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# STANDARDIZATION OF SIDDHA POLYHERBAL FORMULATION "KOMOOTHIRA SILASATHU PARPAM"

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## ABSTRACT

**Aim:** The aim of the study was to assess the Physicochemical and Phytochemical analysis of Siddha herbal formulation "Komoothira Silasathu Parpam."(KSP)

**Materials and Method:** The Siddha Polyherbal formulation Komoothira Silasathu Parpam was prepared in accordance with good manufacturing practises (GMP) guidelines,], Physicochemical and phytochemical analyses were performed.

**Results and Discussion:** Physicochemical analysis shows the Loss on drying, Total cash value, Acid insoluble ash , Water soluble ash , Water soluble extraction , Alcohol soluble extraction. The microbial and biochemical analysis have done.

**Conclusion:** According to the results, it can be stated that the MNC has a comprehensive understanding of the presence of Physicochemical properties, Phytochemical components and has the ability to evaluate the quality profile of Komoothira Silasathu Parpam as the basis for the creation of a pharmaceutical product, which has been standardized.

**Keywords:** Siddha Herbal medicine, Physicochemical and Preliminary Phytochemical analysis, Komoothira Silasathu Parpam.

## INTRODUCTION:

Siddha medicine, one of the oldest traditional systems of healing from South India, has been practiced for thousands of years. This system emphasizes the balance of body, mind, and spirit, using a wide array of natural substances, including herbs, minerals, and animal products, to treat various ailments. With the growing global interest in traditional and alternative medicine, there is a pressing need for scientific validation and standardization of Siddha formulations to ensure their safety, efficacy, and quality.

This study focuses on the comprehensive physicochemical analysis of a specific Siddha formulation. The analysis includes organoleptic properties, physicochemical properties, microbial limits,

biochemical assays, and advanced instrumental techniques such as Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), X-ray Diffraction (XRD), and Scanning Electron Microscopy (SEM).

Organoleptic properties such as color, odor, taste, and texture are essential for preliminary quality assessment. Physicochemical parameters like pH, moisture content, ash values, and solubility provide insights into the stability and storage conditions of the formulation. Microbial limits are assessed to ensure the product's safety by preventing microbial contamination. Biochemical assays help in evaluating the presence and concentration of active constituents, contributing to the standardization of the formulation.

Advanced instrumental techniques offer detailed insights into the formulation’s composition and structure. ICP-OES is used for quantitative analysis of trace elements, ensuring the absence of toxic metals and verifying essential minerals. XRD helps in identifying crystalline phases and purity, while SEM provides high-resolution images of the surface morphology, revealing microstructural details.

The integration of traditional knowledge with modern scientific techniques in this study aims to provide a detailed physicochemical characterization of the Siddha formulation, thereby contributing to the growing body of evidence supporting the safety and efficacy of traditional medicines.

## METHODS AND MATERIAL:

### 1. ORGANOLEPTIC PROPERTIES

The organoleptic properties of KSP were evaluated by visual inspection and sensory perception. Characteristics such as color, odor, taste, and texture were recorded.

### 2. PHYSIOCHEMICAL ANALYSIS

**Ash test:**

A small amount of sample was mixed with the cobalt nitrate solution. A filter paper was soaked into the mixture. Then it was introduced into the Bunsen flame and ignited. If yellow colour flame appears, it reveals the presence of sodium.

**Determination of Total Ash:**

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450º until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug.

**Determination of Acid Insoluble Ash:**

Boil the ash obtained in above test for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

**Determination of Moisture Content (Loss on Drying):**

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowdered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not

more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

## 3. MICROBIOLOGICAL ANALYSIS

## EVALUATION OF TOTAL AEROBIC BACTERIAL COUNT:

### Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

### Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as $10^{-1}$ dilution. From this dilution, one ml was taken from that mixture is added to 9 ml, and designated as $10^{-2}$ dilution. The same procedure was repeated up to $10^{-4}$.

## ISOLATION OF TOTAL VIABLE AEROBIC MICROBIAL COUNT

### Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45ºC, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of $10^{-1}$ and $10^{-2}$. After inoculations, all the plates were incubated at 37ºC for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected.

### Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

## EVALUATION OF SPECIFIED MICROORGANISMS

### Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

### Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

### Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a waterbath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas.

Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

**Isolation & Identification of *Salmonella* sp.**
One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

**Primary Test**
From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

**Secondary test**
The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

**Isolation and Identification of *Pseudomonas aeruginosa***
From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

**Isolation and Identification of *Staphylococcus aureus***
From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

**4. BIO CHEMICAL ANALYSIS**
100mg of the drug was wighted accurately and placed into a clean beaker added a few drops of cons. hydrochloric acid and evaporated it well. After evaporated cooled the content and added a few drops of concentrated nitric acid and evaporated it well. After cooling the content add 20ml of distilled water and dissolved it well. Then it is transformed it to 100 ml of volumetric flask and made upto100 ml with distilled water. Mix well. Filtered it.Then it taken for analysis.

**QUALITATIVE ANALYSIS FOR BASIC RADICALS:**
**Test for Calcium:**
2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.
**Test for Iron (Ferric):**
The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.
**Test for Iron (Ferrous):**
The extract is treated with concentrated Nitric acid and ammonium thiocyanate solution. Formation of blood red colour indicates the presence of ferrous iron.
**Test for Zinc:**
The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the absence of zinc.

**QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:**

**Test for Sulphate:**

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

**Test for Chloride:**

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

**Test for Phosphate:**

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of no yellow precipitate indicates the absence of phosphate.

**Test for Carbonate:**

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

**Test for starch:**

The extract is added with weak iodine solution. Formation of no blue colour indicates the absence of starch.

**Test for albumin:**

The extract is treated with Esbach`s reagent. Formation of no yellow precipitate indicates the absence of albumin.

**Test for tannic acid:**

The extract is treated with ferric chloride. Formation of nobluish black precipitate indicates the absence of tannic acid.

**Test for unsaturation:**

The extract is treated with potassium permanganate solution. The discolourization of potassium permanganate indicates the presence of unsaturated compounds.

**Test for the reducing sugar:**

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes.

Any colour change indicates the presence of reducing sugar.

**Test for amino acid:**

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

5. **INSTRUMENTAL ANALYSIS**

**SCANNING ELECTRON MICROSCOPE (SEM)**

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross sections. The test sample powder was viewed under SEM (FEI Quanta 200 FEG, Berlin, Germany) to determine the morphology at ×100,000 magnification and the particle size at ×200,000 magnification. The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *Komoothira silasathu parpam*. SEM results of *Komoothira silasathu parpam* were represented in results section.

**INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETER (ICP-OES)**

Accurately weighed quantity of about 25 mg of the sample was taken in in the Teflon container. To this 6 ml of concentrated HNO3 and 3 ml of concentrated HCL was added and the contents were allowed to react for approximately 5 minutes prior to sealing the material the sample was thoroughly filtered and the difference in weight was calculated. The sample was preferably stored in plastic container to prevent loss of elements by absorption and quantitatively determined by PE optima 5200 DV ICPOES vessels. Followed by the microwave digestion. The vessels were then heated to the

required temperature. After digestion cooled and made upto a known volume in a standard flask with deionized water.

**X-RAY POWDER DIFFRACTION**

The XRD spectrum of test sample were analyzed using Bruker discover D8 X ray diffractometer. Cu K Alpha radiation was used for recording the spectra. The range of diffraction angle 10-70o operatinf at 30kV and 20 mA. The pattern was recorder from the angle 5 to 80 degree at a scanning rate of 3 degree/second. The XRD studies were carried out at IIT madras, Chennai, Tamil Nadu, India.

**RESULT:**

**PHYSICO CHEMICAL ANALYSIS**

The following characters have been noted in *KSP***.**

**Table No -1.** organoleptic character

| | |
|---|---|
| Colour in day light | White |
| Odour | Pleasant odour |
| Taste | Astringent |
| Appearance | Fine powder |
| Sense of Touch | Fine |

**Table No –2.** Physicochemical properties

| Sl. No | Parameters | Values | Normal Values |
|---|---|---|---|
| 1 | Water soluble ash | 7.65±0.011 | 7.85 % |
| 2 | Acid insoluble ash | 0.85±0.011 | 7.45% |
| 3 | Loss on drying at 70 C | 7.10±0.240 | 5 – 8 % |
| 4 | pH Analysis | 8.540 | > 7 % |

**MICROBIAL LIMIT TESTS**

**Table No: 3.** Results for Microbial limit test :

| **S.No** | **Microbes** | **Colony measurements** | **Normal limits** |
|---|---|---|---|
| 1. | Total viable aerobic count | 4 x$10^4$ col/g | 1 x$10^5$ col/g |
| 2. | Total fungal count | Nil | 1 x$10^4$ col/g |
| | **Test for specific pathogen** | | |
| 1. | *Salmonella sp* | Nil | Nil |
| 2. | *Staphylococcus aureas* | Nil | Nil |
| 3. | *E.coli* | Nil | Nil |
| 4. | *Pseudomonas aeruginosa* | Nil | Nil |

**BIO-CHEMICAL ANALYSIS OF *KOMOOTHIRA SILASATHU PARPAM***

**Table No:4.** Result of Preliminary Basic and Acidic Radicals studies

| S.No | Test | Inference |
|---|---|---|
| 1 | Test for calcium | Present |
| 2 | Test for sulphate | Present |
| 3 | Test for chloride | Present |
| 4 | Test for carbonate | Absent |
| 5 | Test for starch | Absent |
| 6 | Test for Iron ferric | Absent |
| 7 | Test for iron ferrous | Present |
| 8 | Test for phosphate | Absent |
| 9 | Test for Albumin | Absent |
| 10 | Test for Tannic acid | Absent |
| 11 | Test for unsaturation | Present |
| 12 | Test for the reducing sugar | Absent |
| 13 | Test for amino acid | Present |
| 14 | Test for zinc | Absent |

**INSTRUMENTAL ANALYSIS**
**SEM (SCANNING ELECTRON MICROSCOPE):**

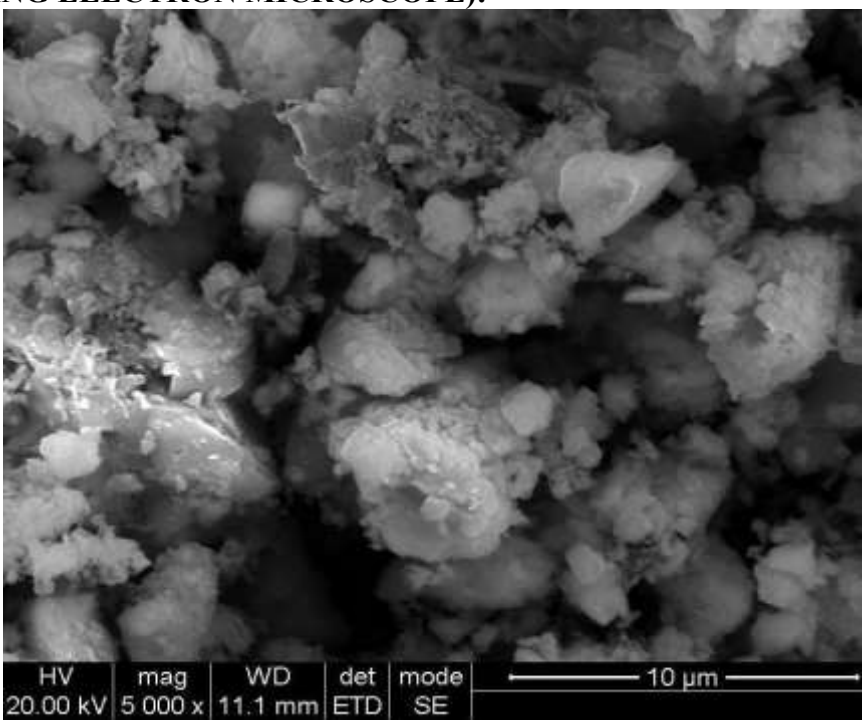



**Fig.No.1.** SEM Picture 30,000 Magnification.

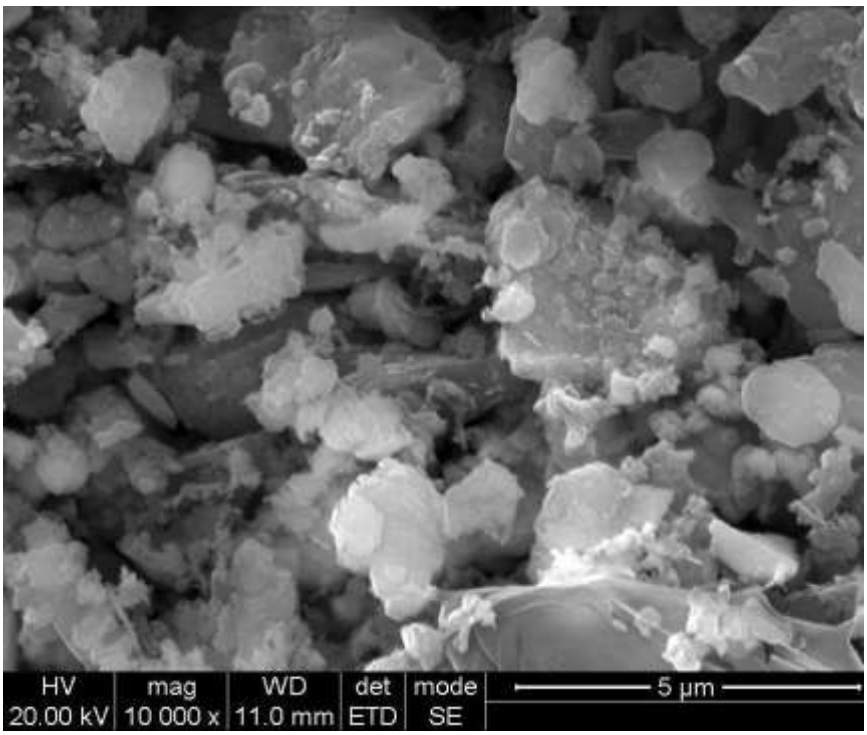



**Fig.No.2.** SEM Picture 80,000 Magnification.

**ICP-OES of *Komoothira Silasathu Parpam***

**Table No. 5**

| Sl.No | Elements | Wavelength (nm) | Concentration |
|---|---|---|---|
| 1 | Al | 396.152 | BDL |
| 2 | As | 188.979 | BDL |
| 3 | Ca | 315.807 | 821.170 mg/L |
| 4 | Cd | 228.802 | BDL |
| 5 | Cu | 327.393 | BDL |
| 6 | Fe | 238.204 | BDL |
| 7 | Hg | 253.652 | BDL |
| 8 | K | 766.491 | 03.071 mg/L |
| 9 | Mg | 285.213 | 01.104 mg/L |
| 10 | Na | 589.592 | BDL |
| 11 | Ni | 231.604 | BDL |
| 12 | Pb | 220.353 | BDL |
| 13 | P | 213.617 | 176.307 mg/L |
| 14 | S | 180.731 | 501.254 mg/L |

**BDL: Below Detectable Limit(Normal-1ppm)**

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

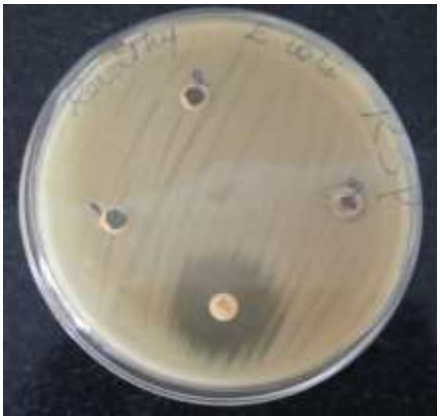
**Table No 6.**Toxic metals and the permissible limits

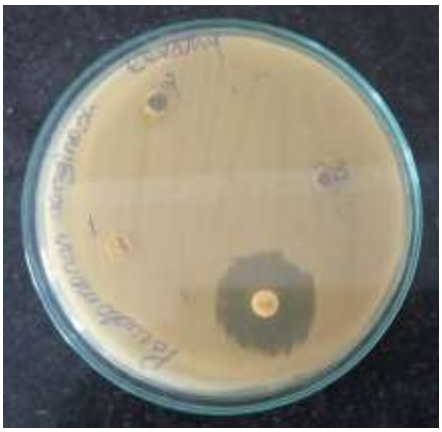
| Heavy metals | WHO & FDA limits |
|---|---|
| Pottasium (K) | 25.621 mg/L |
| Sodium (Na) | 24.310 mg/L |
| Phosporus (P) | 126.341 mg/L |

**Table 7.** Antimicrobial Activities of Various Samples by Agar Well Diffusion Method

| S.No. | Bacterial Pathogens | Zone of Inhibition (mm) | | | | Result |
|---|---|---|---|---|---|---|
| | | Positive C-30ontrol | Sample Load (µl) | | | |
| | | | 10 µl | 20 µl | 30 µl | |
| 1. | *Escherichia coli* | Chloramphenicol-30mcg(24 mm) | R | R | R | Resistant |
| 2. | *Klebsiella sps.* | Chloramphenicol-30mcg (24 mm) | R | R | R | Resistant |
| 3. | *Pseudomonas aeruginosa* | Chloramphenicol-30mcg (23 mm) | R | R | R | Resistant |
| 4. | *Staphylococcu aureus* | Chloramphenicol-30mcg (25 mm) | R | R | R | Resistant |
| 5. | *Candida sp* | Ketoconazole-30mcg (24mm) | R | R | R | Resistant |

**Fig No: 3** Microbiological Analysis of *KSP*

| S.No. | Bacterial Pathogens | Plates |
|---|---|---|
| 1. | *Escherichia coli* |  |

| | | |
|---|---|---|
| 2. | *Klebsiellasps.* |  |
| 3. | *Pseudomonas aeruginosa* |  |
| 4. | *Staphylococcu aureus* |  |
| 5. | *Candida sp* |  |

## DISCUSSION:

Standardizations of the drug is more essential to derive the efficacy and potency of the drug, which was analysed by the various methods. The results of physicochemical and biochemical analysis have been done and tabulated. Pharmacological activity and toxicological results of the drug were derived. The result reveals the effectiveness of the trial drug KSP has been proved by the following scientific parameters.

## ORGANOLEPTIC CHARACTER:

Organoleptic character indicates that the test drug KSP has the following characters, fine powder, White in colour, Pleasant odour, astringent in taste, fine to touch.

## LOSS ON DRYING:

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The percentage loss on drying 7.0% was within acceptable range (5%-8%), thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

## ASH VALUE:

### Determination of ash value:

The Ash Limit Tests are designed to measure the amount of the residual. Substances when a sample is ignited under the conditions specified in the individual monograph. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the drug. The total ash values of KSP were 68.2% .The value of total ash in the formulation is comparatively high. The value of total ash indicates that the inorganic contents of the formulation are below the limits. This signifies the ash value determination as an important parameter to standardize the herbal drugs.

### The Acid-Insoluble Ash :

Limit Test is designed to measure the amount of ash insoluble to diluted hydrochloric acid. Acid-insoluble ash value of the prepared formulation (22.08%) shows that a very small amount of the inorganic component is insoluble in acid.It indicates that adulteration of raw ingredients by substances, such as silica and rice husk, is very less, and a low acid-insoluble ash value may also affect the amount of the component absorbed in the gastrointestinal canal when taken orally.

### Determination of Alcohol-soluble and water-soluble extractive values:

Alcohol-soluble and water-soluble extractive values of ingredients and formulation are depicted in table 1 which shows 81.2% alcohol-soluble extractive value and 97.7% water-soluble extractive value of the formulation. Higher water-soluble extractive value implies that water is a better solvent of extraction for the formulation than ethanol. acid-insoluble ash, and water-soluble ash were found to be 68%,22.08%,71.2% respectively; the value of total ash indicates that the inorganic contents of the formulation are below the limits. The results of Alcoholic and water soluble extracts of the formulation show that alkaloids of the formulations are more soluble in water than alcohol and a higher water soluble extractive value of the formulation depicts that water is a better solvent of extraction for the formulation than alcohol.

**Microbial limits:**
Total viable aerobic counts within the normal level. Total fungal count within the normal level. Specific pathogens like Salmonella sp., Staphylococcus aureus, E.coli and Pseudomonas aeruginosa are Nil. Hence, the test drug is free from any microbial contamination.

**Biochemical analysis**
From the test for basic and acidic radicals studies shows the presence of
1. Calcium,
2. Sulphate,
3. Chloride.
4. Ferrous Iron.
5. Amino acid

**1. Calcium:**
It is an essential component of intracellular process that occur within insulin responsive tissues like skeletal muscle and adipose tissue. A very narrow range of calcium concentration is needed for optimal insulin mediated functioning.
Concentrated levels of calcium out of optimal range may contribute to peripheral insulin resistance.

**2. Sulphates:**
Sulphate has anti bacterial activity and it is one of the macronutrient of cells. Sulphate important role for the anti – microbial activity.
It is needed to start the cascade of digestive enzymes released from the pancreas. Without proteases, lipases and amylases, food is not digested efficiently.

**3. Chloride :**
Chloride regulates the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids.

**4. Ferrous Iron :**
Iron is easily soluble and readily absorbed from intestine and involved.

**5. Amino acid:**
In diabetes mellitus atherosclerotic changes occur earlier ,Amino acids, like arginine, help the arteries of the body retain elasticity, which prevents them from stretching out and allowing fluid to collect. They also help support the expansion and contraction of the arteries with each heartbeat.
The tissues in the body -- muscles, skin, connective tissues, etc. -- need amino acids for repair when injured or damaged. They're especially beneficial after exercise or difficult training, when the muscles can tear. Taking amino acids like arginine after exercise can help the muscles recover and heal properly and more quickly.

**MICROBIOLOGICAL ANALYSIS**
Both Gram positive and Gram negative bacteria *E.coli, Klebsiella pneumoniae* and *Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi* were found to be resistance when compared to the standard drug Gentamycin (Broad spectrum) . Hence the trail drug *KSP* has not anti microbial activity.

**INSTRUMENTAL ANALYSIS**
**SEM:**
SEM analysis of the KSP shows the particle size varies between 5µm to 10µm. The surfaces of the sample grains is uniformly arranged in agglomerates.

Smaller sized particles enhance the absorption and bio availability resulting efficacy of the drug will be increased.

The microparticles

- Increase drug therapeutic efficacy.
- Increase bioavailability.
- Reduces side effect.

In SEM analysis the micro particle present in the test drug results in a better bioavailability and facilitates absorption. So very minimal quantity of the medicine is enough to treat the disease, resulting better efficacy of the drug. Larger particles could not enter in to the target cell because of their size, and it will requires extra digestive and absorptive load, resulting they easily cleared from the blood

**ICP-OES**

ICP-OES reveals high concentration of Sulphate in KSP (0.50120 mg/L). It also has physiologically important minerals like Calcium, Phosphorus, megnesium, sulfer, sodium and Potassium. In KSP , the heavy metals like Arsenic, Mercury, Lead, Cadmium and trace element like nikkal were below detectable level. This reveals the safety of the drug and it has free from toxic substances and has no side effects.

1. Sodium:

Sodium regulates the acid-base balance of the body fluids. Sodium is required for the maintenance of osmotic pressure of the body fluids. It is necessary for the normal muscle irritability and permeability of cells. Sodium maintains extracellular osmotic pressure.

2. Potassium:

Potassium is required for the regulation of acid-base balance and water balance of the body fluids. Potassium maintains intracellular osmotic pressure. Potassium is required for the transmission of nerve impulse.

3. Calcium:

Calcium influences the membrane structure and transport of water and several ions across it. The release of certain hormones (insulin, PIH, calcitonin) from the endocrine glands is facilitated by ca2+

4. Sulphur:

Early research suggests that drinking water from a sulfurous spring three times daily for 4 weeks reduces total cholesterol,low- density lipoprotein (LDL or Bad) cholestrol, and triglyceride levels. However ,it's not clear from this study alone if sulfur might reduce cholestrol.

5. Magnesium:

Eating a diet with more magnesium is linked with a reduced risk of developing diabetes in adults and overweight children. research on the effects of magnesium for people with existing type 2 diabetes shows conflicting results.

6. Phosphorus :

Phosphorus is important for energy transfer in cells as part of ATP and is found in many other biological important molecules. Thus it relieves fatigue.

**X- RAY POWDER DIFFRACTION:**

These XRD fingerprints shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo mineral medicines. Mordern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature. The different peaks shows the presence of minerals in the sample.

**CONCLUSION:**

The comprehensive physicochemical analysis of the Siddha formulation, KSP, underscores the importance of standardization in ensuring the efficacy and potency of traditional medicines. The detailed evaluation included organoleptic properties, physicochemical parameters, microbial limits,

biochemical assays, and advanced instrumental techniques such as ICP-OES, XRD, and SEM. The organoleptic characterization revealed that KSP is a fine, white powder with a pleasant odor and astringent taste, indicating its consistency and quality. The low moisture content (7.0%) observed in the loss on drying test suggests excellent stability and an extended shelf life, as higher moisture levels can lead to deterioration and microbial growth. The ash value analysis indicated that the total ash content of KSP was 68.2%, with an acid-insoluble ash value of 22.08%, suggesting minimal adulteration and high purity of the raw materials used. The high water-soluble extractive value (97.7%) compared to the alcohol-soluble extractive value (81.2%) demonstrated that water is a more efficient solvent for extracting the active constituents of KSP. Microbial limit tests confirmed that KSP is free from pathogenic contamination, making it safe for consumption. Biochemical assays detected essential components such as calcium, sulphate, chloride, ferrous iron, and amino acids, each playing a vital role in various physiological processes and contributing to the therapeutic efficacy of KSP. Instrumental analyses provided further insights into the formulation. SEM analysis revealed that the particle size of KSP varies between 5µm and 10µm, which enhances bioavailability and therapeutic efficacy. ICP-OES confirmed the presence of essential minerals and the absence of toxic heavy metals, ensuring the formulation's safety. XRD analysis confirmed the presence of specific mineral components, aiding in quality control and standardization.

Overall, the findings of this study validate the safety, stability, and therapeutic potential of the Siddha formulation KSP, affirming its use in traditional medicine. The integration of traditional knowledge with modern scientific methods provides a robust framework for the standardization and validation of herbal formulations, ensuring their effective and safe use in contemporary healthcare.

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