal analysis lead, cadmium, arsenic and mercury were found below detectable limit. By these analysis they concluded the traditional value of the chooranam.

- ✓ **Prakash.D *et.al.,(2017),*** Gowthamar chooranam has high potent medicinal value which is used for hepatic disease without any adverse effect. The characterization was done using FTIR and SEM, from these analysis the functional group like amide, phenols, alcohols, alkanes, aldehyde, amine, alkanes, alkenes, ester, ether, alkynes were identified.

- ✓ **Yamini.k *et.al.,(2013),*** Yelaathi chooranam is a polyherbal formulation which is `widely used for the treatment of peptic ulcer. They used hydroalcoholic extract for the evaluation of antiulcer activity against aspirin and pyrolus ligation induced gastric ulcer in albino rats. These rats were killed and their stomach were removed for microscopic and macroscopic index determination. The result data were subjected to one way ANOVA followed by student test. On undergoing these analysis they found that hydroalcoholic extract of this chooranam at 230mg/kg showed anti ulcer effect inhibition. By this they conclude that Yelaathi chooranam has gastroprotective effect on aspirin and pyrolus ligation induced gastric ulcer rats.

- ✓ **ushakanthan.*s et.al.,*(2017)** In Indian Siddha system vasambu chooranam is used as an remedy for *kuruthi azhal noi* which resembles cardiovascular diseases . The analysis on this medicine was done on OPD of ayothidoss pandiar hospital national institute of Siddha. About 1g of the chooranam with warm water was given twice a day before meals for four weeks. The blood pressure was monitored on 7^{th} , 14^{th}, 21^{st} ,28^{th} day respectively. The drug treatment data analysis was carried out using Bonferroni post Hoc test by GraphPad Prism 5.0. The pressure and the mean arterial blood pressure (MAP) were 150.00 mmHg, 93.80 mmHg and 113 mmHg respectively. The mean value of these parameters SBP, DBP and MAP decreased to 135 mmHg, 86.40mmHg and 103 mmHg respectively at end of study. It was found that there was a significant reduction in systolic blood pressure, diastolic blood pressure and mean arterial blood pressure (P value < 0.0001).From the above experiment they concluded that Vasambu chooranam is effective on cardiovascular disease.

- ✓ **Nithya T.G *et.al., (*2011)** Thaaleesaadhi chooranam is a polyherbal formulation which is used for the kapha disease. For the preparation of this drug 23 herbs were collected dired in shade, powdered and mixed thoroughly in equal proportion. The mixture was subjected to the extraction and distillation. Then the filterate was evaporated and dried, then the sample was studied for antibacterial activity. The study concluded that the chooranam had good inhibitory activity against the pathoges.

- ✓ **Priyabrata pattanayak *et.al.,*(2010)** Vaisvanara chooranam a herbal formulation was standardized in order to know the quality of the drug and to identify the therapeutic value. Vaisanara chooranam consisted of four botanical ingredients . The standardization was done using two samples from different manufactures and they were subjected to various physiochemical analysis like HPTLC fingerprinting and botanical characterization along with house formulation using authentic microscopic study. Organoleptic

evaluation, total ash value, physicochemical investigation, loss of dryning were also carried out. From the above analysis the quality and purity of the samples were confirmed.

- ✓ **Mullaicharam. AR *et.al.*,(2010)** said about the medicinal values and chemical constituents of *Trigonella foenum-graecum L* (fenugreek) which comes under the family *fabaceae.* The consumption of 20-100 grams of fenugreek seeds daily will diminish hyperglycemia in diabetic patients. Fenugreek seeds also helps in blood formation, lacting mothers to increase flow of milk. Diosgenin, a steroid sapogenin found in fenugreek was the starting compound for over 60% of the total steroid production by the pharmaceutical industry. Other sapogenins found in fenugreek seed include yamogenin, gitogenin, tigogenin, and neotigogens. Fenugreek seeds contain alkaloids, including trigonelline, gentianine and carpaine compounds and contain fiber, 4-hydroxyisoleucine and fenugreekine, the component having hypoglycemic activity. Other constituents of fenugreek include mucilage, bitter fixed oil, volatile oil, and the alkaloids choline and trigonelline were also identified.

- ✓ **Buba. F *et.al.*,(2015)** Fenugreek (Trigonella foenum-graecum L.) is one of the most promising medicinal herbs known from ancient times having nutritional value. Physicochemical properties of fenugreek seeds in order to identify its quality as a food and medicinal agent was done by **Buba. F *et.al.*,** . The proximate analysis was carried out based on standard methods of Association of Official Analytical Chemists (AOAC), while the vitamin composition was analyzed based on the method described in CODEX. Vitamin C was determined by titration method. The moisture and ash contents of the sample had average values of 10.91±0.85% and 2.99±0.48% respectively. The protein content had an average value of 2.74±0.35%, while fat and carbohydrate contents were 6.33±0.52% and 77.04±0.63% respectively. The vitamin B_1, B_2, B_6 and B_{12} gave average values of 0.1137mg/g, 0.0366 mg/g, 0.0495 mg/g and 0.8710 mg/g respectively, whereas vitamin C and folic acid contents had values of 10.5400 mg/g and 0.0386 mg/g respectively. From the study it was concluded that aqueous

extract of fenugreek seeds contained vitamins, carbohydrates, proteins and fats which is a very good source of food supplement and has potential for treating diverse medical ailments which supports its traditional use.

- ✓ The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Trigonella foenum-graecum was used as a traditional remedy for the treatment of various diseases. **(Nathiya. S *et.al.*,(2014)** reported the chemical constituents, the biological and pharmacological actions of fenugreek such as Anti-diabetic activity, Hypocholesterolaemic properties, Immunomodulatory activity, Anti-toxic activity, Anti-cataract activity and Anti-oxidant activity . The results of these studies provide a complete understanding of the biological action of fenugreek. Fenugreek has different pharmacological attributes such as a hypoglycemic, hypercholesterolemia, gastroprotective, chemo-preventive, an anti-oxidant, and laxative and appetite stimulation. The plant contains alkaloids, flavonoids, salicylate, and nicotinic acid. The authors conclude that Fenugreeks are harmless for human consumption and the anti-oxidant activity could be associated with the polyphenolic components present in the extract and also Fenugreek seeds could modulate the activity of glyoxalase system SOD.

- ✓ **Jit Narayan *et.al.*,(2013)** *Picrorhiza (family Scrophulariaceae)* is a small genus of two important endangered medicinal plant species. These species contain several bioactive compounds that have therapeutic properties. They are medicinally revered herbs used extensively for various immune-related diseases. *Picrorhiza* is used for the treatment of liver disorder, gastrointestinal and urinary disorders, fever, asthma and jaundice and possess anti periodic, cholagogue, stomachic, laxative and antiasthmatic activities. The part of the root *Picrorhiza scrophulariflora* is used in traditional Chinese medicine for the treatment of damp-heat dysentery, jaundice and steaming bone disorder. One hundred thirty-two chemical constituents belonging to different class of compounds were illustrated. The chemical study on the *Picrorhiza kurrooa* rhizomes shows the presence of iridoids, acetophenones and cucurbitacins. It is known to be rich source of picroside-I and II as major bioactive compounds. *Picrorhiza kurrooa* also contains pikuroside, veronicoside, phenol glycosides,

a number of cucurbitacin glycosides and 4-hydroxyl-3-methoxyacetophenone, whereas P. scrophulariiflora contains cyclopentanoid monoterpenes, caffeoyl glycosides, phenylethanoid glycoside and plantamajoside.

- ✓ **Pharamchand *et.al.*,(2016)** Picrorhiza kurrooa is a herbal species. Drugs like Picroliv, Picroside-I, II, III, V and Kutkoside were extracted from dried stolons and roots of Picrorhiza kurrooa . Due to large demand in national and international markets, exploitation of this species in wild is going on. In Western Himalaya (India), the conservation status of this species is either rare or threatened. The main goal is to protect and maintain the evolutionary viability of this species and to maximize the chances of its survival and persistence in the changing environment. Here they conclude the technique for the protection and conservation this species.

- ✓ **Milimitha padhi *et.al.*,(2013)** Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and other ailments. Argyreia nervosa belongs to family-convolvulaceae is an important herb used extensively in traditional systems of medicine. The pharmacological properties include antimicrobial, analgesic, anti-inflammatory, antiulcer, immunomodulatory, hypoglycemic, anticonvulsant etc. The seeds of Argyreia nervosa yielded fatty oil, which was found to contain the glycosides of palmitic, oleic, stearic, behenic, linoleic and linolenic acid Gas liquid chromatography (GLC) of the seed oil revealed the presence of myristoleic, myristic, palmitic, linoleic, linolenic, oleic, stearic, nonadecanoic, eicosenoic, heneicosanoic and behenic acids. Presence of branched fatty acids 12methylmyristic acid and 15-methylstearic acid was also reported. The ethanolic extract of the seeds revealed a mixture of three alkaloids, out of which only one was characterized as ergometrine.

  The other constituents isolated were caffeic acid and ethyl caffeate. By various analysis the presence of ergoline alkaloid, such as includes ergometrine, ergometrinine, lysergic acid-α-hydroxy ethyl amide ,

agroclavine, chanoclavine-I, chanoclavine-II, festuclavine, lysergene, lysergol, isolysergol, setoclavine, iso-setoclavine, ergine and isoergine were identified.

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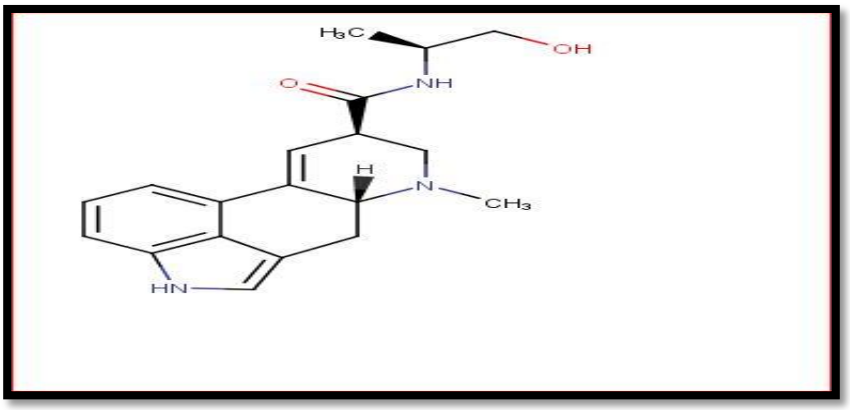

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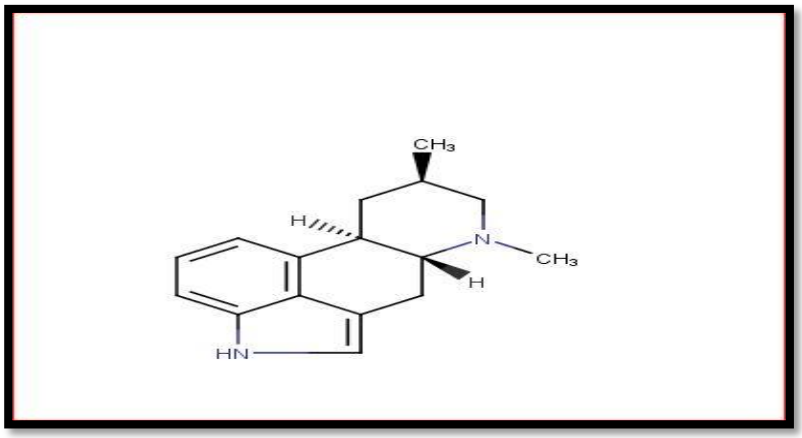

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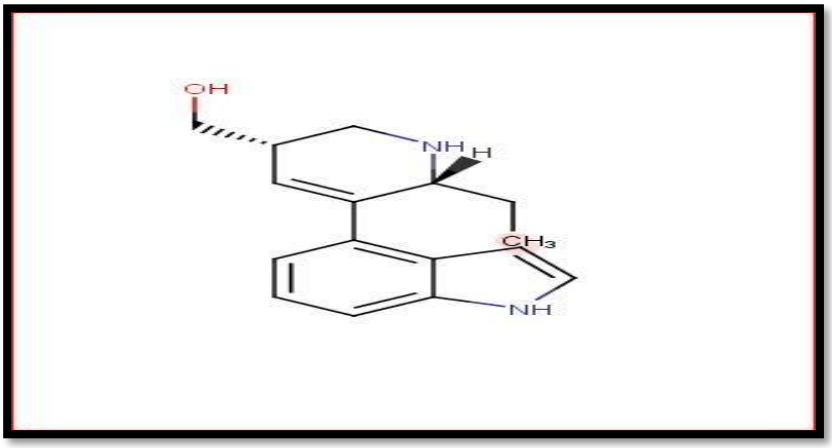

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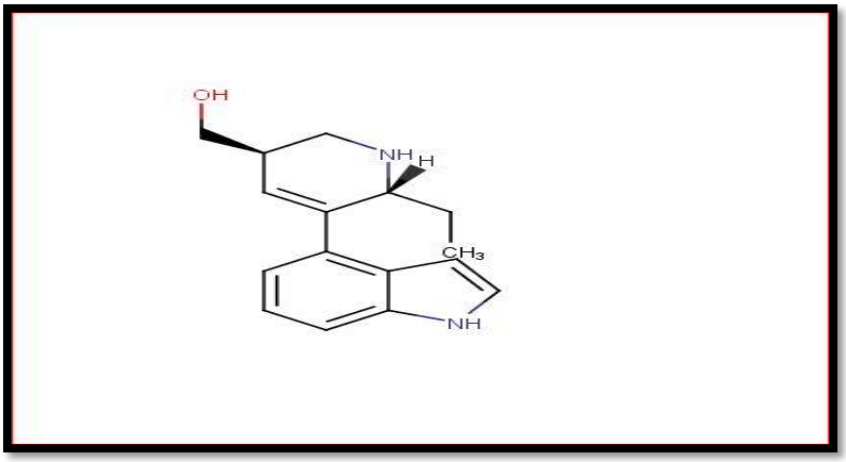

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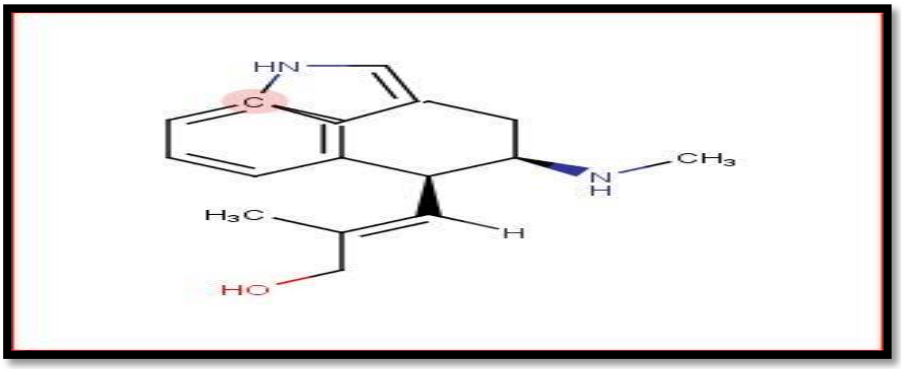

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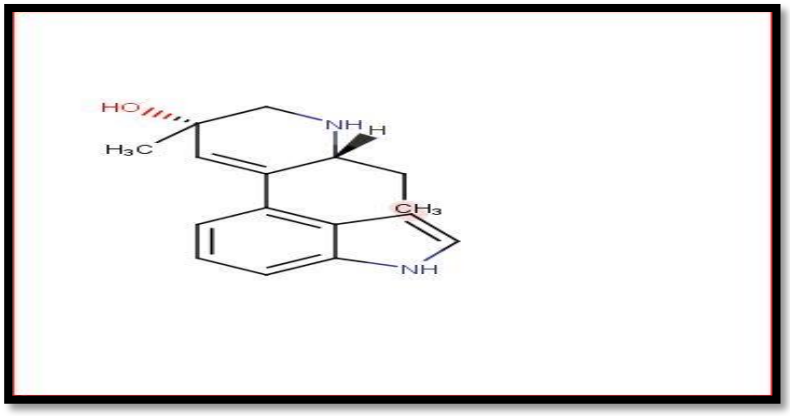

- ✓ **Sareedenchai *et.al.*, (2014)** used 80% ethanolic extract of Argyreia nervosa Bojor (Convolvulaceae) leaves for the identification of phytochemical constituents by chromatographic techniques. Three compounds: 1-hexacosanol , scopoletin and ethyl caffeate were isolated. The biological activities of ethanolic extracts were investigated for anti-HIV and free radical scavenging activities. From the results of preliminary screening, the ethanolic extract it was shown that the inhibited syncytium reduction assay for 80.45% at the concentration of 200 µg/ml. Further investigation for anti-HIV activities was carried out with HIV reverse transcriptase (RT) and HIV protease (PR). Anti-HIV RT activity was studied by radiometric method and anti-HIV PR was studied by spectrophotometry. They conclude that the ethanolic extract could not inhibit HIV RT and HIV PR, but three compounds could inhibit HIV PR. 1-Hexacosanol, scopoletin, and ethyl caffeate at concentrations of 200 µg/ml could inhibit HIV PR by 78.71%, 43.35% and 43.15%, respectively. The free radical scavenging activity was studied using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). The ethanolic extract showed free radical scavenging activity with an EC50 of 21.43 µg/ml. 1-Hexacosanol and scopoletin were inactive, whereas ethyl caffeate was almost twice as active as butylated hydroxyanisol (BHA).

- ✓ Argyreia is traditionally used herb . The antibacterial, antifungal, antipyretic, analgesic, anti-inflammatory activity of the herb were studied. Studies were carried out on healthy wistar strain albino rats weighing about 140-250 g, using carrageenan induced paw edema. It was observed that the ethyl acetate extract and methanol extract produced significant anti-inflammatory activity. Qualitative tests for the carbohydrates, alkaloids, tannins, flavonoids, proteins, saponins and glycosides were carried out. The anti-inflammatory activity of ethyl acetate extract and methanol extract of whole aerial part from Argyreia nervosa was studied using carrageenan induced paw edema. From preliminary phytochemical studies such as ethyl acetate extract of whole aerial part of Argyreia nervosa showed the presence of fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds while methanol extract showed the presence of carbohydrates, protein, amino acids, fixed oil, fats, phytosterols, glycosides, flavonoids, alkaloids, tannins and phenolic compounds **Kamal Jeet *et.al.*,(2012)**.

- ✓ **Shiva kumar *et.al.*, (2010)** This study was based on the hypoglycemic effect of alcoholic extract of Argyreia nervosa roots (500 mg/kg body weight orally) in normal and glucose loaded, streptozotocin (STZ) induced diabetic rats. The extract produced decrease in blood glucose level in normal glycaemic rats (82.6 + 2.6 vs 61.3 + 2.8 mg/dl at 6th hr). It is observed that glucose loaded rats has reduced blood glucose levels from 118.4 + 5.4 to 96.4 + 4.2 mg/dl 2h after oral glucose load. When given orally for 7 days in STZ diabetic rats, it produced significant antihyperglycemic effect and also reversed the changes in total hemoglobin and glycosylated hemoglobin content. The present study concluded the beneficial effect of Argyreia nervosa roots in the control of blood glucose level in normal and diabetic rats. The study confirmed the rational basis for its use in traditional medicine for the treatment of diabetes.

- ✓ **Kartik ch Patra, *et al;*(2009).** Standardized a polyhebral Siddha formulation, Amukkara choornam. Four samples of Amukkara choornam from different manufacturers were collected and subjected to various analysis. The different parameters such as total ash, water soluble ash, acid soluble ash, ethanol soluble extractive value, water soluble extractive value and loss on drying at 70^{0C}, fluorescence analysis, phytochemical analysis and HPTLC chromatogram showed the presence of diagnostic identifying characters for the presence of each ingredient. The ingredients were fine powders of *Lavangam-Syzygium aromaticum, Sirunaga poo-Cinnamomum wightii, Ela arisi- Elettaria cardamomum, Milagu- Piper nigrum, Thippili-Piper longum, Chukku- Zingiber officinale, Amukkara- Withania somnifera, Sarkarai-Saccharum officinarum.* And these parameters can be used for the evaluation of this Siddha formulation.

- ✓ **Rahul Raj Surisetty, *et al;*(2014).** Standardized the Siddha formulation Surya sakthi churna available in market, using different parameters like organoleptic characters, physical characters, physiochemical properties and phytochemical screening etc. The colour, odour, taste of the formulation were tested in the organoleptic evaluation and the P^H, LOD, Ash value, Extractive value was determined for the physicochemical parameters. And also evaluated the bulk density, angle of repose, compressibility Hausner ratio, particle size distribution, fluorescence analysis. The results obtained from this parameters were found to be within the standards. In this standardization of herbal formulation confirmed that these preliminary tests can be prescribed as standards to fix the quality control test of the churna and this can be used in routine analysis of the same.
- ✓ **Sharma .V, *et al;*(2012).** Developed a standardization technique to mingle the traditional system of medicine in the main stream of health science. The authors standardized the traditional formulation, Vatari churna used for rheumatoid arthritis. The organoleptic and physicochemical studies like water soluble extract , ether soluble extract, total ash,water soluble ash, acid insoluble ash, bulk density, tap density, Hausner's ratio, carr's index, ph of suspension were carried out. Also they prepared Vatari churna based on a

traditional method and found that there were no uniformity in the preparation of formulations. It was concluded that it could be very useful for comparative pharmacological studies.

- ✓ **Kokila .N, *et al;*(2013).** Developed a polyherbal formulation (polyherbal capsule) for rheumatoid arthritis and standardized to set up its quality control by evaluating its physicochemical, phytochemical and formulation parameters .Rheumatoid arthritis is a painful inflammatory condition, where the immune system targets and attacks the joints. For the formulation of polyherbal capsules to treat rheumatoid arthritis four active ingredients were selected. The four ingredients were used as crude herbal drugs such as *Cappris decidua, Dioscoraalata, Imbatiens balsamina and Onosma bracteatum.* The capsule powders were evaluated by bulk density, tapped density, compressibility index and Hausner ratio and angle repose. Also standardized the finished capsule formulation were evaluated using parameters like physicochemical parameters, phytochemical studies, heavy metal analysis, microbial load analysis, fluorescence analysis and pesticide residue. Confirmed the presence of alkaloids, flavanoids, tannins, terpenoids, carbohydrates and also exhibits the pharmacopoeial limits for its stability.

- ✓ **Nitin V. Kokare, *et al;*(2014).** To assure the quality, purity and safety of the herbal drug in its standardization physical, chemical, biological and analytical parameters were carried out. Physical parameters such as colour, odour, appearance, fluorescence analysis, ash value, crude fibre, moisture content, extractive value, swelling index, density and determined the presence of tannins. Chemical parameters included limit test, chemical test etc., chromatographic analysis were done using TLC, HPLC, HPTLC, GC, UV, GC-MS, fluorimetry etc.

- ✓ **Chamundeeswari .D, *et al;*(2007).** Traditional medicines are very effective for the gastrointestinal problems due to the safety and efficacy. Churna were defined as a fine powder of drug or drugs in Ayurvedic system of medicine. *Triphala churna, Trikatu churna, Drakeshadi churna and Sudharsana* churna

are some of examples. Hence the churna was formulated by standard procedures using raw materials such as *Rhizomes of Zingiber officinale, Fruits of Foeniculum vulgarae, Barks of Cinnamomum zeylanicum and Fruits of Trachyspermum ammi.* The physical parameters like P^H , moisture content, ash value, extractive value, crude fibre content, heavy metals and presence of microbes in churna were determined.

- ✓ **Sangram keshari panda, *et al;* (2012).** Using various parameters, a poly herbal formulation, *Sitopaladi* churna were standardized for the quality assurance study mostly on the plant drugs for their primary health care needs. Prepared Sitopaladi churna with *Sitopal, Vamsolochana, Pippali, Ela, Tvak* and also evaluated organoleptic properties, fluorescence analysis. Determined the total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, LOD, P^H, bulk density, tap density, angle of repose, Hausner ratio, HPTLC fingerprinting. HPTLC fingerprint profile identified that the presence of all the ingredients were in proportional quantity in the Sitopaladi churna formulation.

- ✓ **Priyanka gupta, *et al;*(2015)**. Reported the comparative standardization of a poly herbal Ayurvedic formulation, Trikatu churna. They evaluated organoleptic study, physical characteristics and physicochemical screening for the two marketed formulation samples and prepared the churna in-house. It was concluded that due to varied geographical locations where these plants grow, coupled with the problem of different vernacular names these plants are known by, there is no uniformity in preparation of these formulations. The physicochemical parameters such as the water soluble, alcohol soluble, moisture content, bulk density, tapped density, carr's index, Hausner ratio, P^H, water soluble ash, acid insoluble ash and organoleptic characterisation can be efficiently used for the standardization of poly herbal formulation. It was also confirmed that the results obtained from this study could be utilised as a reference, for setting limits for the reference standards for the quality control and quality assurance of these drugs.

- ✓ **Kartik Chandra patra, *et al* (2010).** Amukkara choornam is a most effective formulation used during gastric troubles, spleen enlargement, leucorrhoea, hiccup, anaemia, tuberculosis and kappa diseases. Correlation of therapeutic activity of a Siddha formulation with its antioxidant activity was established. The samples were collected from different manufacturers of Tamilnadu India, labelled A, B, C and in-house preparation as D. Compared the methanolic extracts of samples for their total phenolic contents and invitro- antioxidant activity by electrochemical measurement, total antioxidant capacity, iron(III) to iron(II) reduction assay, 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and reducing power. This study indicated that samples showed good antioxidant activity but sample C showed more activity as compared to other samples.

- ✓ **Garg S, *et al;*(2013).** Carried out an Ayurvedic preparations like churna, Avaleha asava, Arishta, Vati, Rasa, Taila, Ghritas and herbal capsules etc., for the development of fingerprint profile for evaluating the purity and quality of Ayuvedic formulations. Standardization and analysis of the chemical marker of the Ayurvedic and other poly herbal formulation has been concerned. The authors concluded that the fingerprint profile was quite helpful in setting up of standards and thus to keep a check on intentional or unintentional adulteration.

- ✓ **Caroline .R, jeba, *et al;*(2013).** Investigation of thirteen samples of Indian medicine and Siddha drugs were done by Caroline .R, *et al*. Microbial and biochemical tests were carried out to ascertain the antimicrobial activity. Antimicrobial activities of the medicinal herbs were found to satisfactory towards the microbes of investigation. Pathogens were identified by using biochemical test and microbiological assay methods for antibiotics. To discover new antimicrobial compounds many efforts have been done in various kinds of sources such as soil, microorganisms, animals and plants. In this study, concluded that the systematic screening of them may result in the discovery of novel effective compounds.

- ✓ **Thanigavelan .V, *et al;*(2012).** Siddha holistic herb-sphaeranthus amaranthoides burm(shivakaranthi) was subjected to pharmacological including pharmacognostical study particularly on leaf and influorescence of sphaeranthus amaranthoides by Thanigavelan .V, *et al*. In this study, included the quality control test and phyto constituents estimation. The presence of calcium, ferrous, tannin, protein and phenols were noted. The results of elemental concentration level indicated the presence of toxic metals withn the tolerance level. The in-vitro antibacterial activity evaluation confirmed the good antimicrobial activity at the dilution of 50 microlitre/disc against the bacteria. This pharmacological study of a Siddha holistic herb added the scientific knowledge for the development of formulations for treating various diseases using this herb.

- ✓ **Arvindkumar shakya (2016).** Medicinal plants are a potential source for the development of new herbal drugs. The pharmacological effects of medicinal plants have been considered as a promising future drug or medicine for the management of health care. Classified various phytochemicals such as alkaloids, glycosides, polyphenols, saponins, terpenes and anthraquinones. From this review we can understand that the knowledge of the medicinal plants as a future source of herbal drugs.

- ✓ **Mubarak H and G Masilamani(2011).** Palagarai is a Siddha marine drug, formulations prepared out of palagarai are the choice of drug for many indications in Siddha medical practice, the chemical constituents of Palagarai was evaluated by Mubarak H and G Masilamani. The pharmacological activities indicated the presence of minerals that contributes to its medicinal value, such as high content of calcium confirms its medicinal role in bone formation an extracellular cation, sodium involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction. Iron plays important role in haemopoeisis, control of infection and cell mediated immunity. All these informations served as an evidence to establish current research in traditional medicinal systems.

- ✓ **Nitu singh, *et al;* (2015).** Evaluated the Pharmacognostical and physical parameters of Ayurvedic formulations containing *Trachyspermum ammi.* Standardization method based on the Pharmacognostic and physicochemical parameters of Trachyspermum ammi and its marketed poly herbal formulation 'Ajamodadi churna' of four different companies were developed. Organoleptic evaluations such as colour, odour , taste and determined the physical characteristics of powder like bulk density, tap density, angle of repose, Hausner's ratio and carr's index for different formulations were evaluated. And also the physicochemical parameters like ash values, extractive values, LOD etc, were determined. The study showed significant difference in their values and was concluded that this study can improve the quality of drugs.

- ✓ **Neeraj choudhary and Bhupinder singh sekhon, (2011).** Standardization is an important step for the establishment of a consistent biological activity, a consistent chemical profile or simply a quality assurance program for production and manufacturing herbal drugs. Various techniques employed in extraction and characterizations of herbal medicines as well as herbal nanomedicines standardization were reported. In this study, various spectroscopic, chromatographic and thermogravimetric techniques individually/ or in combination were discussed. For the extraction of herbals, techniques like Super critical fluid extraction (SFE), Microwave-assisted extraction (MAE) and Solid phase extraction were used (SPE). For the identification and characterization of herbal drugs, techniques such as HPLC, HPTLC, LC-MS, LC-NMR, GC-MS, Supercritical fluid chromatography (SFC), Capillary electrophoresis (CE), Metabolomics technique, IR etc. All these techniques served as a rapid and unambiguous tool in the herbal research , thereby, benefiting the entire pharmaceutical industry.

- ✓ **Rakshitaa .S, *et al.*,(2015).** Evaluated physicochemical and Antioxidant properties of a Siddha formulation, *Panchadeepakini* choornam used to cure various gastro-intestinal problems. The physicochemical, antioxidant and antimicrobial properties of this Siddha formulation using in-vitro methods were analysed. The physicochemical analysis such as particle size analysis,

bulk density, pH value, Ash value, LOD, Extractive value, Total phenolic content were determined. The water extract of *Panchadeepakini* choornam revealed higher level of total phenolic concentration and antioxidant property based on various in-vitro assays. The water extract of this choornam exhibited high antioxidant property that provides scientific support for employing this herbal drug for therapeutic use in Indian system of medicine.

- ✓ **Rajalakhmi P, *et al.*,(2016).** Evaluated the Antioxidant and Anti-inflammatory potentials of selected Siddha herbal drugs such as Cardamom(*Elletaria cadamomum L.),* Ginger(*Zingiber officinale Roscoe),* Arrow root(*Maranta arundinacea L.),* Yew leaves(*Abies webbianaspach),* Indian rose chestnut(*Mesuaferra L),* Pepper(*piper nigrum L)* and Clove(*syzggium aromaticum L).*The phytochemical profile of herbal drugs extract and Total phenol content were determined. Antioxidant activity using DPPH radical method and anti-inflammatory activity of selected herbal drugs were also carried out. This in-vitro studies indicated that the combination of these drugs could exhibit both antioxidant as well as anti-inflammatory activities and provides the therapeutic effect against arthritis.

- ✓ **Sangeeta, *et al.*,(2017).** Characterised Ayurvedic preparation, Amrtadi churna, a poly herbal formulation comprising three ingredients. Based on pharmacognostic, physicochemical, pharmaceutical, microbiological, toxicological, spectroscopic and chromatographic parameters its identity, quality and purity were confirmed. In this study, all ingredients in the formulation had unique R_f values. This results could also be utilised for FTIR Spectroscopy and HPTLC Chromatography based rapid authentication of the formulation.

- ✓ **Elizabeth Thomas, *et al.*,(2013).** Prepared the ethanol, ether and methanol extract of rhizomes of the plant Nervilia aragoana by simple maceration process and soxhalation method were identified. Biochemical components present in the plant using GC-MS analysis. The presence of different fatty acids, heterocyclic compounds etc were also detected by GC-MS

analysis.Therefore the plant was recommended as a plant of phytopharmaceutical importance.

- ✓ **Singh, *et al.*,(2009).** Ras-sindoor a mercury based Indian traditional drug, administered for the various ailments such as syphilis, genital disorders and for rejuvenation. Synthesis and systematic characterization of Ras-sindoor were done using various techniques like X-ray diffraction (XRD), Transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), Far infrared spectroscopy (FIR), Fourier transform infrared spectroscopy (FTIR), Differential thermal analysis (DTA), Thermogravimetry analysis (TGA), Energy dispersive X-ray analysis (EDAX) and Atomic absorption spectroscopy (AAS). Several macro/trace elements were also found to be present in different amounts, which were bio-available and responsible for adding to the medicinal value of Ras-sindoor.

- ✓ **Tambur pavani, *et al.*,(2015).** Synthesized Lauha bhasma by modern methods by an eco friendly technique. Synthesized iron oxide nano particles by various physical and chemical methods which are toxic and harmful for the environment. The iron oxide nano particles obtained were characterized by various characterization techniques such as XRD, UV-Visible spectroscopy, Thermo gravimetric and differential thermal analysis, Average particle size and Atomic force microscopy. The TG/DTA graph indicated that the obtained sample has extreme purity and very small weight loss. AFM represented morphology and particle size within nano range.
- ✓ **Lagad C.E, *et al.*,(2013).** Vanga bhasma is used for genitor-urinary disorders since long in Ayurveda. Bhasma was analyzed using ICP-AES, X-ray diffraction and revealed that the Vanga bhasma contains major compound SnO_2 and TG/DTA showed no weight loss and no physical or chemical changes.

- ✓ **Lakshmi Chandra, *et al.*,(2000).** Study about the Withania somnifera (ashwagandha) indicated that it possesses anti-inflammatory, anti-tumor, anti-stress, anti-oxidant, immunomodulatory, hemopoetic and

rejuvenatingproperties. Toxicity studies revealed that Withania somnifera is an ingredient used in many formulations and prescribed for a variety of musculoskeletal conditions (eg: arthritis, rheumatism). It is recognized that Withania somnifera may be effective not only in isolation, but may actually have a potentiating effect when given in combination with other herbs or drugs.

i. **Jayaprakasam .B, *et al.*,(2003).** Various Ayurvedic medicines were prepared containing Withania somnifera roots as one of the main ingredients. Isolated twelve Withanolides such as withaferin A, sitoindoside IX, 4- **(**1-hydroxy 2,2-dimethyl cyclopropanoe), 2,3-dihydro withaferin A etc, from the leaves of this species. Withanolides possessing the withaferin A unit was the most active. The anti proliferative activity of 2,3-dihydro withaferin A was considerably decreased as compared to withaferin A. This indicated that the double bond in withaferin A significantly contributed to its anti proliferative activity to other withaferin A type Withanolides

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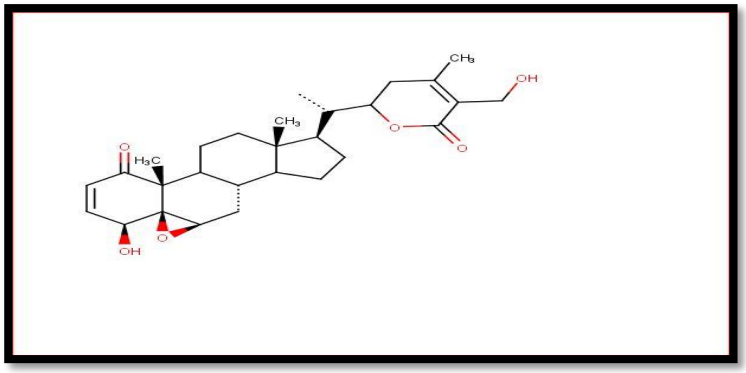
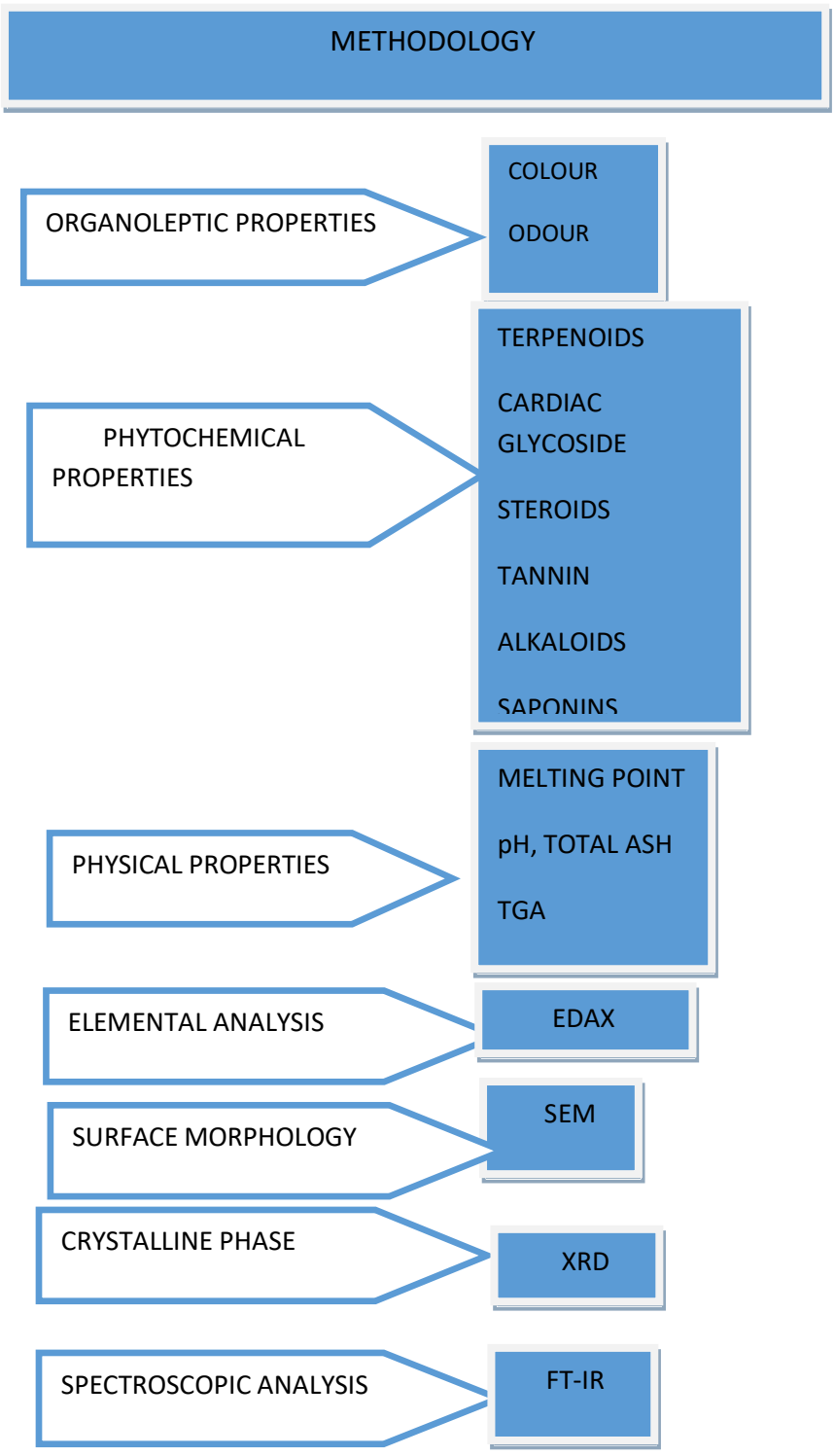


ii. **Malik .F, *et al.*,(2007).** Investigated in-depth the immunomodulatory activity of the chemically standardized aqueous alcoholic (1:1) root extract of Withania somnifera (AGB). Result of this study indicated that AGB was rich in withanolide A content and as such is highly efficient in augmenting the immune responses to T-dependent antigen. Several lines of evidence suggested that DTH is an important compound of the defense system. It was concluded that AGB is a potent immuno stimulating agent, which can be therapeutically used in enhanceing the immune response in diseases like tuberculosis , cancer, leprosy and AIDS.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

In the present study evaluation of **Vatari Chooranam** was carried out. The drug was collected from Siddha drug suppliers of Tuticorin District. It is a polyherbal formulation that is used for treating Rheumatic arthiritis . the methodology used for standardisation of the drug is as follows,



3.1 ORGANOLEPTIC PROPERTIES

An 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 and the characteristic changes were observed.

3.1.1 Test with concentrated hydrochloric acid

A small amount of the sample was treated with concentrated hydrochloric acid.

3.1.2 Test with concentrated nitric acid

A small amount of the sample was treated with concentrated nitric acid.

3.1.3 Test with concentrated sulphuric acid

A small amount of thesample was treated with concentrated sulphuric acid.

3.14 Test with 5% aqueous sodium hydroxide

A small amount of the sample was treated with 5% aqueous sodium hydroxide.

3.15 Test with iodine solution

A small amount of the sample was treated with iodine solution.

3.16 Test with 5% aqueous potassium hydroxide solution

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

3.17 Test with Glacial acetic acid

A small amount of the sample was treated with glacial acetic acid solution.

3.18 Test with 5% aqueous potassium hydroxide solution

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

3.2 PHYTOCHEMICAL COLOUR TESTS

3.2.1 TEST FOR ALKALOIDS

Hager's test

A few grams of sample is treated with dil. HCl and dissolved in saturated picric acid.

3.2.2 TEST FOR CARDIAC GLYCOSIDE

Keller- Killani test

2 ml of sample in water extract is treated with 2 ml of glacial acetic acid and 1 ml of concentrated H_2SO_4.

3.2.3 TEST FOR STEROIDS

The ethanol extract of the sample is treated with 2 ml of acetic anhydride and 2 ml of concentrated H_2SO_4.

3.2.4 TEST FOR FLAVONOIDS

Test with Sodium hydroxide

The water extract of the sample is treated with sodium hydroxide.

Test with Lead acetate

The water extract of the sample is treated with lead acetate.

3.2.5 TEST FOR SAPONINS

Froth test

The water extract is dissolved in water and shaken in a graduated test tube for 15 minutes.

3.2.6 TEST FOR QUINONES

The water extract of the sample is treated with the alcoholic potassium hydroxide.

3.2.7 TEST FOR CARBOHYDRATES

Molisch's Test

Few drops of Molisch's reagent were added to each of the herbal drug dissolved in distilled water and 1 ml of concentrated sulphuric acid was added along the sides of the test tube.

3.2.8 TEST FOR TERPENOIDS

The water extract of sample is mixed with 2ml of chloroform and treated with 3ml of concentrated sulphuric acid.

3.2.9 TEST FOR TANNINS

The herbal durg was dissolved in water and heated on a water bath for one hour. It was then treated with Ferric chloride.

3.3 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 **(Meenu Sharma, et al 2013).**

3.3.1 Melting point of the drug

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

3.3.2 pH values

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

3.3.3 Determinations of ash values

i. Silica crucibles
ii. Muffle Furnace - Furnace was fitted with an indicating pyrometer, to maintain the temperature. **(Genuine equipment manufactures)**
iii. Analytical balance – with to 0.001 mg sensitivity. **(Shimaozu corporation, type AY220)**
iv. Desiccator

v. Drying oven - with temperature control of 105 ± 2°C. **(sigma scientific instruments Chennai, Pvt.ltd)**

3.3.4 (A) Total ash (TA) value:

Accurately 2 to 3 g of air–dried samples of the **VC** 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.

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

W = Weight of air – dried drug.

3.3.5 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.

3.3.6 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.

3.4 ELEMENTAL COMPOSITION

3.4.1 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.

3.5 SURFACE MORPHOLOGY

3.5.1 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.

3.6 CRYSTALLINE PHASE

3.6.1 XRD

To determine the different crystalline phase present in the sample,XRD patterns were obtained using an X-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.

3.7 SPECTROSCOPIC ANALYSIS

3.7.1 FT-IR

The fuctional groups of the phytochemical constituents present in the sample was characterised by using FT-IR (Model IR Affinity -1 Shimatzu).

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

In the present study quantitative analysis of the herbal drug sample VC was carried out using modern techniques. The results of the study are illustrated below.

4.1 ORGANOLEPTIC PROPERTIES

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

Table 1

S.No	Parameters	Observation
1	Colour	Pale green
2	Odour	odourless
3	Taste	Tasteless
4	State 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 sample VC was partly soluble in concentrated Hydrochloric acid and concentrated Sulphuric acid .The sample was insoluble in concentrated nitric acid, iodine solution , glacial acetic acid and 5% aqueous sodium hydroxide.

Table 2

Behaviour of VC with different reagents

S.No	Chemical treatment	Observation
1	Drug powder treated with Concentrated Hydrochloric Acid	Partly soluble
2	Drug powder treated with Concentrated Nitric Acid	Insoluble

3	Drug powder treated with Concentrated Sulphuric Acid	partly soluble
4	Drug powder treated with 5% aqueous Sodium Hydroxide	Insoluble
5	Drug powder treated with Iodine Solution	Insoluble
6	Drug powder treated with5% aqueous Potassium Hydroxide Solution	Insoluble
7	Drug powder treated with Glacial Acetic Acid	The sample was found to insoluble &settled down slowly.

2. 4. PHYTOCHEMICAL SCREENING

The phytoconstituents present in the herbal drug used for rheumatoid arthritis were identified by the colour test. The results revealed the presence of alkaloids, flavonoids, terpenoids, carbohydrates and tannins. The results of the preliminary studies are summarized in **Table-3.**

Table-3

Phyto-chemical screening of Herbal Powder

Phyto Constituents	**Results**
Tannin	+
Steroids	-
Terpenoids	+
Flavinoids	+
Saponins	+
Quinines	+
Carbohydrates	+
Cardiac glycosides	-

4.3 PHYSICAL PROPERTIES

Physical properties of sample VC like solubility, ash value, and loss on drying, pH values and stability were determined as per standard procedures **(Meenu Sharma, et al 2013).**

4.3.1 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 sample VC was checked with water, dichloromethane, chloroform, DMSO, ethyl acetate ,ethyl alcohol, petether and acetone. Of these, the sample was partially soluble in DMSO and dichloromethane and completely insoluble in other solvents.

4.3.2 The melting point of the drug was determined by melting point apparatus **Ajay R**. the melting point of the drug was in the range of 260°C - 280°C.

4.3.3 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 **7.17**.

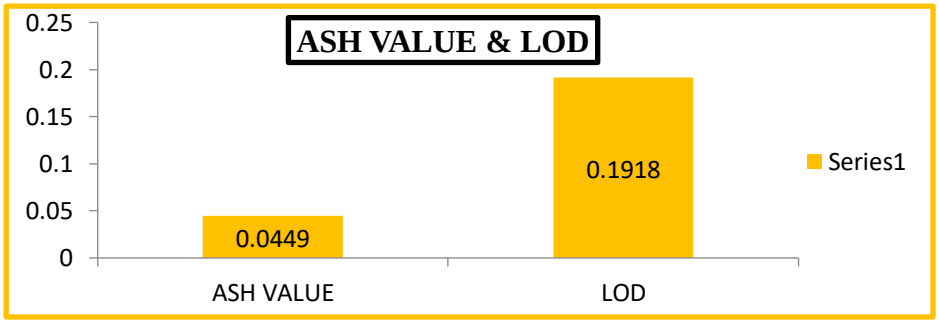
4.3.4 Ash value

The Ash value was found to be **0.0449** , the minimum ash value showed that the sample contained greater percentage of inorganic salts.

4.3.5 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 sample VC.

Figure-8



4.3.6 TG/DTA ANALYSIS

Thermal analysis encomposes a group of technique in which a property of the sample is monitored against time and temperature in a specified atmosphere. **Figure 9 and 10** represents the TG curve of sample VC. The weight loss of VC at 131^{0}C was found to be 14% and weight loss at 339^{0}C was found to be 29%. This may be due to the loss of carbon dioxide, oxygen, fluorine and bromine present in the mixture. DTA **(Figure 11 and 12)** showed two decomposition peaks at 67^{0}C and 376^{0}C. DTG curve showed exothermic peak which indicates the breakdown reactions of the metallic mixture.

Figure-9 TG curve of VC

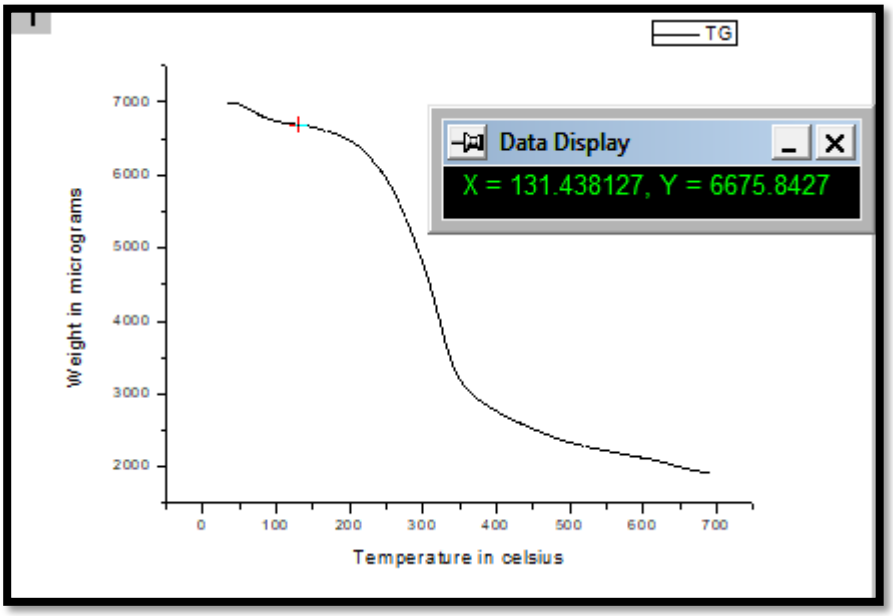


Figure- 10 TG curve of VC

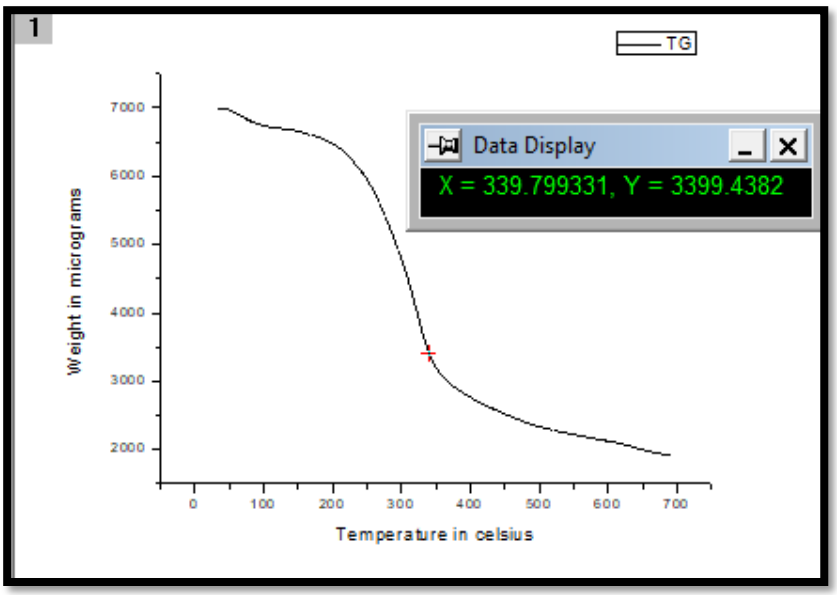


Figure- 11 DTA curve of VC

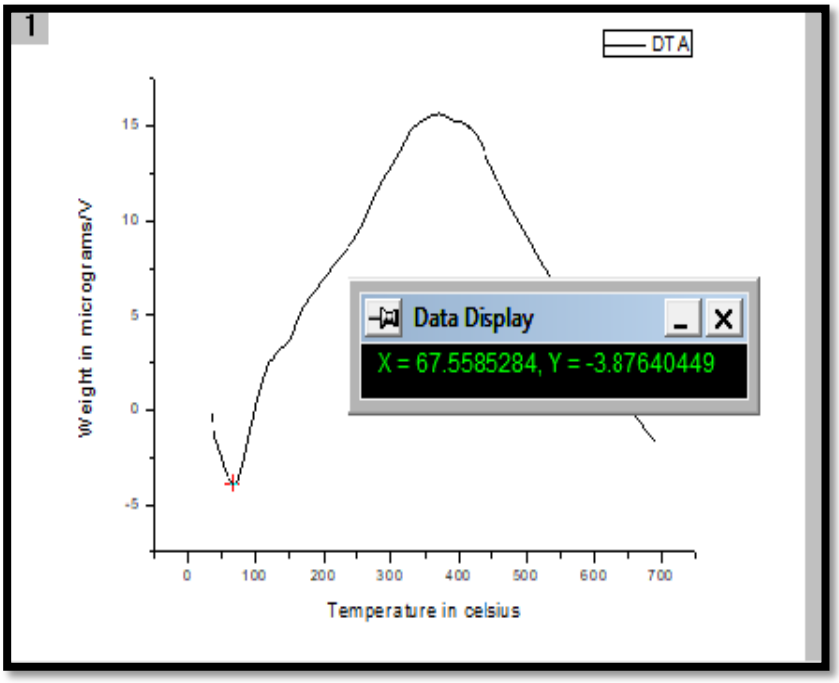


Figure- 12 DTA curve of VC

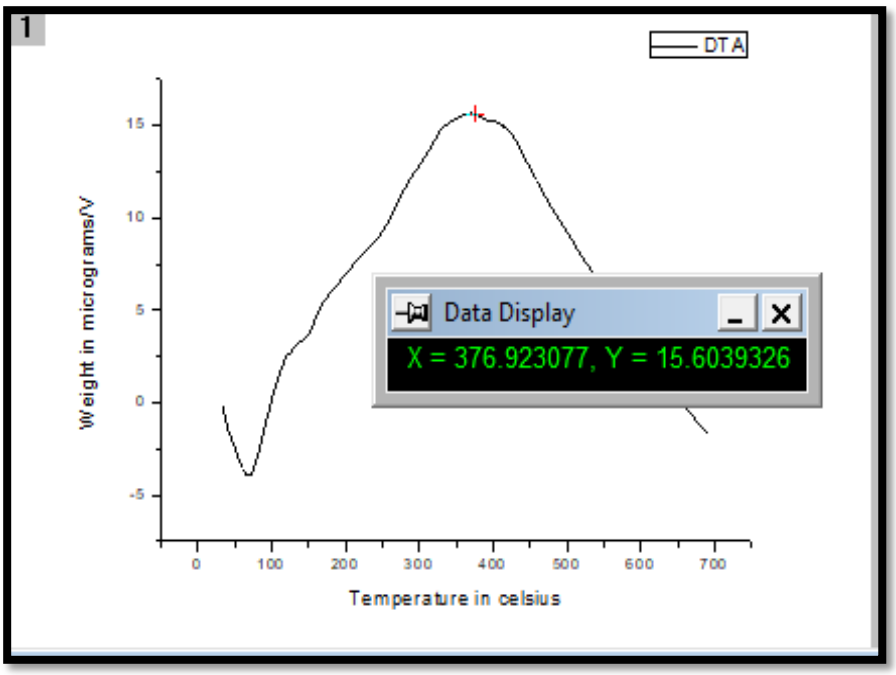
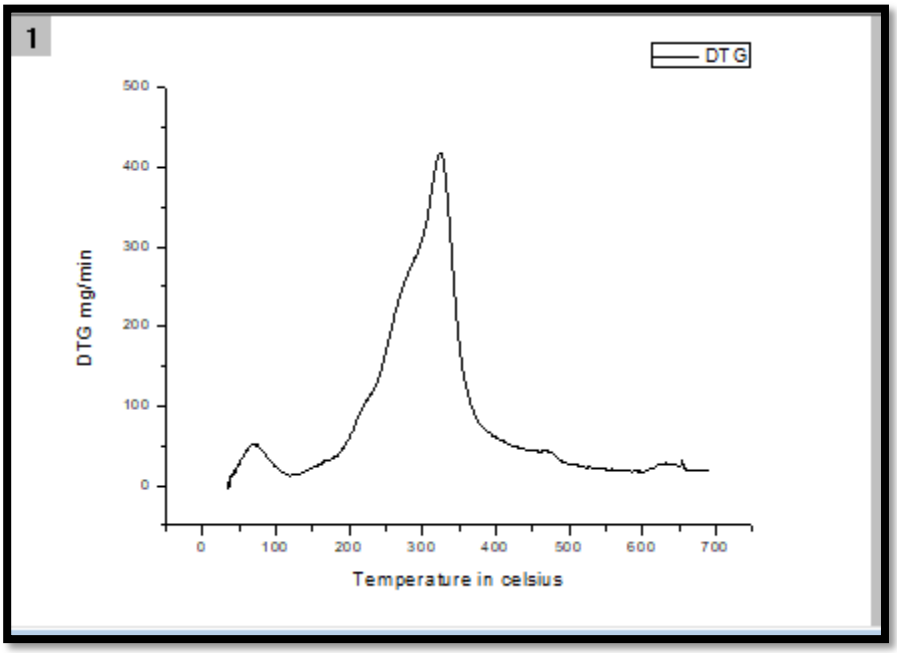


Figure- 13 DTG curve of VC



4.4 ELEMENTAL ANALYSIS

4.4.1 EDAX

Elemental analysis of the VC showed the presence of Ytterbium, Bromide, Aluminium, Pottasium and Oxygen **(Table 4 & 5)** Calcium was found to be the major element in its oxide form (85.47 %) **(Figure 14 and 15)**

Table 4

ELEMENTAL COMPOSITION OF VC

Element	Weight%	Atomic%	Compd%	Formula
C a	C a	C a	C a	C a
Al K	Al K	Al K	Al K	Al K
Br L	Br L	Br L	Br L	Br L
Yb L	Yb L	Yb L	Yb L	Yb L
O	O	O	O	O
Total	100.00			

Figure-14 EDAX spectrum of VC

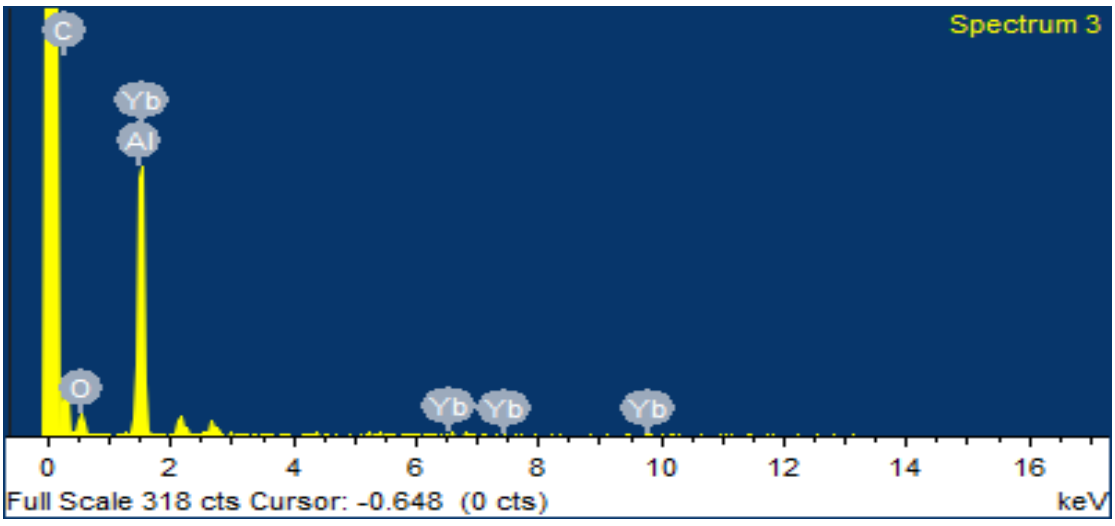
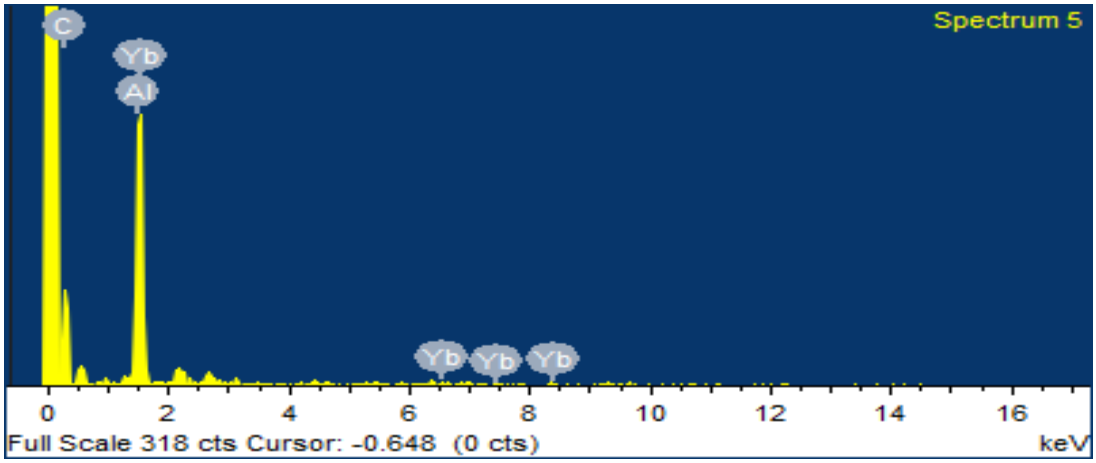


TABLE-5

ELEMENTAL COMPOSITION OF VC

Element	Weight%	Atomic%	Compd%	Formula
C a	23.33	27.55	85.47	CO2
Al K	15.08	7.93	28.49	Al2O3
Br L	-14.55	-2.58	0.00	
Yb L	0.52	0.04	0.59	Yb2O3
O	75.62	67.06		
Totals	100.00			

Figure-15 EDAX spectrum of VC



5 SURFACE MORPHOLOGY

4.5.1 SEM

The particle size of sample VC was assessed by SEM.**(Figure 16 and 17)** Particle size, shape and surface area affects homogeneity, efficiency and granules and also the stability. Through SEM analysis the particle size of sample VC formulation was found to be near 10µm-200µm. Surface was found to be porous and the sample VC 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**. (Arun sudha*et al*2009)**.

Figure-16

SEM IMAGE OF VC

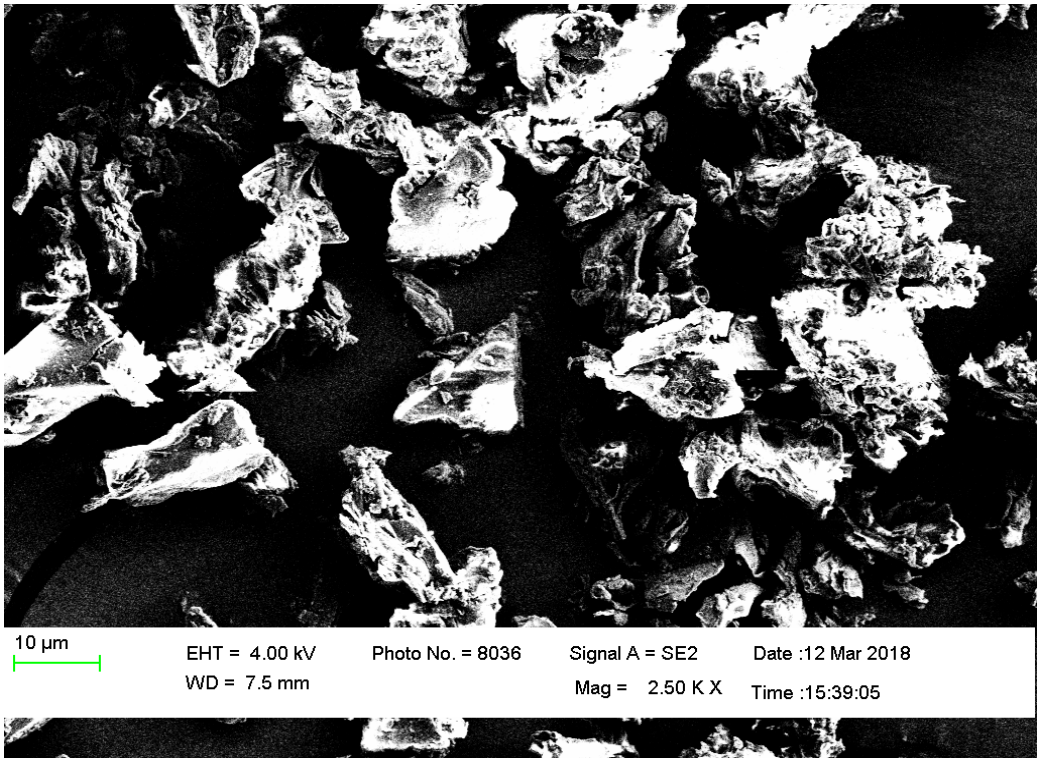
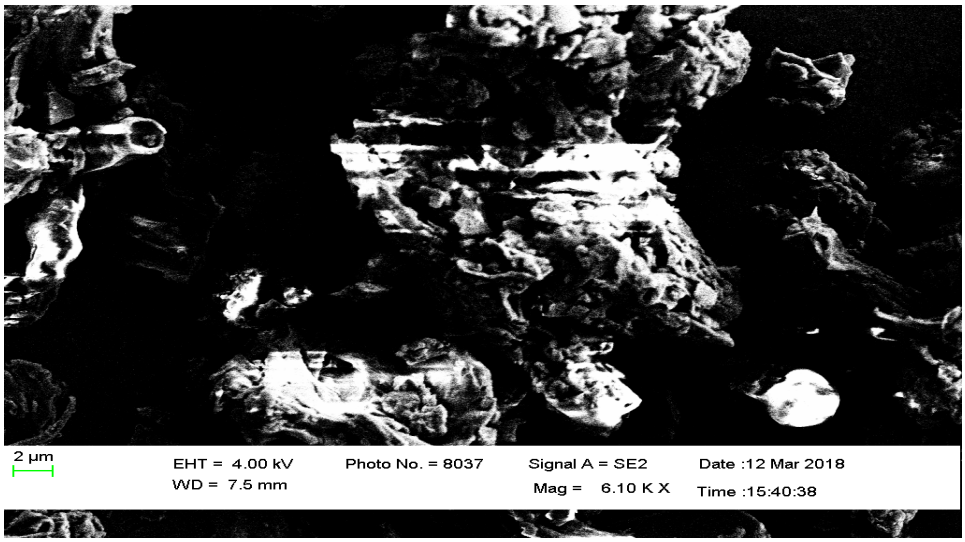


Figure-17

SEM IMAGE OF VC



4.6 CRYSTALLINE PHASE

4.6.1 XRD ANALYSIS

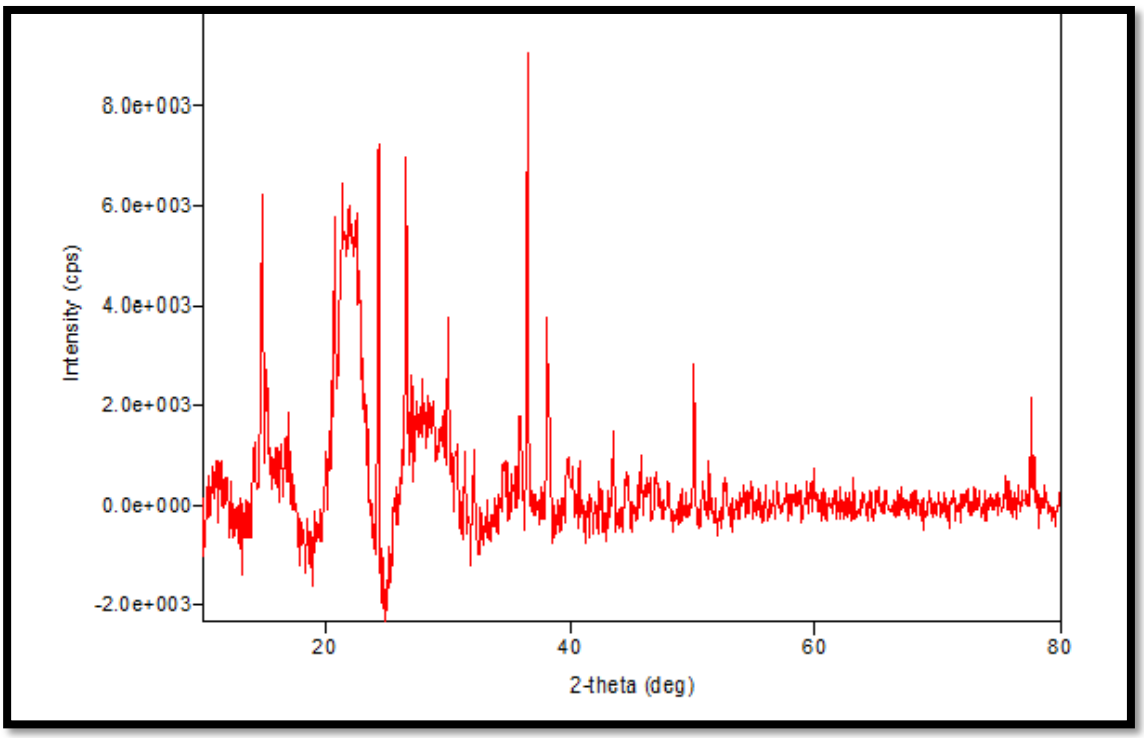
The analysis of the XRD illustrated the presence of $CaCO_3$ and YbF_3 in the mixture **(Figure-18) .** This was confirmed by comparing the XRD patterns of the sample with that of $CaCO_3$ and YbF_3. The peaks of the $CaCO_3$ and YbF_3 were found at angles 30(2θ),24(2θ), respectively.

The average crystalline size was calculated using Debye-Scherrer equation

$$D = \frac{0.9\,\lambda}{\beta \cos\theta}$$

D = shape factor, λ = x-ray wavelength, β = FWHM of diffraction peak, θ = Bragg angle. The grain size of $CaCO_3$ was found to be **21.34 nm** from XRD analysis **(Manjusha Hariharan *et.al.*, 2014.**

Figure – 18 XRD of VC



4.7 SPECTROSCOPIC STUDIES

4.7.1 FT-IR

Based on the peak assignment from IR spectrums **(Figure-19 and Table -6)** the following groups viz amines, alcohols and bromides were found to be present in the sample VC. This may be due to the phytochemical constituents of the plant ingredients viz., fenugreek, ginger, ashwagnadha,kutki, vidhara.

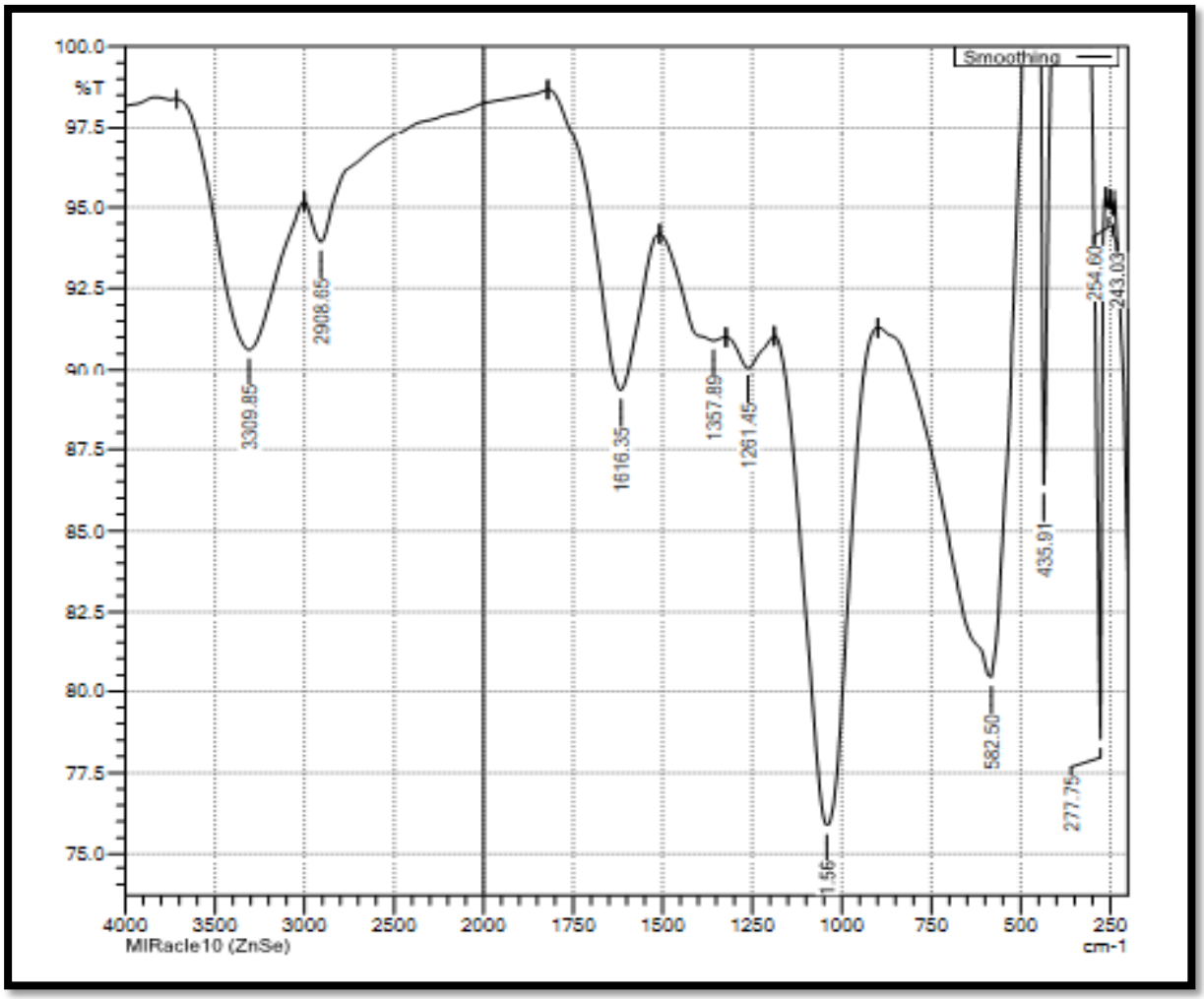
Table- 6

IR values of sample VC

PEAK ASSIGNMENT	FREQUENCY	POSSIBILITY
N-H (stretching)	3309.85	Amines
O-H (sharp)	3309.85	Alcohol
N-H(broad)	1616.63	Amines
C-N(stretching)	1357.89	Amines
C-O (stretching)	1261.45	Alcohol
C-Br(stretching)	1050.60	Bromides

Figure-19

IR SPECTRUM OF VC



SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

To get knowledge regarding the science behind traditional formulations it is very essential to validate these formulations using modern techniques. The findings of the present study of standardisation of VC is summarized as follows

- The preliminary phytochemical analysis showed the presence of tannins, terpenoids, flavinoids, saponins, quinones and carbohydrate.
- Ash value was found to be 0.0449%. The minimum ash value showed that the sample contained greater percentage of inorganic salts.
- TG-DTA curves indicated the Exothermic break down reactions of the metallic mixture.
- The metals aluminium, calcium, potassium and ytterbium were found to present as their oxide, calcium being present in greater percentage.
- SEM analysis revealed that the surface of VC were porous and size of the particle was found to be 10µm-200µm.
- XRD analysis confirmed the presence of $CaCO_3$ and YbF_3 and also the particle size was found to be 21.34nm.
- FTIR analysis indicated indicates the presence of amines, alcohols and bromides in the mixture.

The result of the present study revealed the physicochemical and phytochemical parameters of vatari chooranam which have not been reported. The above findings will help in the standardization of vatari chooranam.

Calcium was found to be the main ingredient of the chooranam. Calcium is an essential constituent of all living cells and is present in the body to a large extent(>1%) than any other mineral element. It is considered as a structural element, which plays an important role in the correction of bone metabolic disorders such as osteoporosis. Hence this choorana may help to increase the strength of the bone in the Rheumatoid arthritis patients

BIBLIOGRAPHY

6. REFERENCE

- Anitha John, Jayachandran.R, Sasikala Ethirajulu, Sathiyarajeswaran.P (2015) **Analysis of Kabasurakudineern chooranam-a Siddha formulation**, vol.3, pp.2915-2920.
- Anitha John, Sasikala , Jayachandran , Shakila and Sathiyarajeswaran (2015) Ariamuthu saraswathy (1994)**Standardisation of siddha drugs**,Vol No. XIV Nos. 1 & 2, pp. 53 – 60.
- Anupam Kr Sachan, Garima Vishnoi, Roopak Kumar, (2016), **Need of standardization of herbal medicines in modern era**, Vol.8(3), pp. 300-307.
- Arjun Ram, Duraisamy Arul Joseph, Selvakumar Balachandar, Vijay Pal Singh, (2009), **Medicinal plants from Siddha system of medicine useful for treating respiratory diseases**, Vol.1(2), pp. 20-30.
- Arvind Kumar Shakya, (2016), **Medicinal plants: Future source of new drugs**, Vol.4(4):59-64.
- Beristain C. I, GarcmHa H. S and Vernon-Carter E. J, (2001), **Spray-dried Encapsulation of Cardamom (*Elettaria cardamomum*) Essential Oil with Mesquite (Prosopis juli-ora) Gum,** Vol.34,pp.398-401.
- Bolleddula Jayaprakasam, Yanjun Zhang, Navindra P Seeram, Muraleedharan G Nair, (2003**), Growth inhibition of human tumor cell lines by Withanolides from *Withania Somnifera* leaves**, Vol.74, pp. 125-132.
- Buba F., Ngura U. and Abdulrahman A. A,(2015), **Studies on the physicochemical properties of fenugreek (Trigonella Foenum-Graecum L.) seeds**,vol. 7 (3),pp.104-107.
- Caroline jeba R & Rameshkumar G, (2013), **Antimicrobial activity of chosen medicinal herbs and sidda drugs,** Vol.3, pp.5-18.
- Chamundeeswari D, Kanimozhi P, Vasanthkumar, Umamaheswara Reddy C, (2007), **Formulation and evaluation of churna for digestive property**, pp. 39-43.
- Divya .A, (2015), **Research & Reviews: Journal of Pharmacognosy and Phytochemistry**, Vol.3(1), pp. 11-12.
- Dr Rajesh Kumari, Dr Mita Kotecha, (2016), **A review on the Standardization of herbal medicines,** Vol.7, pp. 97-106.

- Dr. Nath. S Dr. Rudra A, (2002), **Herbal medicines and their importance to anaesthesiologists,** Vol.46(3), pp. 224-225.
- Fayaz Malik, Jaswant Singh, Anamika Khajuria, Krishan A Suri, Naresh K Sati, Surjeet Singh, Maharaj K Kaul, Arun Kumar, Aruna Bhatia, Ghulam N Qazi, (2007), **A Standardized root extract of *Withania Somnifera* and its major constituents withanolide – A elicit humoral and cell-mediated immune responses by up regulation of Th 1- dominant polarization in BALB/c mice,** Vol.80, pp. 1525-1538.
- Fei guo, fengchang wu, Yunsong mu, Yan hu, Xiaoli zhao, Wei meng John P.Giesy,Ying lin (2016). **Characterization of organic matter of plant from lakes by thermal analysis in N_2 atmosphere.**
- Garg S, Mishra A and Gupta R, (2013), **Fingerprint Profile of Selected Ayurvedic Churnas/Preparations: An Overview,** Vol.2(6).
- Gurdip Singha, Kapoor I P S, Pratibha Singha, Carola S. de Heluanib, Marina P. de Lampasonab, Cesar A.N. Catalanb, (2008), **Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale,*** Vol.46, pp. 3295-3302.
- Indu Sasidharan, Nirmala menonA,(2010),**Comparative chemical composition a nd antimicrobial activity fresh & dry ginger oils (*Zingiber officinale roscoe*)**, Vol.2(4), pp. 40-43.
- Jit Narayan Sah, Vinay Kumar Varshney, **Chemical constituents of Picrorhiza genus: a review**,vol.1 (2),pp. 22-37.
- Juliet.L , Sivakumar.S (2015), **standardization of polyherbal Siddha medicine eladhi chooranam,** vol.4, issue.6,pp.33-37.
- Kamal jeet, Raneev thakur(2012), **Evaluation of anti-inflammatory activity of whole aerial part-argyreia nervosa,**vol.3(4),pp.150-154.
- Kapoor I P S, Bandana singh, Gurdip singh, Carola S. De heluani, De lampasona M P and Cesar A. N. Catalan,(2009), **Chemistry and in Vitro Antioxidant Activity of Volatile Oil and Oleoresins of Black Pepper *(Piper nigrum*),** Vol.57, pp. 5358-5364.
- Kartik ch patra, Jayaram kumar K, Suresh P, (2009), **Standardization of a polyherbal formulation, *Amukkara choornam,*** Vol.8(3), pp. 449-452.

- Kartik chandra patra and jayaram kumar K, (2010),**Establishing correlation of therapeutic activity of a siddha formulation with its antioxidant activity. A comparative study,** Vol.1(2).
- Kokila1 N, Radha1 R, Jayshree1 N,Selvaraj B, Selvakumar S, (2013), **Development, standardisation and accelerated stability studies of a polyherbal formulation,** Vol.1(2), pp. 29-51.
- Kwang-Geun Lee,Takayuki Shibamoto, (2001), **Antioxidant property of aroma extract isolated from clove buds *[Syzygium aromaticum* (L.) Merr. et Perry],** Vol.74, pp. 443-448.
- Lakshmi-Chandra Mishra, Betsy B. Singh, (2000), **Scientific Basis for the Therapeutic Use of Withania somnifera (Ashwagandha): A Review,** Vol.5(4), pp. 334-346.
- Lokhande Smita, Chougule Avinash, Patil Swapnil, Patil Vishal(2015),**Need of herbal drug standardization,**Vol 3, Issue3,pp.874-876.
- Maciej J Bogusz, Huda Hassan, Eid Al-Enazi, Zuhour Ibrahim, Mohammed Al-Tufail, (2005), **Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies,** Vol.41, pp. 554-564.
- Mahalakshmi.K, Subhashini.E (2015), **Phytochemical Screening of a Polyherb: Seeraga Chooranam,**vol.7(4),pp. 265-268.
- Mary Suja R, Christudhas .W and Christudhas Williams B (2017), **Antioxidant Potential of Paavu Chooranam Prescribed to Cure Breast Cancer by the Cheruthikonam Traditional Siddha Medicinal Practitioner of Kanyakumari District, India,**vol.1,pp.2573-2609.
- Meena R, Ramaswamy R.S, (2015), **Treatment of Uthiravatha Suronitham (Rheumatoid Arthritis) with a Siddha Compound Formulation - A Case Study**, Vol.5(9), pp. 50-52.
- Meena Sharma. Sharma R.G, Aakanksha Sharma,(2013), **study of physic chemical properties of drug and physiological variation in leaves of andrographis paniculata (burm.F) Nees,** vol3(1),pp.52-64
- Mehmet Unlua, Emel Ergeneb, Gulhan Vardar Unlua, Hulya Sivas Zeytinoglub, Nilufer Vuralc, (2010), **Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae),** Vol.48, pp.3274-3280.

- Michael heinrich, Anita ankli, Barbara frei, Claudia weimann and Otto sticher, (1998), **Medicinal plants in mexico: Healers' consensus and cultural importance**, Vol.47, pp. 1859-1871.
- Mi-Jin Park, Ki-Seob Gwak, In Yang, Won-Sil Choi, Hyun-Jin Jo, Je-Won Chang, Eui-Bae Jeung, and In-Gyu Choi, (2007), **Antifungal Activities of the Essential Oils in *Syzygium aromaticum* (L.) Merr. Et Perry and *Leptospermum petersonii Bailey* and their Constituents against Various Dermatophytes,** Vol.45(5), pp. 460-465.
- Milimita Padhi, Sujata Mahapatra, Jnyanaranjan Panda, Nikunja Kishor Mishra(2013), **Traditional uses and Phytopharmacological Aspects of Argyreia nervosa,**vol. 4(1), pp.23-32.
- Mishra Pramod Kumar, Rai N P, (2013), **A clinical study on Ajmodadi churna and Eranda taila in the management of Amavata (Rheumatoid Arthritis),** Vol.4(6), pp. 211-213.
- Mohamed A Farag, Andrea Porzel, Ludger A Wessjohann, (2012), **Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques,** Vol.76, pp. 60-72.
- Mubarak H and Masilamani G, (2012), **Siddha marine drug Palagarai (*Cypraea moneta Linn*.)- A review,** Vol.41(2), pp. 121-123.
- Mullaicharam AR, Geetali Deori , and Uma Maheswari R,(2013), **Medicinal Values of Fenugreek – A Review,**vol.1,pp.1304-1311.
- Nandhagopal.K, kanniyakumari.M, Anbu.Jand .J,Velpandian.V (2013) **Antidiabetic activity of karchure chooranam on alloxan induced diabetic rats,**pp.434-439.
- Nathiya S, Durga M, Devasena T(2014), **Therapeutic role of Trigonella foenum-graecum [Fenugreek] – A Review,** Article No. 12, pp.74-80.
- Neeraj Choudhary and Bhupinder Singh Sekhon, (2011), **An overview of advances in the standardization of herbal drugs,** Vol.2(2), pp. 55-70.
- Nithya.T.G,Dahineeswari.V, Shirlie Roy Chowdhury, Sivakumar.S (2011), **Antibacterial Activity of Thaaleesaadhi Chooranam Against Human Pathogens**, Vol.1(4) ,pp. 224-230.

- Nitin V Kokare, Kiran A Wadkar, Manish S Kondawar, (2014), **Review on standardization of herbal Churna,** Vol.5(3), pp. 397-401.
- Nitu singh, Ramanjaneyulu K, (2015), **Evaluation of pharmacognostical and physical parameters of Ayurvedic formulations containing *Trachyspermum ammi Linn***,Vol.6, pp. 156-161.
- Nobuji Nakatani, Reiko Inatani, Haruko Ohta, and Atsuko Nishioka, (1986), **Chemical Constituents of Peppers (*Piper spp*.) and Application to Food Preservation: Naturally Occurring Antioxidative Compounds**, Vol.67, pp. 135-142.
- Periyasami1.D, Ganapathy.G, MuthukumarN.J, Banumathy.V, (2016**), Physico chemical and phytochemical analysis of Vidathari chooranam, a Siddha herbo-mineral formulation,**vol.3,pp.72-79.
- Prakash.D, Anitha.G, Velpandian.V, Muthukumar.N.J, Banumathi.V (2017) **Analysis of the Siddha drug gowthamar chooranam using FTIR and SEM technique,** vol.4,pp. 11-13.
- Priya F, Shakila R, Sathiyarajeswaran P, Pitchiahkumar. M (2014) **Standardization of Milagathi Choornam**, vol.1(6),pp.69-74.
- Priyabrata Pattanayak, Danendra Kumar Hardel, Prithwiraj Mohapatra (2010) **Standardization of Vaisvanara Churna: A Poly-herbal Formulation**, Vol. 2,Issue 5,pp.53-58.
- Priyanka Gupta, Deepak Nagwansi and Surya Prakash Gupta, (2015), **Comparative Standardization of a polyherbal Ayurvedic formulation trikatu churna,** Vol.7(12), pp. 191-196.
- Rahul Raj Surisetty, Fatima Grace Xavier, Shanmuganathan Seetharaman, Chamundeeswari Duraipandian, (2014), **Standardization of Marketed Churna an Ayurvedic Polyherbal Formulation,** Vol.28(2), pp. 108-110.
- Rajalakshmi P*, Vadivel V, Abirami K, Brindha P, (2016), **Evaluation of Antioxidant and Anti-inflammatory Potentials of Selected Siddha Herbal Drugs – An In vitro Study,** Vol.8(3), pp. 519-523.
- Rajeev kurele (2015), **Drug standardization of ayurveda , unani and Siddha drug**, vol.6(2),pp.192-193.
- Rajesh Kumari and Mita Kotecha,(20116), **A review on the Standardiz+ation of herbal medicines,**vol.7,pp.97-104.

- Rakshitaa S, Vadivel V, Brindha P, (2015**), Evaluation of Physico-Chemical and Antioxidant Properties of an Indian Siddha Formulation: *Panchadeepakini choornam,*** Vol.35(1), pp. 67-73.
- Ramakrishnan.G, Gayathri.V, Sathiya.S, Parameswari.R.P, Saravana Babu.C (2015) **Physiochemical and Phytochemical Standardisation of Thraatchathi Chooranam- A Polyherbal Siddha Formulation,** Vol. 7(6),pp. 305-313.
- Rivera JO, Loya AM and Ceballos R, (2013), **Use of Herbal Medicines and Implications for Conventional Drug Therapy Medical Sciences,** Vol.2(6), pp. 2-6.
- Samraj.K, Thillaivanan.V,Padmanathan,S, Parthiban.P(2014) **Phytochemical and Physico-Chemical Analysis of Siddha Preparation Magizham Pattai Chooranam,** Vol.1, Issue 1,PP. 29-33.
- Sangeeta mukhi, Bose A, Ray A and Swain P K, (2017), **Analytical Standards of Amrtadi Churna: A Classical Ayurvedic Formulation,** Vol.76(2), pp. 227-240.
- Sangram Keshari Panda, Snehasis Das, Manaswini Beher, Bichitra Tripathi and Debasis Pati, (2012), **Standardization of Sitopaladi Churna: A Poly-Herbal Formulation,** Vol.4(1), pp. 205-216.
- Sanjay Jain, Sweta Koka, Asim Gupta, Rakesh Barik, Neelesh Malviya(2010), **Standardization of "Chopchiniyadi Churna": An Ayurvedic polyherbal formulation,**vol.2,pp.62-63.
- Sareedenchai1.V, Wiwat.C,.Wongsinkongman.P and Soonthornchareonnon.N (2014), **In vitro Testing of Anti-HIV and Antioxidative Activities of Argyreia nervosa (Burm.f) Bojor Leaves** vol. 41 (4), pp.47-53.
- Selene Maia de Morais, Valdir Alves Facundo, Luciana Medeiros Bertini, Eveline Solon Barreira Cavalcanti, Jo ~ ao Francisco dos Anjos Ju ´nior, Silane Aparecida Ferreira, Edy Sousa de Brito, Manoel Alves de Souza Neto, (2007), **Chemical composition and larvicidal activity of essential oils from *Piper* species**, Vol.35, pp. 670-675.
- Sharma V, Agarwal K, Maithani J and Nanda D, (2012), **Development of quality standards of Vatari churna: A polyherbal formulation,** Vol.3(8), pp. 2870-2872.

- Shirin Adel P. R.* and Jamuna Prakash, (2010), **Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*),** Vol.4(24), pp. 2674-2679.
- Shiv Kumar and Alagawadi.K.R (2010), **Hypoglycemic effect of Argyreia nervosa root extract in normal and streptozotocin-diabetic rats** ,Vol. 2(2),pp.333-337.
- Siddheshwar K. Chauthe, Ram Jee Sharma, Farrukh Aqil, Ramesh C. Gupta and Inder Pal Singh, (2012), **Quantitative NMR: An Applicable method for quantitative analysis of medicinal plant extracts and herbal products**, Vol.23, pp. 689-696.
- Siva Saravanan KS, Velpandian V, Musthafa MD, Anbu N, Abdul Kadher AM (2013**), Clinical Evaluation of Thelkodukku Chooranam (Heliotropium Indicum) in the Treatment of Scabies,**vol.4,pp.2249-9571.
- **Standardization of chuntaivatral chooranam- a polyherbal Siddha formulation**, vol.2(7), pp. 156-160.
- Suchandra Chatterjee, Zareena Niaz, Gautam S, Soumyakanti Adhikari, Prasad S. Variyar, Arun Sharma, (2007), **Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace *(Myristica fragrans*),** Vol.101, pp. 515-523.
- Thanigavelan V, Lakshmanakumar V, Kaliyamurthi V, Pitchiah Kumar M and Victor Rajamanickam G, (2012), **Pharmacological Study of a Siddha Holistic Herb *Sivakaranthai - Sphaeranthus Amaranthoides* Burm for Analgesic and Anti-Inflammatory activities,** Vol.02(01), pp. 95-101.
- Thenmozhi.P, Pitchiah Kumar.M, Velpandian.V, Sandhiya.D, Banumathi. (2014) **Antibacterial activity of aavaraivithaadhi chooranam in animal model**, vol. 3, issue 11, pp. 550-559 .
- Trivedi Atal Bihari, Mahajan Nitin, Malakay Ritu, Khatotra Sukhdev, (2015), **Clinical evaluation of Ajmodadi churna in the management Amavata W.S.R to Rheumatoid Arthritis,** Vol.3(10).
- Ushakanthan, S., Visweswaran, S., Sivakkumar, S., Mariyappan, A and Banumathi, (2017) **clinical pilot study of vasambu chooranam for the mamagement of kuruthi Azhal noi (primary hypertension),**vol.9,pp.61125-61127.

- Vedavathy S, (2003), **Scope and importance of traditional medicines,** Vol.2(3), pp. 236-239.
- Verma S K, Vartika jain, Katewa S S, (2009), **Blood pressure lowering, fibrinolysis enhancing and antioxidant activities of Cardamomum (*Elettaria cardamomum*),** Vol.46, pp. 503-506.
- Vinotha Sanmugarajah, Ira Thabrew and Sri Ranjani Sivapalan (2014) **standardization of ammukkirai chooranam, a traditional herbal preparation in Siddha system of medicine,** vol.5(2), pp. 134-144.
- Wnanbyr Tabuneng, Hideo Bando and Takashi Amiya, (1983), **Studies on the constituents of the crude drugs '*Piperis Longi Fructus*'. On the Alkaloids of fruits of *Piper Longum* L,** Vol.31(10), pp. 3562-3565.
- Yamini.K,Onesimus.T, Mubarak.H (2013), **Anti-Ulcer Effect Of Yelaathi Chooranam-A Polyherbal Siddha Formulation,**Vol.3(4),pp.3026-3028.
- Yi – Zeng Liang, Peishan Xie, Kelvin Chan, (2004), **Quality control of herbal medicines,** Vol.812, pp. 53-70.