NMR AND GC – MS ANALYSIS OF AMUKKARA CHOORNAM

By

SHILPA.S

(16PCH017)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher

Education for Women, University

(Estd.u/s of UGC Act 1956)

Coimbatore- 641043

Partial Fulfilment of the Requirements for the Degree of

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SHILPA.S

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LIST OF ABBREVATIONS

AMC	Amukkara choornam
GC-MS	Gas chromatography-Mass spectrometry
LC-MS	Liquid chromatography-Mass spectrometry
1D NMR	One dimensional neutron magnetic resonance
HPTLC	High performance thin layer chromatography
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
LOD	Loss on drying
UV	Ultraviolet- visible spectroscopy
LC-NMR	Liquid chromatography-nuclear magnetic resonance
IR	Infrared spectroscopy
FTIR	Fourier transform infrared spectroscopy
FIR	Far infrared spectroscopy
DTA	Differential thermal analysis
TGA	Thermogravimetry analysis

SFC	Supercritical fluid chromatography
SPE	Solid phase extraction
CE	Capillary electrophoresis
MAE	Microwave – assisted extraction
DPPH	2,2-diphenyl-1-picryl hydrazyl
XRD	X- ray diffraction
TEM	Transmission electron microscopy
XPS	X-ray photoelectron spectroscopy
EDAX	Energy dispersive x-ray analysis
AAS	Atomic absorption spectroscopy
BHA	Butylated hydroxyl anisole
BHT	Butylated hydroxyl toluene
TBA	Thiobarbituric acid
PCA	Principal component analysis
DMSO	Dimethyl sulfoxide

INTRODUCTION

1. INTRODUCTION

India has one of the oldest, richest and most diverse cultural living traditions associated with the use of medicinal plants. In the present scenario, the demand for herbal products is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential**(Adithan, 1996; Tandon et al.,2004).**

Plants have provided mankind a large variety of potent drugs to alleviate suffering from diseases in spite of spectacular advances in synthetic drugs in recent years, some of the drugs of plant origin have still retained their importance. The use of plant-based drugs all over world is increasing. In spite of the tremendous advances made in the modern medicine there are still a large number of ailments for which suitable drugs are yet to be found. Today, there is an urgent need to develop safer drugs for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Hence, there is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment **(Morgan, 2002).**

The preparations of traditional medicines have been widely used for thousands of years in many countries. However, preparation of herbal medicines either as single herb or as collection of herbs, are extracted using boiling water. This is one of the characteristics of oriented herbal medicines. Due to this, the quality control of oriented herbal medicines is more difficult as compared to western drugs. One or two markers or pharmacologically active compounds in herbs or herbal mixtures were currently employed for evaluating the quality and authenticity of herbal medicines and in assessing the quantitative herbal composition of a herbal product. The kind of determination, however, does not give a complex picture of a herbal product, because multiple constituents are usually responsible for its therapeutic effects **(Yi. Zeng Liang *et al,* 2004).**

India’s traditional medicinal system is associated with the natural derived preparations for the treatment of various diseases. It explores the utilization of herbs, metals and minerals for medicinal purposes. Herbal medications are widely believed to be beneficial. The popularity and availability of the traditional remedies have generated concerns regarding the safety, efficacy and responsibility of practitioners using traditional remedies. Recent literatures show that Ayurvedic processing of metallic formulations may bring down its shape to nanometre size. Herbal products can be purchased without a prescription and might not recognize any potential hazards in an inferior product. In varied industrial processes significant metals are also used. The herbs are combined with metals that facilitate in assimilation and delivery of the ingredients into the human body. Despite the poor risk-benefit analysis for herbs, it may be reasonable to use certain herbs for patients who have conditions where there are no known effective treatments, or when standard therapies have not been tolerated or have failed to lead to improvements **(Divya.A 2015).**

1.1 IMPORTANCE OF SIDDHA MEDICINE

Siddha system of medicine is one of the oldest traditional systems of medicine, which has been originated from India and is practiced mostly in the southern part of this country for treating various diseases including even chronic conditions**(Arjun Ram *et al.*,2009).**

WHO defines traditional medicine asincluding diverse health practises, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well being, as well as to treat, diagnose or prevent illness. WHO has provided some terms related to herbal drugs, according to their definitions. .Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral material)**(Dr Rajesh Kumari et al., 2016).**

According to Siddha, wind predominates in the first third of life, bile in the second third, and phlegm in the last third of life, while in Ayurveda phlegm dominates the first third and wind the last third of life. Most modern Siddha doctors place the greatest emphasis on the examination of the pulse, whereby both diagnosis and prognosis can be obtained through one process **(KENNETH G. ZYSK, 2008).**

1.2 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 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.
- **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.

1.3 WHY STANDARDISATION IS IMPORTANT?

The knowledge about the herbs and herbal medicines by the villagers and tribes is unknown to scientists. To know more about the herbal medicine, standardization is required. The quality, safety and side effects can be determined while standardizing each herbal formulation. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Hence standardization is a tool in the quality control process **(Sachan *et al.*, 2016).**

Modern system of medicine is based on sound experimental data, toxicity studies and human clinical studies. But, pharmacopoeial standards on raw material/ finished products are not available. The lack of quality standards has resulted in mild to serious adverse effects ranging from hepato toxicity of death. Hence, herbal ingredients require tools for determining identity, purity and quality and tools have to be technically sufficient, rapid and cost effective with requirements **(Sachan *et al.*, 2016).**

Standardization of herbal drugs involves pharmacognostic evaluation, physico-chemical parameters, chemical parameters, chromatographic and spectroscopic analysis, microbiological parameters etc.

1.4 TECHNIQUES USED FOR STANDARDIZATION

PHARMACOGNOSTIC EVALUATION	• Colour • Taste • Odour • Size • Shape • Texture • Microscopical characters • Histological parameter
PHYSICO-CHEMICAL PARAMETERS	• Total ash • Acid-insoluble ash • Moisture content • Viscosity • Hardness • Flow capacity • Alcohol content • Sedimentation

CHEMICAL PARAMETERS	• TLC • HPLC • HPTLC • GC • UV • IR • FT-IR • AAS • LC-MS • GC-MS • Fluorimetry
MICROBIOLOGICAL PARAMETERS	• Total mould count • Total coli forms count • Limiters are used as quantitative tool or quantitative to determine and control the amount of impurities.

1.5 AMUKKARA CHOORNAM

Amukkara choornam is a polyherbal Siddha formulation. It is composed of spices and herbs like Syzygium aromaticum, Cinnamomum wightii, Elettaria cardamomum, Piper nigrum, Piper longum, Zingiber officinale, Withania somnifera and Cane sugar.

1.6 INGREDIENTS OF AMUKKARA CHOORNAM

- ❖ Withania somnifera - 25.20%
- ❖ Zingiber officinale - 12.60%
- ❖ Piper nigrum - 3.15%
- ❖ Piper longum - 6.30%
- ❖ Elettaria cardamomum - 1.57%
- ❖ Cinnamomum verum - 0.79%
- ❖ Syzygium aromaticum - 0.39%
- ❖ Saccharum officinarum - 50%

All these ingredients that present in this Siddha formulation have unique properties and they are used to prepare various medicines. Herbs and spices, which are important part of the human diet, have been used for thousands of years to enhance the flavor, color and aroma of food. Scientific experiments since the late 19th century have documented the antimicrobial and antioxidative properties of some spices, herbs and their components (**G. Singh *et al*,2008).**

WITHANIA SOMNIFERA: Ashwagandha is a commonly used herb in traditional medicines. Ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic, and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems.

ZINGIBER OFFCINALE: Ginger is one of the oldest herbs known by the people and is one of the earliest spices to be known in the east. Ginger has been used to treat a wide range of ailments including stomach aches, diarrhea, nausea, asthma, respiratory disorders.

PIPER NIGRUM: Black pepper is native to India and the tropical evergreen forest of the Malabar region of southern India. It is one of the oldest spices used for both culinary and medicinal purposes.

PIPER LONGUM: Indian long pepper is used to improve appetite and digestion, as well as treat stomachache, heartburn, indigestion, intestinal gas, diarrhea and cholera. It is also used for lung problems including asthma, bronchitis and cough.

ELETTARIA CARDAMOMUM: Cardamom is a little known condiment in American cooking, but for Scandinavian, Arab and Indian food products it is one of the most important of all spices. In the pharmaceutical industry, cardamom tinctures are used to add both Flavour and aroma to many patents.

CINNAMOMUM VERUM: Cinnamomum is a tropical evergreen tree and grows wild in Sri Lanka, Madagascar, India and Indochina. The inner bark of the tree has been used in ethno-medicine and flavoring for foods.

SYZYGIUM AROMATICUM: Clove oil (Syzygium aromaticum) is widely used as a perfume and food flavoring, as a medicine for the treatment of asthma and for various allergic disorders.

SACCHARUM OFFICINARUM: It is reported to be antidote, antiseptic, antivinous, bactericide, cardiotonic, laxative, diuretic etc. it is used as a remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhea, hiccups, inflammation, sore throat, spleen, tumors and wounds **(Duke and Wain, 1981).**

1.7OBJECTIVES

Amukkara choornam has anti-arthritic activity and it helps to cure rheumatoid arthritis. Various diseases such as Vata roga, Kapha Roga, splenomegaly (spleen enlargement), leucorrhoea, hiccup, anemia and tuberculosis can also be treated by this Siddha formulation. It supports better digestion and stimulates the liver to secrete bile and also balance Kapha.

Hence the present study is carried out to identify the bioactive phytochemical constituents present in Amukkara choornam by,

Spectroscopic analysis

- ✓ **Fourier transform – Infrared spectroscopy (FT – IR)**
- ✓ **Gas chromatography – Mass spectroscopy (GC – MS)**
- ✓ **Nuclear magnetic resonance (NMR)**

REVIEW OF LITERATURE

2.REVIEW OF LITERATURE

2.1 STANDARDIZATION OF POLYHERBAL SIDDHA MEDICINES

- **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 analyses. 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.

- **Mohamed A Farag, *et al.,(*2012).** Glycyrrhiza glabra is a popular herbal supplement used for the treatment of chronic inflammatory conditions and possesses anticancer and antiviral activities. This study provided the first holistic approach to reveal secondary metabolite compositional difference among Glycyrrhiza species extracts. Metabolite profiling of these extracts provided characteristic metabolic fingerprints of liquorice and its allied species. Primary

metabolites profiling using GC-MS revealed the presence of cadaverine, an amino acid. Both LC-MS and NMR were found effective techniques in sample classification based on genetic and or geographical origin as revealed from derived PCA analysis.

- **Deattu .N, *et al.*,(2012).** Reported the chromatographic analysis of a polyherbal formulation by using HPTLC and GC-MS method. Extract of this polyherbal formulation was prepared from the combined ethanolic extract of *Saraca indica, Symplocos racemosa, Hemidesmus indicus, Aloe Vera, Asteracantha longifolia, Erythrina indica and Tribulus terrestris*. Identified their quality and stability using these methods. The correlation between the extract and formulation wore revealed using HPTLC studies. Using GC-MS analysis the phytoconstituents present in the both extract and formulation were identified.

- **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.

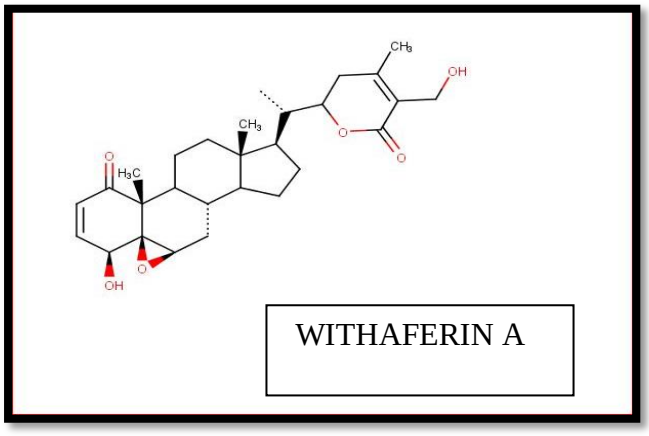
2.2 INGREDIENTS OFAMUKKARA CHOORNAM

2.2.1 WITHANIA SOMNIFERA

- **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 rejuvenating properties. 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.

- **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 typeWithanolides.

- **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 enhancing the immune response in diseases like tuberculosis ,cancer, leprosy and AIDS.

2.2.2ELETTARIA CARDAMOMUM

- **Varma .S.K, *et al.*,(2009).** Evaluated the antihypertensive potential and effect on some of the cardio-vascular risk factor in individuals of Elettaria cardamomum. This study suggested that longterm administration of cardamom has significant blood pressure lowering effect along with fibrinolysis and antioxidant enhancing properties in patients with stage I hypertension.

- **Beristain .C.I, *et al*.,(2001).** About 40g of oil/kg seed were extracted from cardamom seeds by steam distillation. Cardamom-based oil microcapsules were successfully produced by spray drying, using mesquite gum. In the microcapsules,

Total oil retention, Surface oil, Moisture content and Bulk and Particle density were analysed. They confirmed that the interesting emulsifying properties and goods flavour encapsulation ability that qualify mesquite gum as an important alternative encapsulating medium**.**

2.2.3 ZINGIBER OFFICINALE

- **Gurdip singh, *et al*., (2008**). The essential oil and oleoresins (ethanol, methanol, CCl4 and isooctane) of *Zingiberofficinale* were extracted respectively by hydrodistillation and Soxhlet methods and subjected to GC–MS analysis. The antioxidant activity of essential oil and oleoresins were evaluated against mustard oil by peroxide, anisidine, thiobarbituric acid (TBA), ferric thiocyanate (FTC) and 2,20-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods. .The antimicrobial properties were also studied using various food-borne pathogenic fungal and bacterial species. For other tested fungi and bacteriae, the essential oil and all oleoresins showed good to moderate inhibitory effects. Though, both essential oil and oleoresins were found to be effective, essential oil was found to be better than the oleoresins.
- **Shirin and Jamuna (2010).** Determined the chemical composition and antioxidant activity of *Zingiber officinale*. Analyzed polyphenols, vitamin C, beta carotene, flavanoids and tannins as antioxidant components. Antioxidant activity by three different methods showed higher activity in solvent extract than water extract. And concluded that ginger as a good source of antioxidant and most of the antioxidant components exhibit higher activities in alcoholic media as determined by different assays.

2.2.4 PIPER NIGRUM

- **Nobuji Nakatani, *et al*., (1986).** In the structural analysis of the compounds of the genus piper, the authors identified five phenolic amides from *Piper nigrum,* seven compounds from *Piper retrofractum* and two compounds from *Piper baccatum.* All the phenolic amides possess significant anitoxidant activities that are more effective than the naturally occurring antioxidant, alpha-tocopherol at the same concentration (0.01%). At this concentration, one ofthe amides had activity as high

as the synthetic antioxidants, BHA (butylated hydroxy anisole) and BHT (butylated hydroxy toluene).

- **Selene Maia de Morais, *et al.*, (2007**).The larvicidal activity of essential oils of four species of Piper from the Amazon Forest were tested using third-instar larvae of Aedes aegypti. The oils were extracted by steam distillation and analyzed by GC and GC-MS. The main components isolated from each Piper species were as follows: *viridiflorol* (27.50%), *aromadendrene* (15.55%) and *beta-selinene* (10.50%) from *Piper gaudichaudianum; b-selinene* (15.77%) and *caryophyllene oxide* (16.63%) from *Piper humaytanum; dillapiol* (54.70%) *and myristicin* (25.61%) from *Piper permucronatum;* and *asaricin* (27.37%) and *myristicin* (20.26%) from *Piper hostmanianum.* They confirmed that the oils with higher content of arylpropanoids were more active.

2.2.5 SYZYGIUM AROMATICUM

- **K wang-Geun Lee and Takayuki shibamoto, (2001).** Aroma extract from dried clove buds [*Syzygium aromaticum*] was obtained by using steam-distillation under mild conditions (55ºC and 95 mm Hg). The antioxidant property of the aroma extract and its two major aroma chemicals, eugenol and eugenyl acetate were evaluated in two different assays. The authors concluded that the presence of various aroma chemicals may explain the improvement of food stability. Moreover, ingestion of these compounds may help to prevent in vivo oxidative damage, such as lipid peroxidation, which is associated with many diseases, including cancer, arteriosclerosis, diabetes, and immune deficiency.

- **Mi-Jin park, *et al.,(*2007).** Investigated the potential of plant oils derived from *Leptospermum petersonii Bailey* and *Syzygium aromaticum* as natural antifungal agent. Evaluated the antifungal effects of essential oils at concentrations of 0.05, 0.1, 0.15, and 0.2 mg/ml on the dermatophytes *Microsporum canis* (KCTC 6591), *Trichophyton mentagrophytes* (KCTC 6077), *Trichophyton rubrum* (KCCM 60443), *Epidermophyton floccosum* (KCCM 11667), and *Microsporum gypseum* using the agar diffusion method. The major constituents of the active fraction against the dermatophytes were identified by GC-MS and HPLC analysis. The antifungal activities of S. aromaticum oil (clove oil) against the dermatophytes

tested were highest at a concentration of 0.2 mg/ml, with an effectiveness of more than 60%. In this study, they demonstrated that clove and tea tree oils exhibited significant antifungal activities against the dermatophytes.

- 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, 86.40 mmHg 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.

- **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.

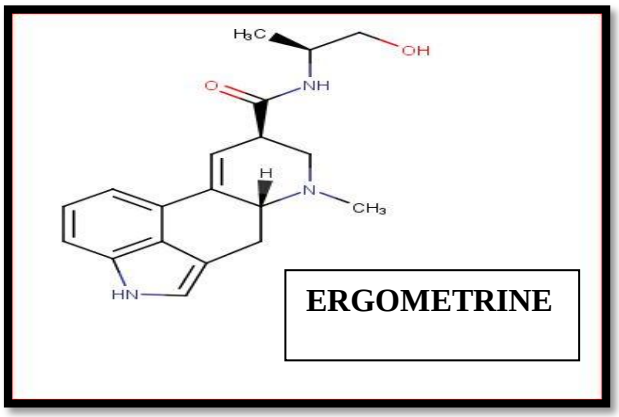
- The species name "foenum-graecum" means "Greek hay" indicating its use as a forage crop in the past. Trigonella foenum-graecum was used as a traditional remedy for the treatment of various diseases. **(Nathiya. S *et.al.*,(2014)** reported the chemical constituents, the biological and pharmacological 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. *Picrorhizakurrooa* 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.

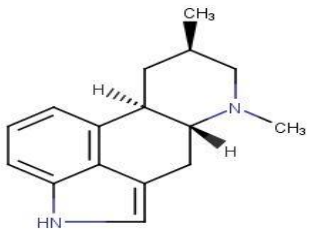
- **Milimitha padhi *et.al.*,(2013)** Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and other ailments. Argyreia nervosa belongs to family-convolvulaceae is an important herb used extensively in traditional systems of medicine. The pharmacological properties include antimicrobial, analgesic, anti-inflammatory, antiulcer, immunomodulatory, hypoglycemic, anticonvulsant etc.

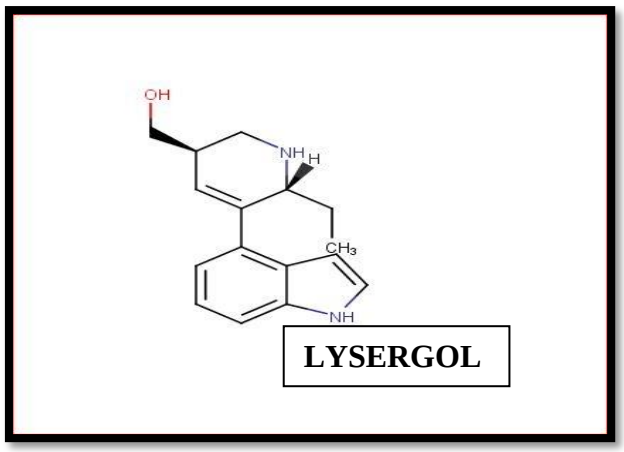
The seeds of Argyreia nervosa yielded fatty oil, which was found to contain the glycosides of palmitic, oleic, stearic, behenic, linoleic and linolenic acid Gas liquid chromatography (GLC) of the seed oil revealed the presence of myristoleic, myristic, palmitic, linoleic, linolenic, oleic, stearic, nonadecanoic, 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.



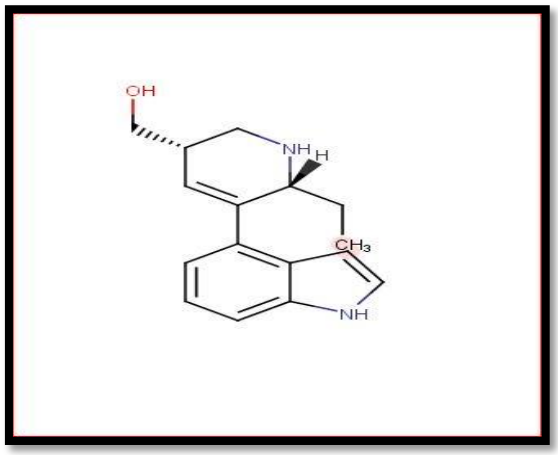
ERGOMETRINE

FESTUCLAVINE



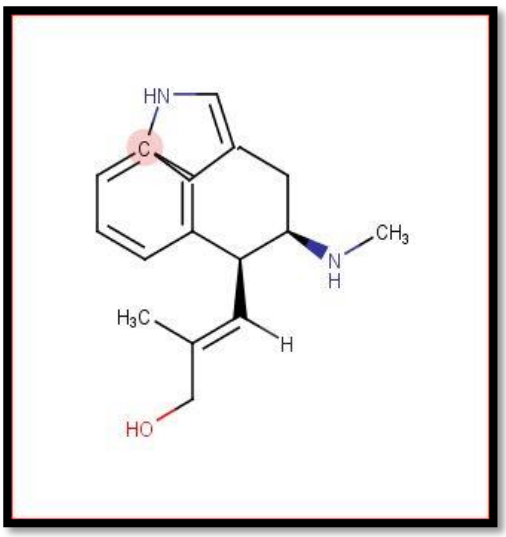


LYSERGOL

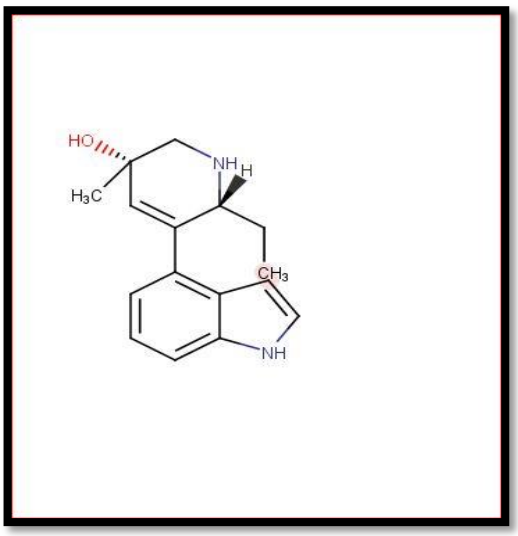


ISOLYSEGOL

CHANOCLAVINE –II



SETOCLAVINE



- **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).

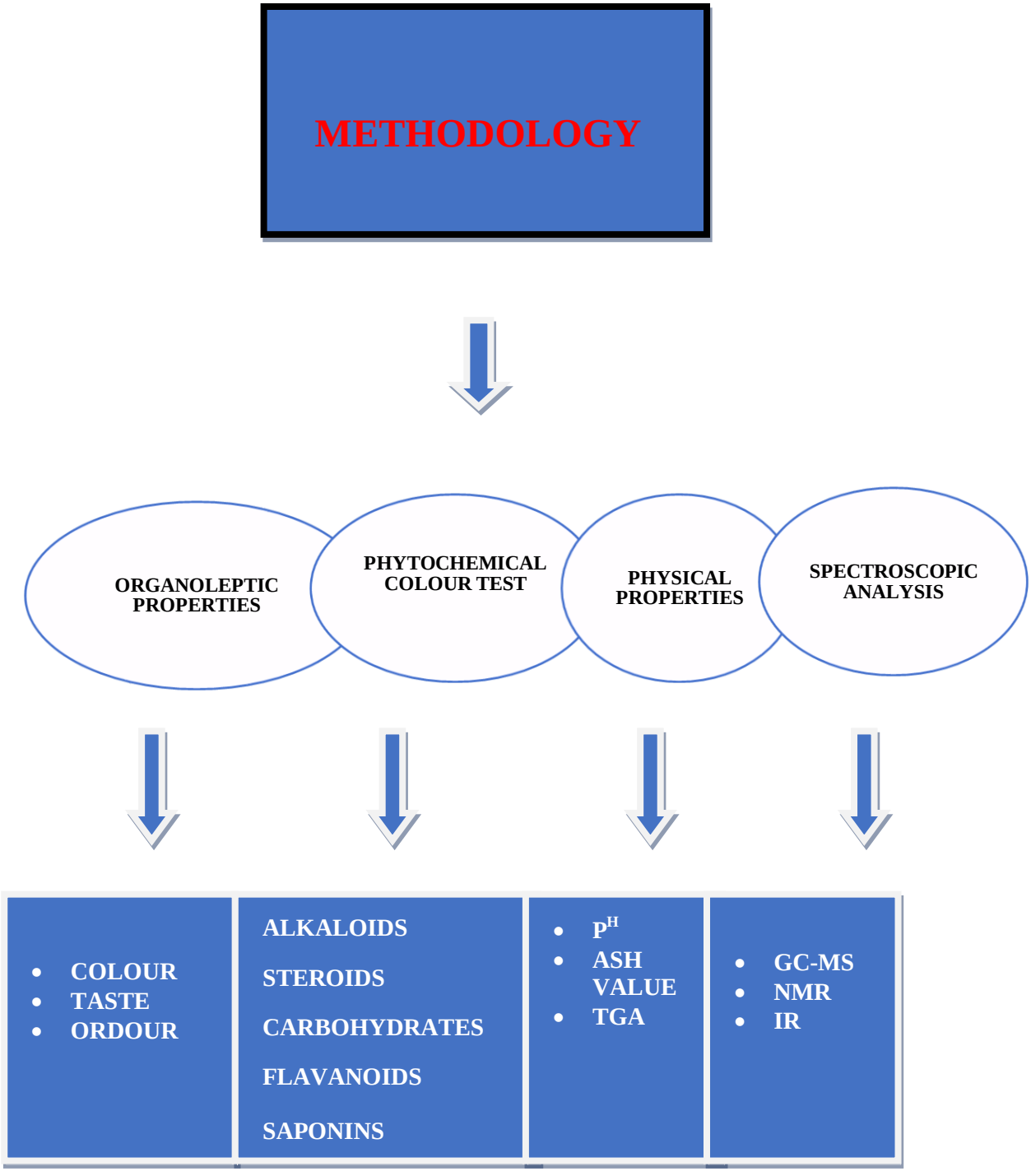
- Argyreia is traditionally used herb . The antibacterial, antifungal, antipyretic, analgesic, anti-inflammatory activity of the herb were studied. Studies were carried out on healthy wistar strain albino rats weighing about 140-250 g, using carrageenan induced paw edema. It was observed that the ethyl 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 anddiabetic rats. The study confirmed the rational basis for its use in traditional medicine for the treatment of diabetes.

MATERIALS AND METHODS

3. MATERIAL AND METHODS

In the present study the evaluation of **Amukkara choornam (AMC)** was carried out. The drug was collected from Siddha drug suppliers, Mettupalayam district. It is a polyherbal Siddha formulation, which helps to cure Rheumatoid arthritis. The detailed methodology adopted is given below.



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, 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 the sample was treated with concentrated sulphuric acid.

3.1.4 Test with 5% aqueous sodium hydroxide

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

3.1.5Test with iodine solution

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

3.1.6 Test with 5% aqueous potassium hydroxide solution

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

3.1.7 Test with Glacial acetic acid

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

3.2 PHYTOCHEMICAL COLOUR TEST

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 ethanolic 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 QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS:

Total flavonoid contents of different extracts were determined by method described by **Ordonez et al.** based on the formation of a flavonoid-aluminium complex. 0.5ml of ethanol extract (1mg/ml) was mixed with 0.5ml of aluminium chloride prepared in (2% in ethanol). The resultant mixture was incubated for 60min at room temperature for yellow colour development which indicated the presence of flavonoid. Absorbance was measured at 420nm using UV-VIS spectrophotometer. Total flavonoid content was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve:

$$Y = (0.217) * (X)$$

Where X is the absorbance and Y is the quercetin equivalent.

3.4 PHYSICAL PROPERTIES

Physical properties like ash value, loss on drying, pH values were determined as per method described in Indian Pharmacopoeia **(Meenu Sharma, et al 2013).**

3.4.1 Melting point of the drug

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

3.4.2 pH values

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

3.4.3 Determinations of ash values

Apparatus

i. Silica crucibles
ii. Muffle Furnace - Furnace was fitted with an indicating pyrometer, tomaintain 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. **(sigmascientific instruments Chennai, Pvt,Ltd.).**

3.4.3 (A) Total ash (TA) value:

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

3.4.4 Loss on drying (LOD)

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

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

Where W = weight of the sample powder in g.

3.4.5 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 6 – 12 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.

3.5 SPECTROSCOPIC ANALYSIS

Spectroscopy is the interaction between matter and electromagnetic radiation. There are different spectroscopic analyses such as IR, UV, NMR, GC – MS for the identification of the constituents present in the sample.

3.5.1 FT - IR

The IR of AMC was recorded using **Affinity – I Shimatzo** FT – IR instrument.

3.5.2 GC-MS Analysis

GC-MS analysis was performed using the **Clarus 680 GC** employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 μm df) and the components were separated using helium as carrier gas at a constant flow of 1ml/min. The injector temperature was set at 260^0C during the chromatographic run. The 1 μL of extract sample injected in to the instrument the oven temperature was as follows: 60^0C (2min); followed by 300^0C at the rate of 10^0C min-1; and 300^0C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240^0C; ion source temperature 240^0C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST library.

3.5.3 NMR

The H^1NMR of AMC was recorded in 500 MHz Bruker Avance NMR instrument, using DMSO as solvent.

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

In the present study phytochemical analysis of a polyherbal formulation AMC was carried out using NMR and GC – MS techniques. The results of the study were deliberatedin 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 1

Organoleptic properties of AMC

S.No	Parameters	Observation
1	Colour	Light Brown
2	Odour	Dried Ginger smell
3	Taste	Sweet
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 was soluble in concentrated hydrochloric acid and it was partially soluble in nitric acid and sulphuric acid. The sample was insoluble in iodine solution and glacial acetic

Table 2.

Behaviour of AMC with different reagents

S.No	Chemical treatment	Observation
1	Drug powder treated with Concentrated Hydrochloric Acid	Soluble
2	Drug powder treated with Concentrated Nitric Acid	Partially soluble
3	Drug powder treated with Concentrated Sulphuric Acid	Partially 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.

4.2

PHYTOCHEMICAL SCREENING

The phytoconstituents present in the polyherbal formulation used for rheumatoid arthritis, were identified by the colour test. The results revealed the presence of alkaloids, flavonoids, carbohydrates. The results of the preliminary studies were summarized in **Table-3.**

Table-3

Phyto-chemical screening of AMC

PHYTO COSTITUENTS	RESULTS

ALKALOIDS	+
CARDIAC GLYCOSIDE	_
STEROIDS	_
FLAVONOIDS	+
SAPONINS	_
QUINONES	_
CARBOHYDRATE	+
TERPENOIDS	_
TANNINS	_

4.3. QUANTITATIVE ESTIMATION OF FLAVONOID IN THE DRUG

Many herbs and plant products have been shown to have hypoglycemic action. Flavonoids are known to be bioactive antidiabetic principles. Flavonoid compounds such as Boswellic acid, Ellagic acid, Quercetin, and Rutin revealed maximum reduction in the blood glucose levels**.(Puchchakayala 2012).**

The quantitative estimation of flavonoid was done by the method described by **Ordonez et al.** based on the formation of a flavonoid-aluminium complex. The results showed that **0.328mg/1g** of flavonoid in the sample by Ordonez method.

4.4. PHYSICAL PROPERTIES

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

4.4.1 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 AMC was checked with solvents like dichloro methane, ethyl acetate, ethyl alcohol, DMSO, acetone, chloroform, pet ether and water. The AMC was found to be partially soluble in ethanol and chloroform, and soluble in DMSO.

4.4.2 The melting point of the drug was determined by melting point apparatus **Ajay R**. The AMC melted at **260^{0}C.**

4.4.3 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 in the range of **7.0 – 7.71.**

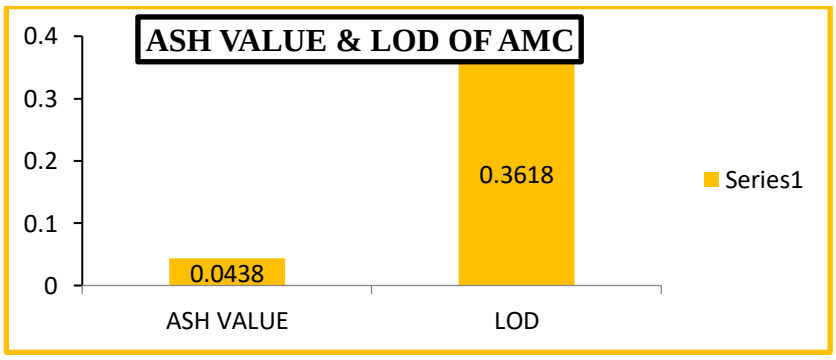
4.4.4 Ash value:

Ash value was found to be **0.0438%(Figure - 1).**At higher temperature the sample charred, which indicated the presence of organic matter and absence of inorganic salts.

4.4.5 Determination of loss of ignition

Loss on drying of the sample was carried out using Muffle Furnace maintained at 105-110°C and the loss on drying was found to be **0.3618%** which indicated the low moisture content of AMC.

Figure - 1



4.4.6 TG/DTA ANALYSIS

Thermal analysis is a technique used to study the properties of a material with change in temperature.**Figure 2 & 3** represents the TG curve of AMC. The weight loss of AMC started at 203^{0}C and completely decreased at 575^{0}C. Above that there was no change

in weight observed in the curve. DTG curve showed exothermic curve of AMC as shown in the **Figure(4 & 5).** It showed two decomposition peaks at 190^0C and 512^0C. DTG of organic matters give three exothermic peaks due to oxidation viz, labile organic matter (200 – 380^0C), recalcitrant organic matter (380 – 475^0C) and refractory organic matter including black carbon **(Fei guo et al, 2016).**In the DTG curve of AMC **(Figure –6),** the region corresponding to the peak at 300^0C, 450^0C are due to aliphatic, carboxylic acids and aromatic groups. 139% of aliphatic and acid compounds present and 75% of aromatic compounds are present.

Figure - 2

TG curve of AMC.

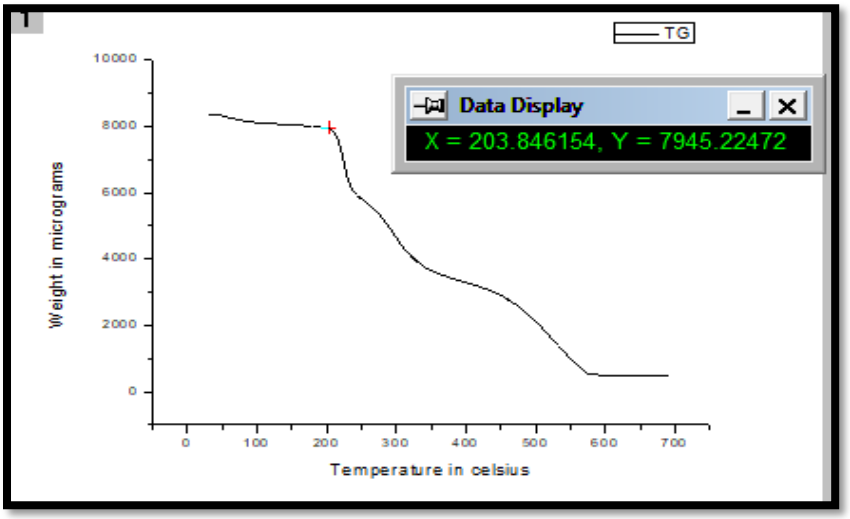


Figure – 3

TG curve of AMC

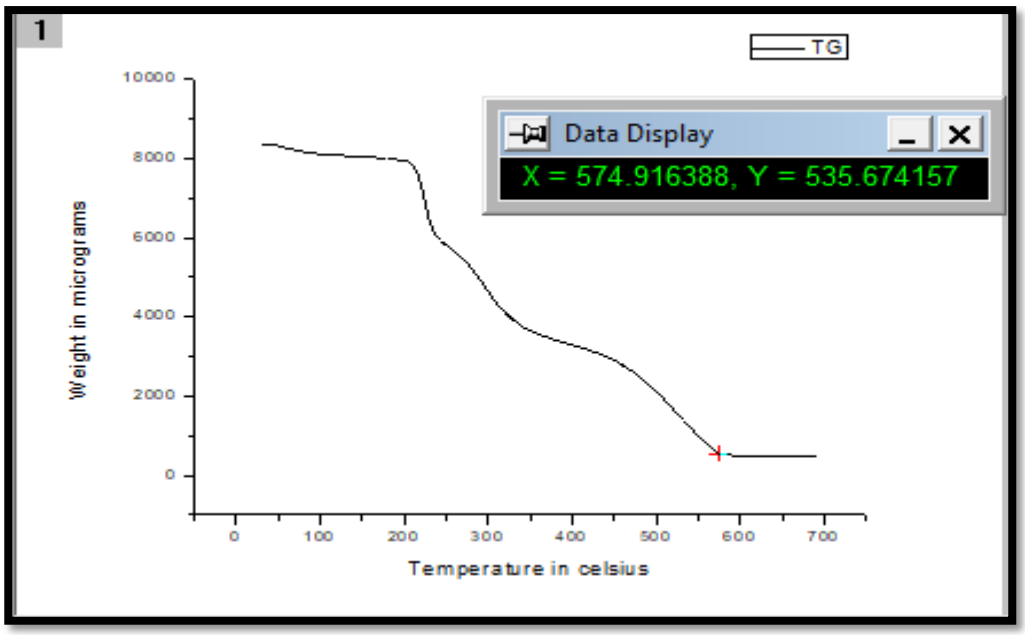


Figure – 4

DTA curve of AMC

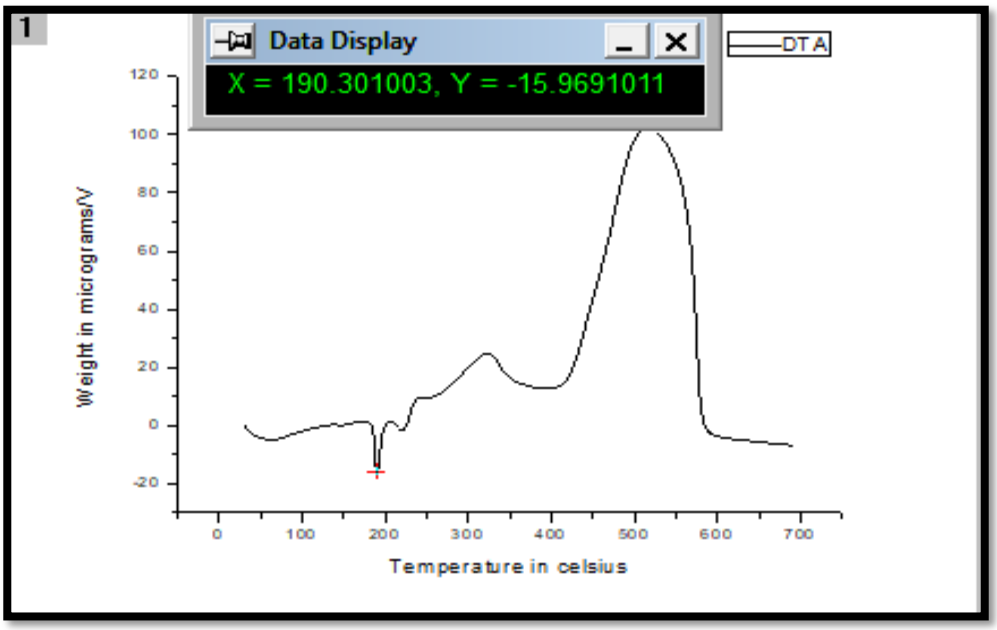


Figure – 5

DTA curve of AMC

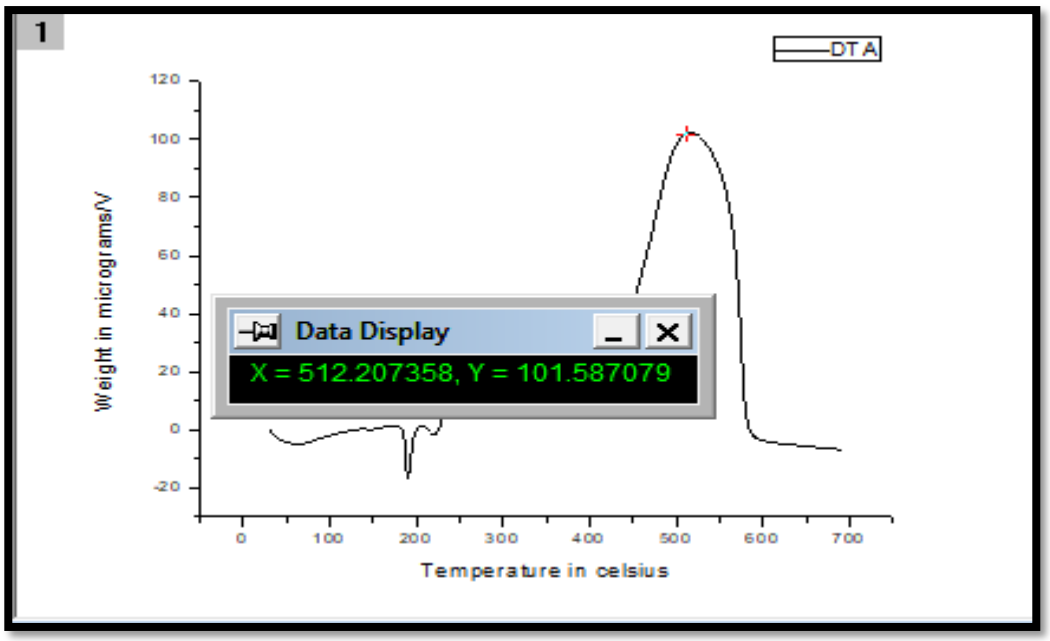
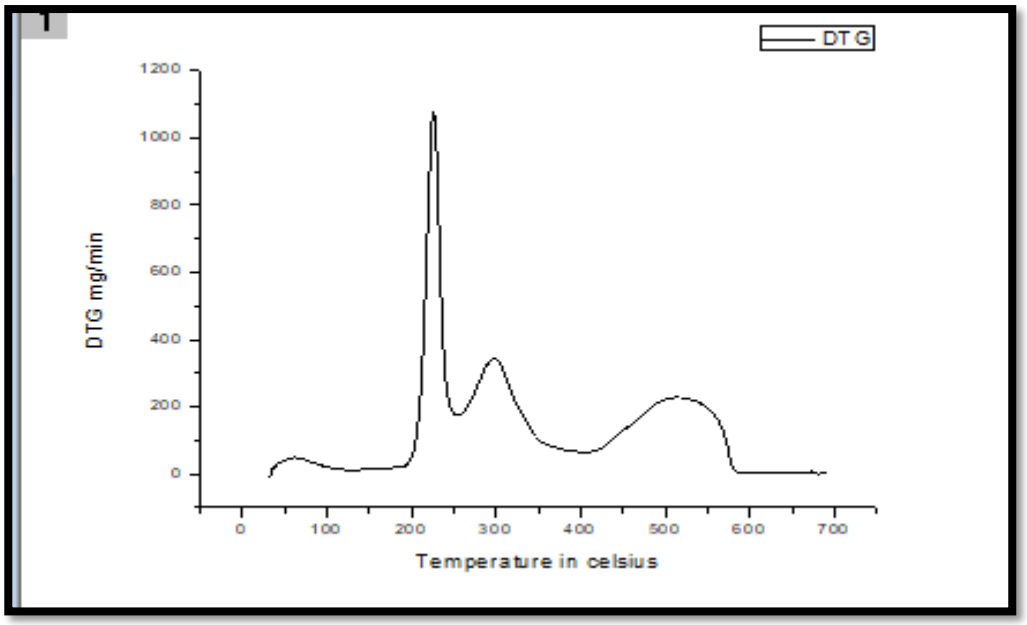


Figure – 6

DTG curve of AMC



4.5 SPECTROSCOPIC ANALYSIS

4.5.1 FT – IR

IR spectrum **(Figure – 7)** indicated the presence of amides, alcohols/phenols, amines and alkyl halides in the AMC sample **(Table – 4)**, which may be due to the presence of plant ingredients used in the AMC sample.

Figure - 7
Fourier Transform Infrared of AMC

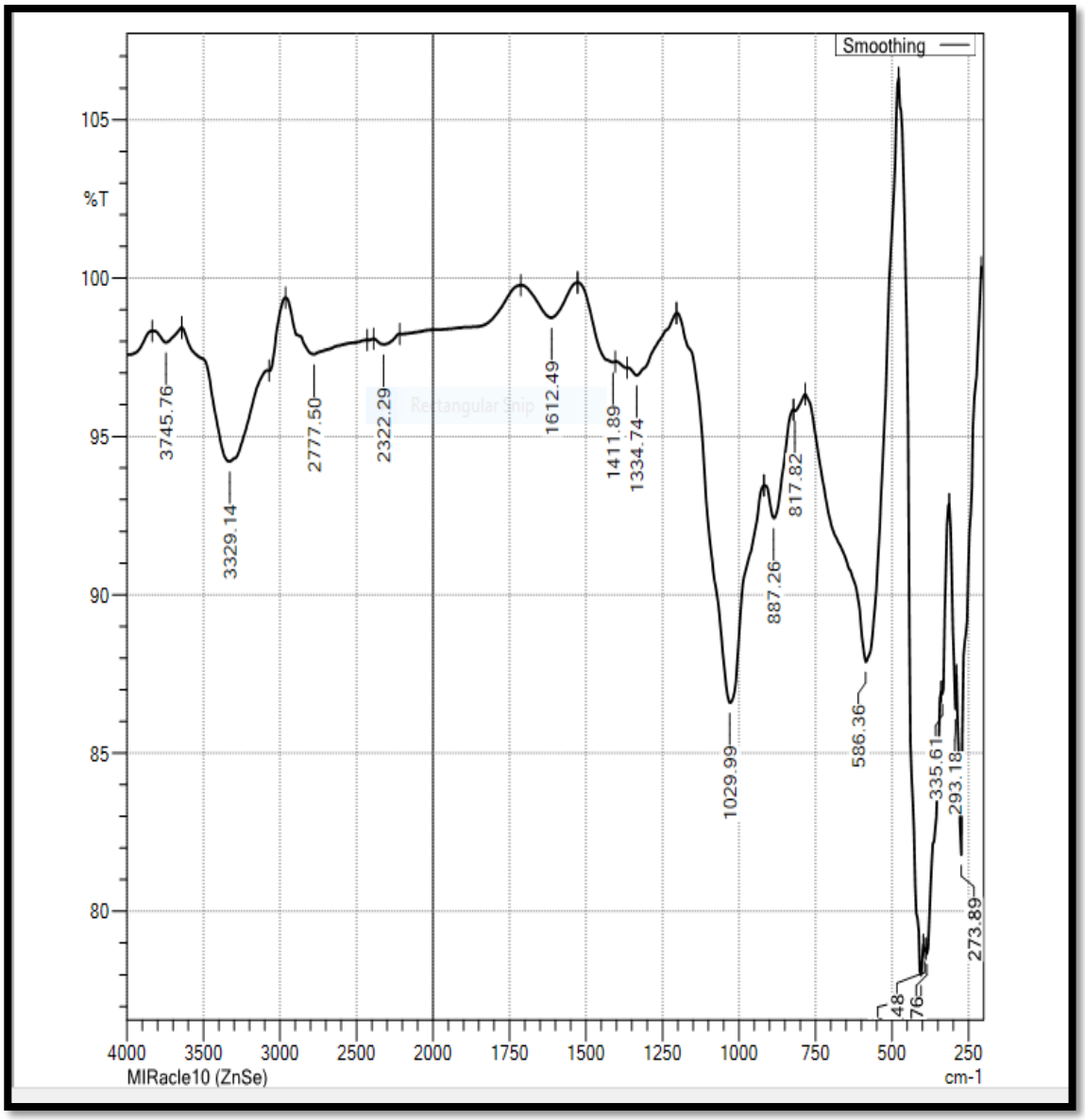


Table - 4

FTIR analysis of AMC

FREQUENCY	PEAK ASSIGNMENT	POSSIBILITY
3329.14 1612.49	N-H Stretching (strong) N-H Bending for 1^0& 2^o amides	Amides
3329.14 1411.87 1029.99	O-H Bond is broad C-O-H Bending appears broad (weak) C-O Stretching	Alcohols & Phenols
3329.14 1334.74	N-H Stretching C-N Stretching	Amines
586.36	C-Br stretching (strong)	Alkyl halide

4.5.2GC – MS

Gas chromatography coupled with mass spectrometry is a technique used for separating and identifying the components of complex volatile mixtures.The phytochemicals present in the formulation AMC were identified by GC – MS method. The GC – MS chromatogram of the formulation are shown in **Figure (8, 9, 10 & 11).** The

spectrum showed nine major peaks. The compounds pertaining to the peaks were identified by comparing with NIST library data of the peaks and mass spectra of the peaks with those reported in literature. The following compounds were identified in AMC viz,

- Apigenin
- Chrysoeriol**(Ana plazonic et al, 2009).**
- Piperine
- Adipic acid**(NISTchemistry webook).**

Figure - 8

GC spectrum of AMC

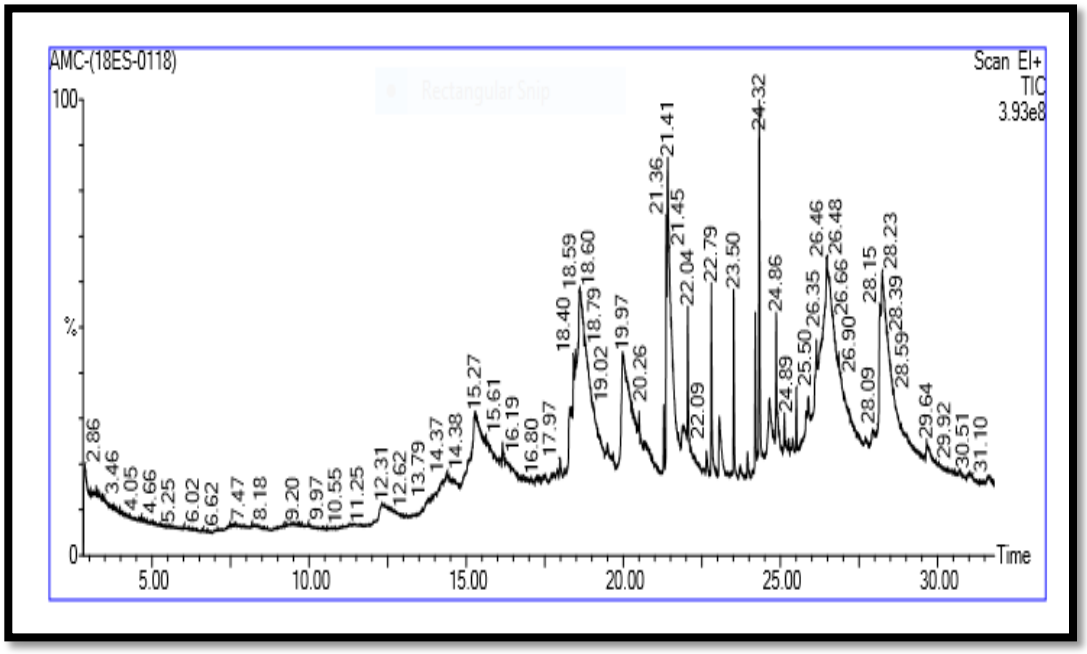


Figure – 9

Mass - Spectrum of AMC - I

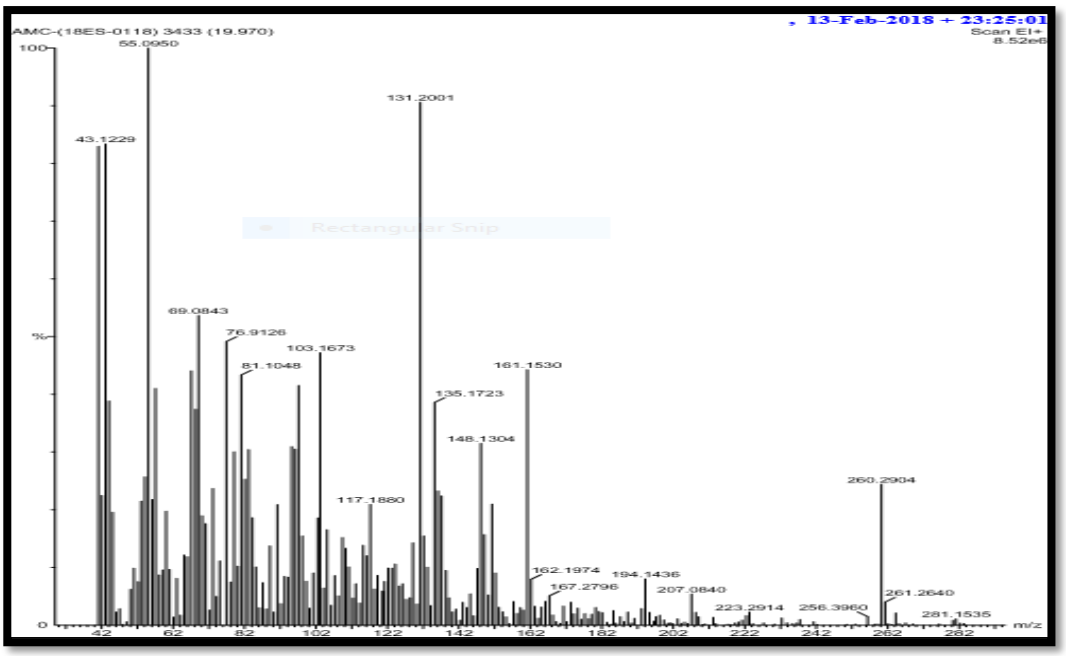


Figure - 10

Mass – Spectrum of AMC - II

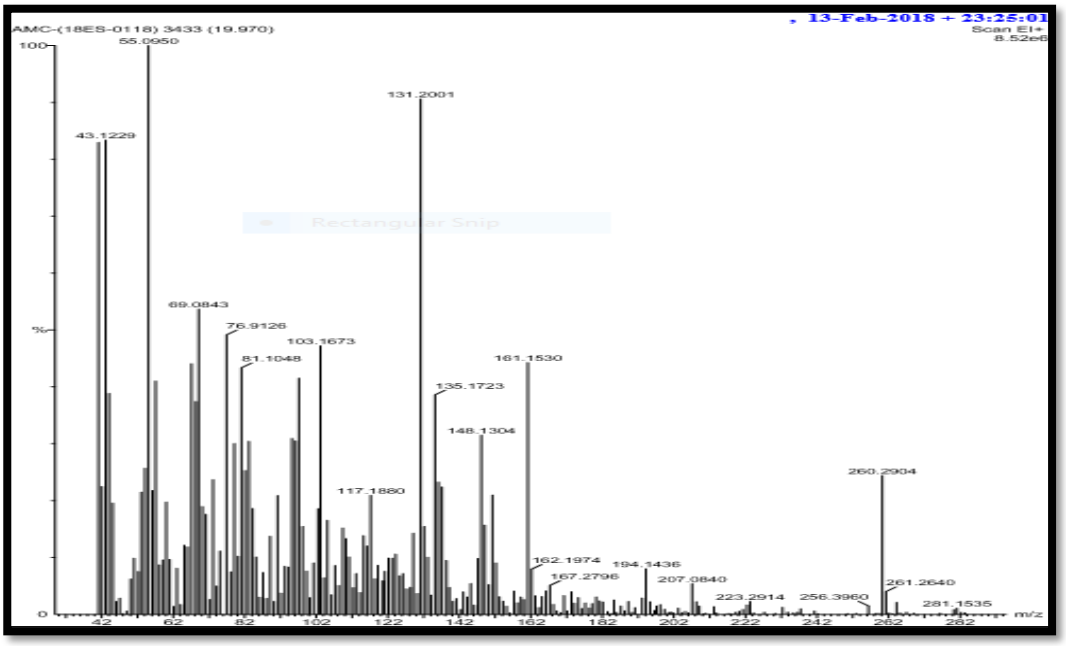


Figure - 11

Mass – Spectrum of AMC - III

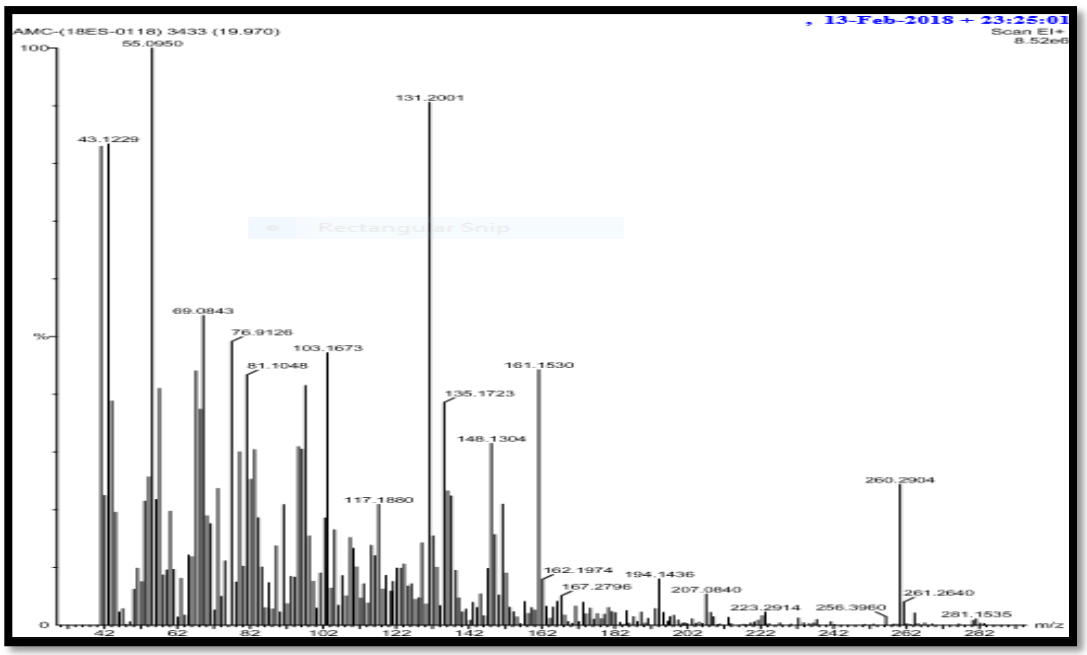


Figure – 12

Mass – Spectrum of AMC - IV

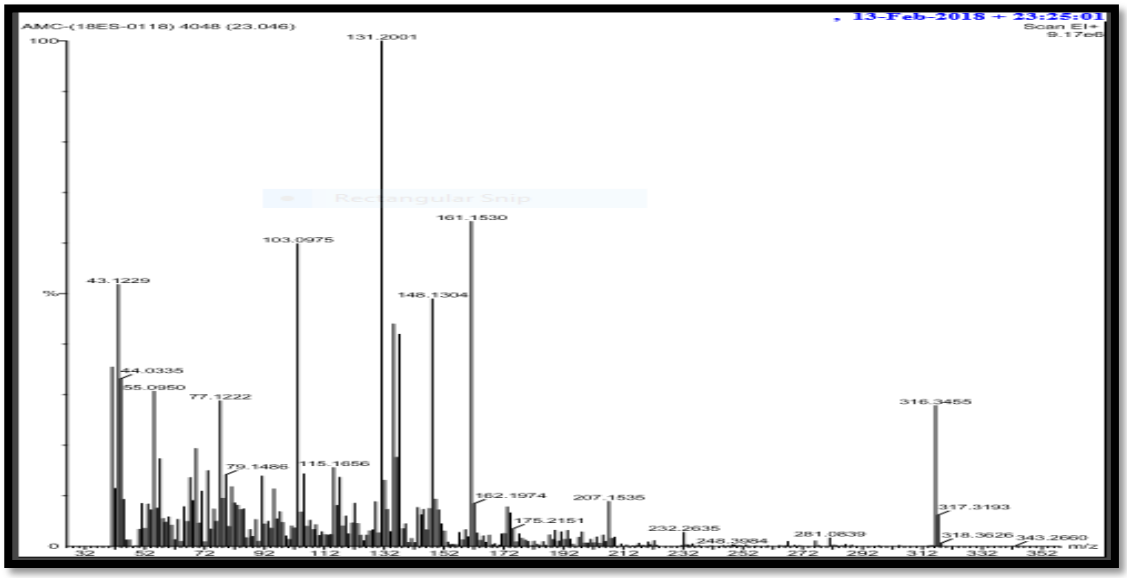


Figure 13

Mass Spectrum of AMC-V

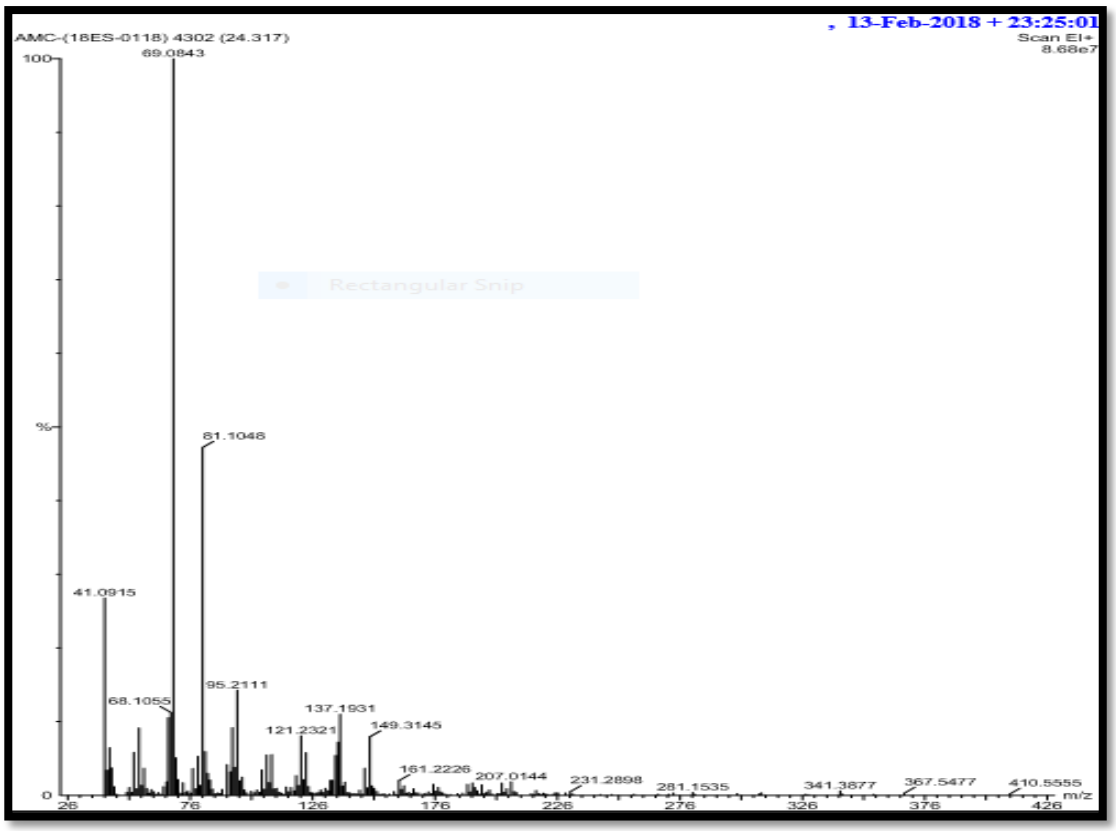
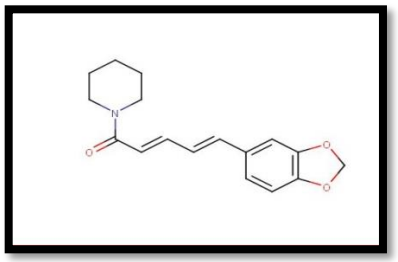
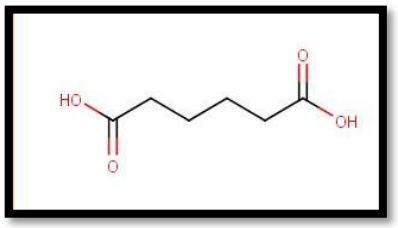
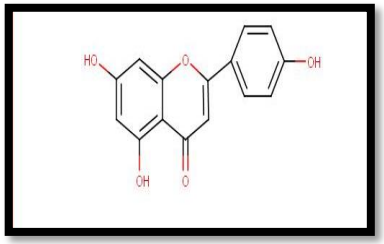
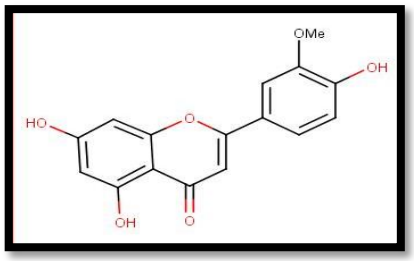
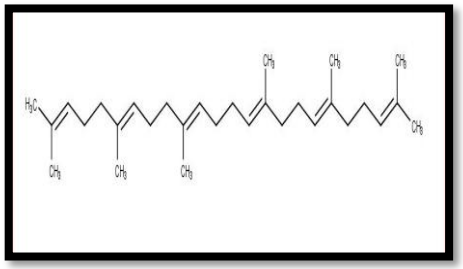

AMC-(18ES-0118) 4302 (24.317)
, 13-Feb-2018 + 23:25:01
Scan EI+
8.68e7
100
%
0
69.0843
81.1048
41.0915
68.1055
95.2111
137.1931
121.2321
149.3145
161.2226
207.0144
231.2898
281.1535
341.3877
367.5477
410.5555
m/z
26
76
126
176
226
276
326
376
426

Table – 5

Structure of identified constituents in the AMC by GC – MS Analysis

RT	AREA %	MOLECULAR WEIGHT	GC – ESI – MS	IDENTIFIED COMPOUND	NATURE & USES
26.478	19.575	285.343 g/mol	**MS:** 285.18, 207.01, 173.12, 137.12, 115.16, 71.11.	**PIPERINE** 	Pungent, increase the bioavailability of nutritional compounds.
21.406	10.537	146.142 g/mol	**MS:** 103.1, 83.1, 77.05, 54.9, 43.05.	**ADIPIC ACID** 	Acidic, used in the production of pharmaceuticals, bactericides.

19.970	7.826	270.24 g/mol	**MS:** 260.29, 256.3, 207.08, 194.14, 161.15, 148.13, 117.18, 103.16.	**APIGENIN** 	Natural flavones, antioxidant, anti - inflammatory & anti – tumor properties.
23.046	1.474	300.27 g/mol	**MS:** 316.34, 232.26, 207.15, 161.15, 148.13, 131.2, 103.09.	**CHRYSOERIOL** 	Flavonoid, antioxidant, anti – inflammatory properties.
24.317	2.812	410.718g/mol	**MS:** 149.31, 137.19, 121.23, 95.211, 81.104, 69.0843, 68.105	**SQUALENE** 	Emollient, antioxidant.

4.5.3 NMR

NMR gives the largest amount of information about the structure of a compound. The comparison of the spectrum showed the presence of chemical constituents namely, Piperine, Apigenin, Zingiberene and Squalene. Hence H^1 NMR analysis was done for the AMC sample to identify the components present in the sample. In the spectrum the peaks were seen, in the regions 5.9 – 7.0 ppm, 3.54ppm and 0.8 – 1.55ppm, indicating the presence of both aromatic and aliphatic regions. The NMR values were compared with the standard spectrum present in the SDBS database.

- Piperine – 5.9 – 7.0
- Apigenin – 3.54
- Zingiberene – 0.8 – 1.55
- Squalene – 0.91 – 5.04

Figure – 14
NMR spectrum of AMC (a)

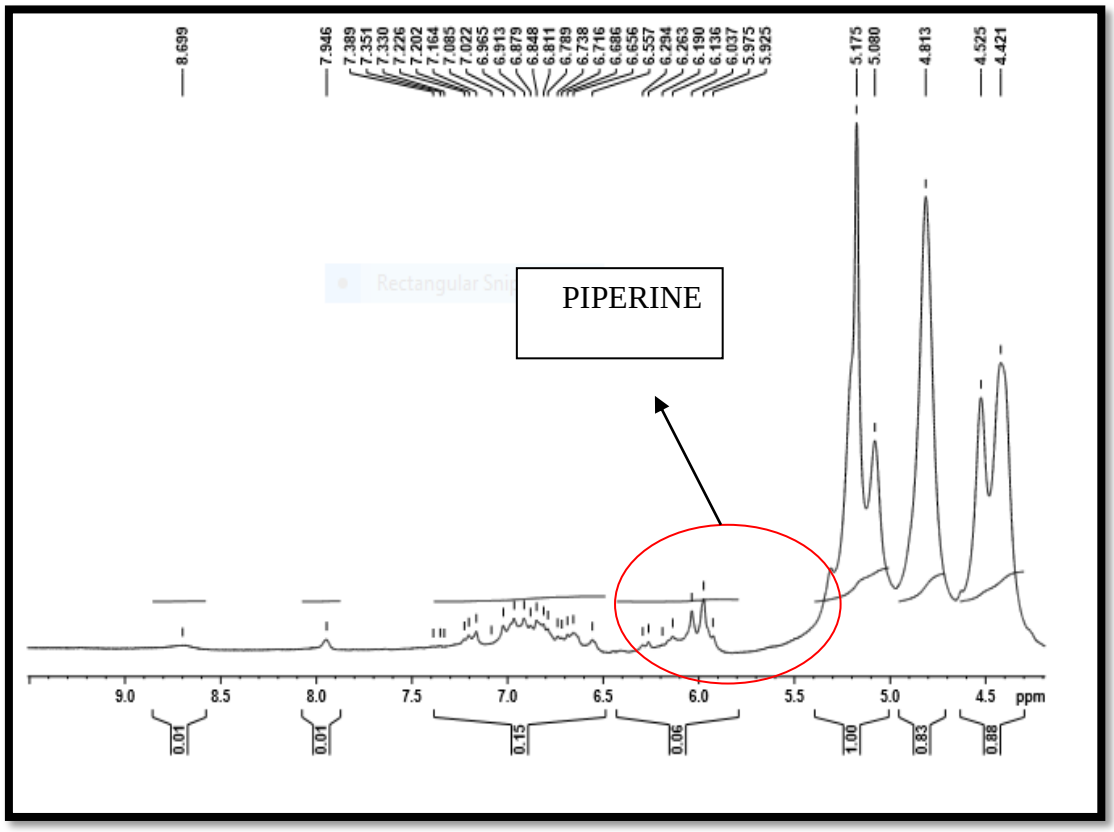


Figure – 15

NMR spectrum of AMC (b)

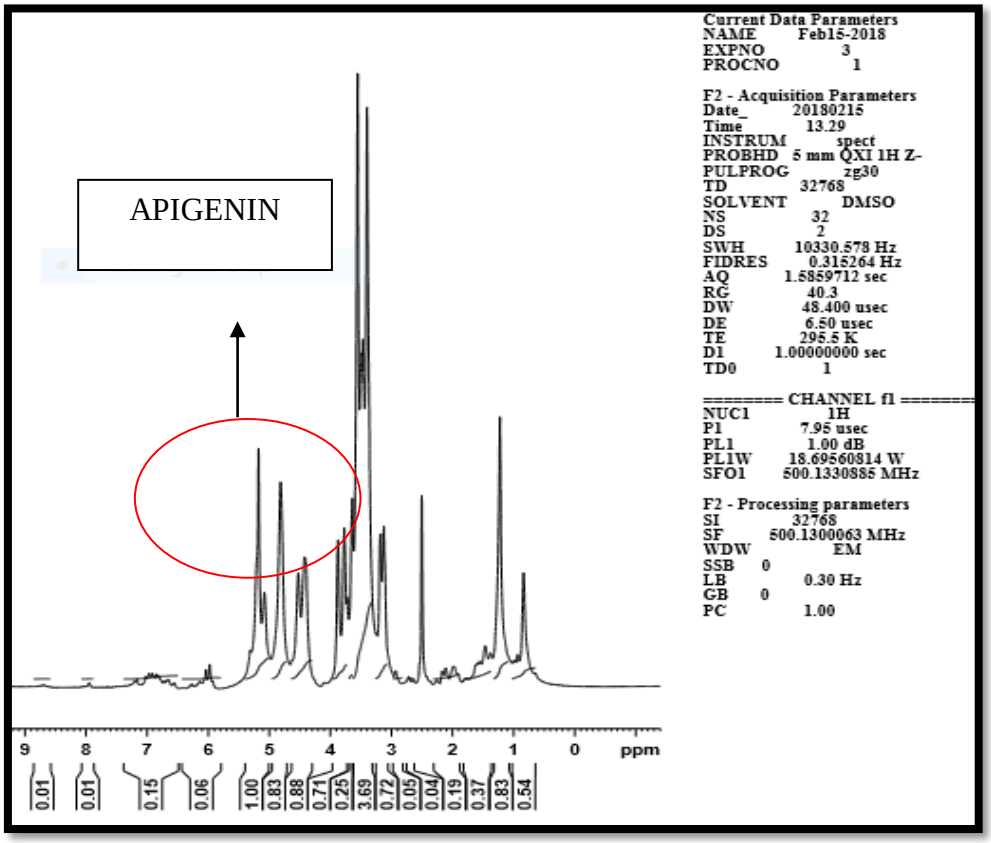


Figure – 16

NMR spectrum of AMC (c)

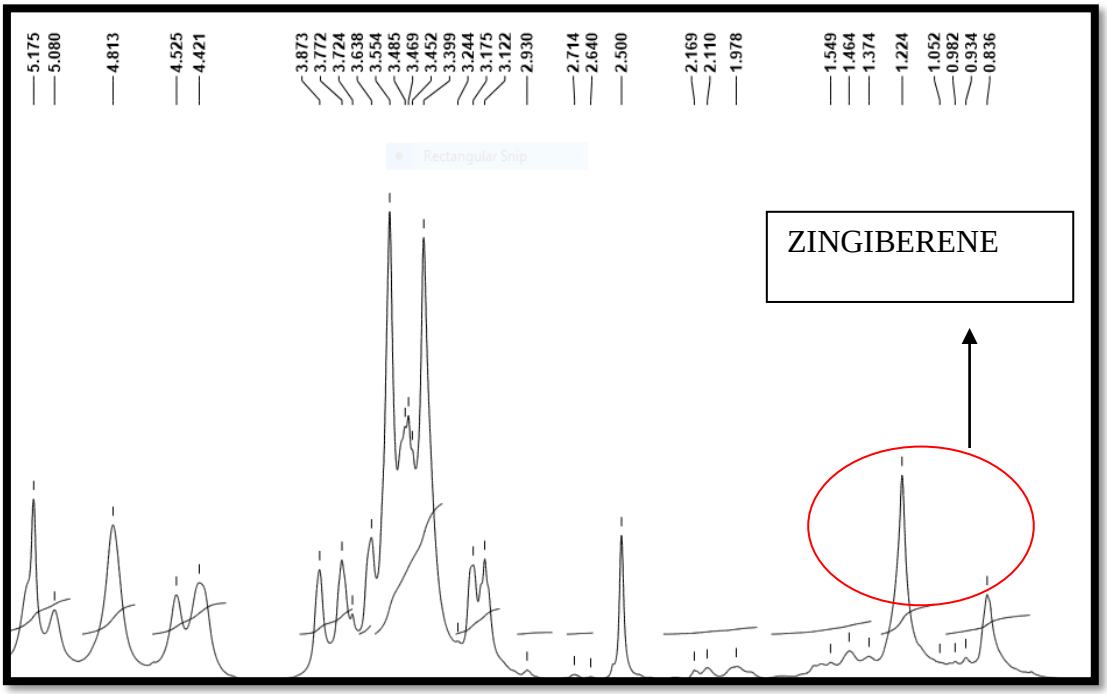
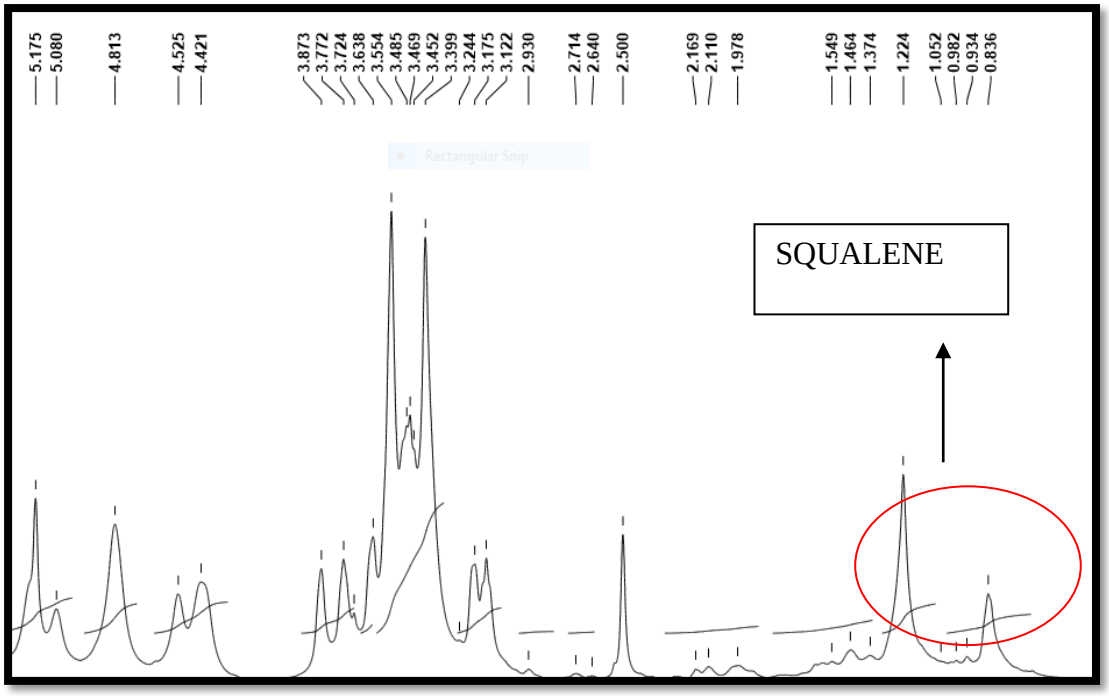


Figure – 17

NMR spectrum of AMC (d)



SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

The combinative approach of FT – IR, GC – MS and NMR techniques help in evaluating the quality, stability and consistency of herbal preparation. In this study, the phytochemical analysis of a polyherbal drug has been carried out, and the summary of the results are given below.

- The preliminary phytochemical analysis of the herbal drug showed the presence of alkaloids, flavonoid and carbohydrates.
- The amount of flavonoid in the herbal drug was found to 0.328mg/1g by Ordonez method.
- Ash value was found to be 0.0438%.At higher temperature the sample charred, which indicated the presence of organic matter and absence of inorganic salts.
- TG – DTA Analysis showed the exothermic nature of the reaction, during heating process.
- The GC – MS Analysis confirmed the presence of Piperine, Adipic acid, Apigenin and Chrysoeriol.
- The NMR results indicated the presence of three compounds Piperine, Apigenin and Zingiberenol.

Thus using combined chromatographic and spectroscopic methods the quality of herbal drugs can be easily assessed. The compound Piperine identified in the AMC, increases the bioavailability of nutritional compounds, and thus enhances the briskness in a person. Apigenin and Chrysoeriol have anti inflammatory activity thus validates the anti rheumatoid arthritic activity.

BIBLIOGRAPHY

REFERENCES

- 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 *WithaniaSomnifera* 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 plants from lakes by thermal analysis in a 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 and a ntimicrobial 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, SelvakumarS, (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.
- Meenu Sharma, Sharma R.G, Aakanksha Sharma, (2013), **Study of physico chemical properties of drug and physiological variation in leaves of andrographis paniculata (burm. F,) Nees,** Vol.3(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 Standardization 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), **Clinicalevaluation 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.