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SOPHISTICATED INSTRUMENTAL ANALYSIS OF POLYHERBAL SIDDHA FORMULATION ASHUWATHI CHOORANAM FOR CURING PCOS

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ABSTRACT

Ashuwathi chooranam (AC) is a polyherbal formulation from the classical Siddha literature. The present investigation was aimed to validate the safety and efficacy of the Siddha poly-herbal formulation AC and standardize the drug by means of physicochemical characters like pH value, ash values, specific gravity, solubility which were analysed. The total ash value was discovered to be 2.64%w/w, water soluble ash value is 1.35%w/w, and acid insoluble ash is 0.185% w/w. The water soluble extractives and alcohol soluble extractives are 46.37%w/w and 46.92%w/w respectively. The pH value is 3.53. The FT-IR spectroscopy in the sample exhibited the presence of functional groups like alkyl halides, aromatics, alkenes, 1º amine, and nitriles. The SEM analysis of the sample displayed that the Nano particles are defined as particulate dispersion or solid particles with a size in the range of 100-800nm in diameter. The XRD analysis of AC concluded in which the major peaks are identified that range 48-75nm in association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. The ICP-OES analysis in AC

revealed the heavy metals such as mercury, lead; cadmium and arsenic are within the limits. The current research is to standardise the AC through modern scientific techniques.

KEYWORDS: Instrument analysis, FT-IR, SEM, XRD, ICP-OES.

INTRODUCTION

Worldwide for the other systems of medicine, the Siddha system and others dated back to an unimaginative days of yore became the parent source in course of time. The Siddha system is based on three humours which represent the modern scientific ideas of diseases such as glandular, endocrine and metabolic activities and their disturbance.[1] The Siddha system is effective in treating various diseases of the liver, gastrointestinal tract, skin diseases, allergic disorders, general fevers, chronic diseases, gynaecological disorders. Many Siddhars were more concentrated in treating various gynaecological disorders including Karpavayu.[2] This Siddha drug Ashuwathi Chooranam (AC) is yet remained unexplored for its exact chemical, pharmacological values in terms of scientific research. To fill these scientific lacunae, the present work was undertaken to standardize Ashuwathi chooranam (AC) to validate through physicochemical analysis, phytochemical analysis, and instrumental analysis such as FT-IR, XRD, SEM, and ICP-OES. World Health Organisation (WHO) has developed and issued a series of guidelines such as Guidelines for the assessment of herbal medicines; Research guidelines for evaluating the safety and efficacy of herbal medicines. The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use Worldwide.[3] The guidelines of AYUSH for the standardization of drugs using scientific and evidence-based methodologies have been persisted. According to Siddha, many formulations have been reported which help to restore the ovulation and minimize the PCO phenotypes. One of such effective formulation mentioned in this system is Ashuwathi Chooranam (AC) for treating Karpa Vayu (PCOS) successfully.[4] Some principal herbal ingredients of this formulation which include Withania somnifera, Zingiber officinale, Piper nigrum, Piper longum, Myristica fragrans, Glycyrizzha glabra, Syzygiumar omaticum, Picrorrizha Scrophularia, are known to possess various beneficial activities. Hyoscymus niger has a stimulatory effect on the ovarian tissue, which may produce an estrogen-like activity that enhances repair of the endometrium and stops bleeding.[5] It is found to be effective in menorrhagia and dysmenorrhea. Even though it is mentioned in Siddha system of medicine for treating PCOS, no scientific data available to confirm its effectiveness in treating various gynaecological disorder especially PCOS.

MATERIALS AND METHODS

Collection of the drug

The ingredients of the drug were collected from around Chennai district in Tamilnadu. The test drug was identified and authenticated by Gunapadam Experts, P.G Gunapadam branch, GSMC, Arumbakkam, Chennai-106. Sample of the ingredients kept in PG Gunapadam department for future reference. All the raw materials here were purified individually as per the Siddha literature.

Purification of the Chooranam

The drugs that are purified by removing the sand, dust particles, and roasted are Withania somnifera, Piper nigram, Myristica fragrans, Picrorhiza scrophulariiflora, Hyoscymus niger; Zingiber officinale (dried ginger)-the outer skin were removed, the other drug was roasted slightly Piper longum, Myristica fragrans, Glycirriza glabra, Syzygium aromaticum -the flower buds were removed and fried slightly.[6]

PREPARATION OF THE DRUG

Ingredients

Amukkura(Withania somnifera)	-35gms
Chukku(Zingiber officinale(dried ginger)	-35gms
Milagu(Piper nigrum)	-35gms
Thippili(Piper longum)	-35gms
Jaadhikaai(Myristica fragrans)	-35gms
Jaadhipathiri(Myristica fragrans)	-35gms
Adhimadhuram(Glycirriza glabra)	-35gms
Krambu(Syzygium aromaticum)	-35gms
Kadugurohini(Picrorhiza scrophulariiflora)	-35gms
Krosaniomam(Hyoscymus niger)	-15.3gms
Sugar(Saccarum officinarum)	-127.5gms

Procedure

Take the equal quantities of all drugs winter cherry, chukka, milagu tippili, jadhikai, jadhipathiri, adhimadhuram, krambu, kadugurogini and krosaniomam were and roasted and grounded into a fine powder. The powder was sieved through a clean white cloth to get a uniform particle size of Chooranam. The Ashuwathi chooranam was purified by pittaviyal method (steam cooking in milk) as per Siddha classical literature. For this process cow's milk

and water were taken in equal ratio and half-filled in mud pot. A clean dry cloth was tied firmly around the mouth of the pot with a depression. Chooranam was placed over the depression on the tied cloth. Another mud pot of similar size was kept over the mouth of the mud pot. The gap between mud and pot was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk reduced to the lower pot. Then the chooranam was taken, dried, powdered finely.[7]

SOPHIASTICATED INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red)

Fourier transform infrared spectroscopy (FTIR) is a form of vibrational spectroscopy technique which is a great tool for chemical identification and to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering is an optical process of excitation of light interacting a solid, liquid or gas. An FTIR spectrometer concurrently collects spectral data in a wide spectral range 450-4000 cm-1, resolution of 1.0 cm-1 and sample 50mg, solid or liquid. Fourier Transform Infrared Spectroscopy (FTIR) determines bonding mechanisms in solids and on surfaces by producing an infrared absorption spectrum. The spectrographic techniques provide an ideal profile from the absorbance of sample, a distinctive molecular fingerprint that can be used to screen and scan samples for many different components. FTIR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information.

In infrared spectroscopy, high IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted) produces a unique signal. This is a dynamic system resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint there is no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for various types of analysis.

Applications

Ashuwathi chooranam (AC) was an herbal drug, FT-IR study was selected to identify the unknown materials of the test drug and determine the amount of components in the sample.[8]

Scanning Electron Microscopy (SEM)

SEM provides complete owing to high images of the sample by restoring a focused electron beam across the surface and detecting secondary or backscattered electron signal. SEM

provides images with magnifications that are vast up to ~X 50,000 allowing sub-micron-scale features to be seen i.e. well beyond the range of optical microscopes. It gives rapid, high resolution imaging with identification of elements present and Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in properties including chemical specification, texture and orientation of materials. The SEM is also capable of performing analyses of selected spots of locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.[9]

ICPOES (Inductively Coupled Plasma Optic Emission Spectrometry)

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) too different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions and intensity of the wavelength is measured. With respect to other types of analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES. Model of the instrument is Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP).

Application

The analysis of major, minor and trace elements in solution samples to identify the intensity of the wavelengths.

OBJECTIVES

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis

- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms.

Mechanism

In plasma emission spectroscopy (OES), a sample solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values. The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form to ensure constant flow through peristaltic pumps for analysis. 100 mg AC was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowed for few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution. The digested sample solution was shifted into plastic containers and labeled properly. It was completed in Bio-chemistry lab, Govt. Siddha Medical College, Chennai-106.[10]

X-Ray Powder Diffraction Method(X-RD)

DEFINITION

X-ray powder diffraction is most comprehensively used for the characterize the unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Applications

- Characterization of crystalline materials
- Advanced superior angular resolution for identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically are found, organized and counted.

- Determination of unit cell dimensions
- With robust systems, XRD can be used to:
- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:

a. determining lattice mismatch between film and substrate and to inferring stress and strain
b. determining dislocation density and quality of the film by rocking curve measurements
c. measuring super lattices in multilayered epitaxial structures
d. determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
e. Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction

Strengths

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations

- Homogeneous and single phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder
- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation

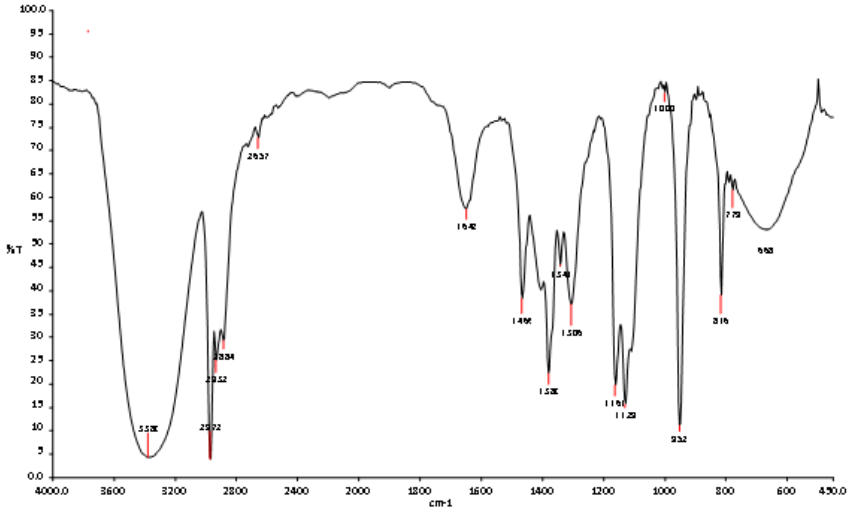
Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible

- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
- Powder less than ~10 μm(or 200-mesh) in size is preferred place into a sample holder or onto the sample surface.[11]

INSTRUMENTAL ANALYSIS

Fourier Transform InfraRed spectroscopy (FTIR)



FTIR ANALYSIS - Ashuwathi chooranam

Test

REF 4000 85 1300 50 700 65

3380 4 2972 3 2932 26 2884 29 2657 74 1648 57.5 1466 39 1380 23

1341 46 1306 37 1161 20 1129 17 1000 83 952 12 816 39 779 63

END: 16 PEAK(S) FOUND.

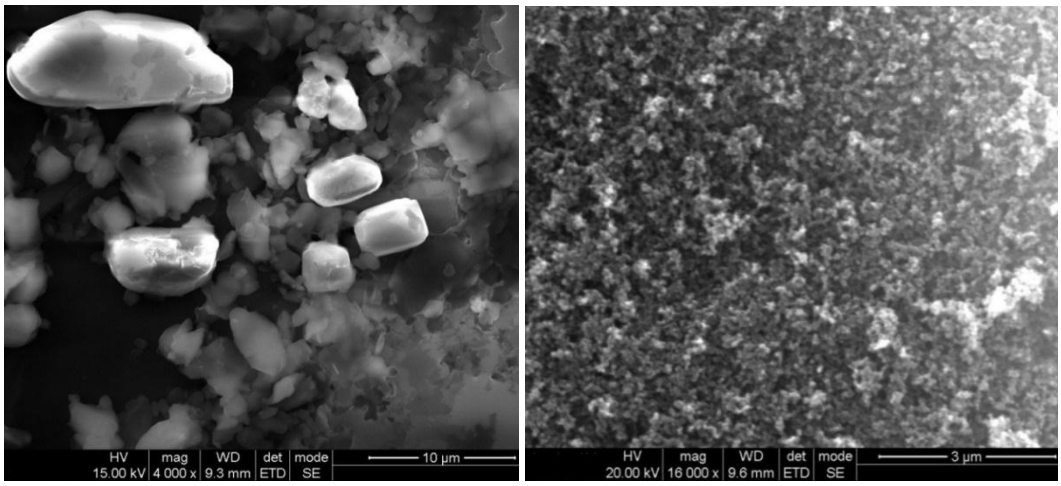
FTIR interpretation

Absorbtion peak cm-1	Stretch	Functional Group
779	C-Cl Stretch C-H "oop"	Alkyl halides Aromatics
816	C-Cl Stretch C-H "oop"	Alkyl halides Aromatics
952	=C-H bend	Alkenes
1648	N-H bend	1°amine
2657	C=N	Nitriles
3380	N-H stretch	1°, 2°amines, amides, alcohol

Interpretation

The trial drug was subjected to FTIR analysis to know the functional groups of the bio molecules, to elucidate the structure and to confirm the active molecules responsible for the therapeutic effect of the drug. It helps to understand the formation of complexes with the phytoconstituents during the processing of herbal medicines. The study revealed the presence of functional groups alkyl halides, aromatics, alkenes, 1º amine, nitriles.

SCANNING ELECTRON MICROSCOPE (SEM)



SEM reveals the nano size (100nm-800nm) particle of the sample.

Interpretation

Nano particles are defined as particulate dispersion or solid particles with a size in the range of 100-800nm in diameter.

They are easily

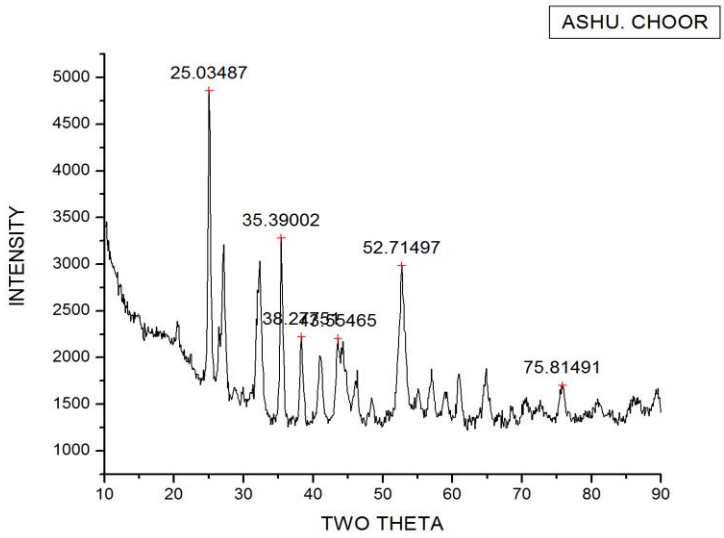
- Absorbable
- Non-antigenic in nature
- Biodegradable
- Biocompatible
- Selective/Targeted/Controlled delivery of drugs to specific site of action in the body even across the blood brain barrier
- Use to extend time window of bioavailability and to protect drug from enzymatic and chemical decomposition
- Result in reduced peripheral side effect of drugs.[12]

SEM analysis of the test drug Ashuwathi chooranam revealed the presence of nano particles of size 100-800 nm. The particles of size nano and near nano size show that the drug may easily enter the cells at the molecular level to treat the disease rapidly and increase the therapeutic effect.

Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale. This examination can yield information about the topography (surface features of an object) morphology (shape and size of the particles making up the objects) composition (the elements and compounds that the object is composed of the relative amounts of them) and crystallographic information (how the atoms are arranged in the object).

Nanoparticles have valuable properties that can be used to improve drug delivery. Where larger particles would have been unfurnished from the body, cells take up these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, together with the ability to get drugs through cell membranes and into cell cytoplasm. Effectiveness is important because many diseases depend upon processes within the cell and can only be impeded by drugs that make their way into the cell.

X-ray Diffraction Method (XRD)



Interpretation

The structure, the size and shape of the particles are highly dependent on the route of synthesis and high lights the efficacy of the drug. The micro particles may enhance bio absorption of the drug. The major diffraction peaks are identified after XRD analysis of AC

concluded that range 48-75nm in association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in AC act as additional supplement and possibly helps in increasing the efficacy of the formulation.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) ICP-OES Interpretation.

AshuwathiChooranam (wt:0.14715g)	**Element symbol** wavelength (nm)
Al396.152	BDL
As 188.979	BDL
Ca 315.807 mg/L	51.160
Cd 228.802	BDL
Cu 327	BDL
Hg 253.652	BDL
K766.491 mg/L	43.114
Mg285.213 mg/L	01.334
Na 589.592	15.310
Ni231.604	BDL
Pb220.353	BDL
P213.617	224.301
Zn206.200 mg/L	01.218

Interpretation

Potassium

A composition and method for stimulating gonadotropin hormone production and maintaining intracellular mineral balance in the reproductive organs of mammals. The composition consists of a mineral mixture containing effective amounts of manganese, iron and zinc as amino acid chelates in appropriate ratios. Preferable the composition also contains effective amounts of one or more minerals selected from the group consisting of copper, magnesium and potassium all of which are present at least in part as amino acid chelates or complexes. When magnesium and potassium are present at least some of these are present in inorganic form. Vitamins, other minerals such as calcium and phosphorus, and fillers may also be utilized. The composition is orally administered to female mammals to both induce estrus and bring about a stronger and more noticeable estrus.[13]

Phosphorus, Calcium and Zinc

Calcium (Ca) plays an important role in gonadotropic regulation of ovarian steroidogenesis. Marginal deficiency of phosphorus cause disturbance in the pituitary-ovarian-axis including

ovulation. Zinc (Zn) deficiency may reduce GnRH secretion that eventually leads to arrest of ovulation. It also suggests that Ca is involved in the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells, which contributes to the process of ovulation.[14]

DISCUSSION

Ashuwathi chooranam (AC) is a polyherbal formulation from the classical Siddha literature given for PCOS. The Siddha system is effective in treating various diseases of the liver, endocrinological disorders, skin diseases, allergic disorders, general fevers, chronic diseases, gynaecological disorders. Many Siddhars were more concentrated in treating various gynaecological disorders including Karpavayu. As stated in Siddha classical literature, AC helps to improve menstrual irregularities in reproductive age. However, scientific evaluation of Ashuwathi Chooranam (AC) for treatment of female infertility has not been attempted till date. In this regard, it would be interesting to examine the role of Ashuwathi Chooranam (AC) in management of Polycystic Ovarian Syndrome (PCOS) and its associated complications. The drugs that are collected and purified by removing the sand, dust particles, and are roasted the flower buds were removed and fried slightly.[15] Phytochemicals are present like alkaloids, carbohydrates, glycosides, phytosterols, proteins and amino acids. Carbohydrate and is appears to be important in many intracellular activities including infection by bacteria and viruses, communication among cells of lower eukaryotes, specific binding of sperm to egg; and recirculation of lymphocytes. In radical analysis presence of calcium which is necessary for the maturation of oocyte as well as in the resumption and progression of follicular development, menstrual regularity. The total ash value was discovered to be 2.64%w/w, water soluble ash value is 1.35%w/w, and acid insoluble ash is 0.185% w/w. The water soluble extractives and alcohol soluble extractives are 46.37%w/w and 46.92%w/w respectively. The pH value is 3.53. The FT-IR spectroscopy in the sample exhibited the presence of functional groups like alkyl halides, aromatics, alkenes, 1º amine, and nitriles. The SEM analysis of the sample displayed that the Nano particles are defined as particulate dispersion or solid particles with a size in the range of 100-800nm in diameter Nanoparticles have valuable properties that can be used to improve drug delivery. Complex drug delivery mechanisms are being developed, together with the ability to get drugs through cell membranes and into cell cytoplasm. The XRD analysis of AC concluded in which the major peaks are identified that range 48-75nm in association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. The

ICP-OES analysis in AC revealed that the heavy metals such as mercury, lead, cadmium and arsenic are present below the detectable limits that enhance the description of the AC drug study.

CONCLUSION

The contemporary study of polyherbal formulation AC concluded through various parameters such as physicochemical standards and safety evaluation that affirm the AYUSH standards for chooranam. The physicochemical and phytochemical studies revealed the compounds such as carbohydrates, phytosterols, proteins and amino acids. The activating protein kinase C, AGEs may impair insulin action, thereby perpetuating insulin resistance in PCOS. Instrumental analysis FT-IR study revealed the presence of functional groups alkyl halides, aromatics, alkenes, 1º amine, nitriles essential for the anti-infertility activity. SEM analysis of AC showed the nano size particles which have valuable properties that can be used to improve drug delivery. Worldwide there is proof for the increasing shift towards the use of traditional medicine.[16] Thus the results explored for providing therapeutic solutions for unfolding health problems by developing standards.

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