PHYSICO CHEMICAL STANDARDIZATION OF SIDDHA PREPARATION KASA SWASA KSHAYA KULANTHAGA MATHIRAI

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ABSTRACT
Owing to longstanding and time proven uses of herbo-mineral drug along with their safety margins, World Health Organisation (WHO) has taken necessary steps to ensure quality control with modern techniques and application of suitable standards for this purpose. The pharmacopoeias of different countries include monographs indicating quality parameters and standards for various herbal drugs and also their products. For the purpose of quality control of herbal drugs, W.H.O. has prepared accordingly the guidelines. The objectives put forth are provisions for recommended general test methods and also the general limits for contaminants for herbal drugs. In the literature of Siddha Research Pharmacopeia, there is a preparation, called “Kasa swasa kshaya kulanthaga mathirai”, which is exclusively indicated for Eraippu noi (Bronchial asthma). The quality assessment of this herbo-mineral formulation is of paramount importance in order to justify their acceptability in modern system of medicine. Standardization of herbo-mineral formulations is essential in order to assess of quality drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical, and standardization.

KEYWORDS: siddha, standardization, Kasa swasa kshaya kulanthaga mathirai.

INTRODUCTION
Nearly 80% of world populations rely on Herbal and herbo-mineral medicines, particularly in developing countries for primary health care needs. The herbo-mineral drugs described in siddha system have been the basic treatment of various human diseases. Standardization of
herbo-mineral formulations is essential in order to assess the quality of the drugs, based on the concentration of their active principles, physical, chemical, phyto-chemical and standardization. The quality assessment of this herbo-mineral formulation is of paramount importance in order to justify their acceptability in modern system of medicine. World Health Organization (WHO) encourage, recommend and promotes traditional remedies in natural health care programmes, because safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of herbo-mineral products by using modern techniques and applying suitable standards.

MATERIALS AND METHODS

Preparation of the Test Drug

*Kasa swasa kshaya kulanthaga mathirai* has been selected from the siddha literature, “Siddha research pharmacopeia”.

Collection of the Raw Drugs

1) *Jathilingam* (Red sulphide of mercury)

Raw mercury was bought from the Gobala aasan store, Nagercoil. The raw material was identified and authenticated by PG Gunapadam department experts, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.

2) *Manosilai* (Arsenic disulphide)

It was bought from the TAMCOL sales counter, Palayamkottai. The raw material was identified and authenticated by PG Gunapadam department experts, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.

3) *Haridharam* (Arsenic trisulphate)

It was bought from the Gobala aasan store, Nagercoil. The raw material was identified and authenticated by PG Gunapadam department experts, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.

4) *Vengaram* (Borax):

It was bought from the TAMPCOL sales counter, Palayamkottai. The raw material was identified and authenticated by PG Gunapadam department experts, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.
5) Thrikadugu, Karunabi
It was bought from the TAMPCOL sales counter, Palayamkottai. Thrikadugu, and karunabi was taken and purification process was the raw material was identified and authenticated by botanist and PG Gunapadam department experts, Govt. Siddha Medical College, Palayamkottai, Tamilnadu.

6) Kuppai meni (Acalypha indica leaf)
It was collected from Govt.Siddha Medical College, Botanical Garden, Palayamkottai. The raw material was identified and authenticated by botanist and PG Gunapadam department experts, Govt. Siddha Medical College, Palayamkottai, Tamilnadu.

Ingredients of Kasa swasa kshaya kulanthaga mathirai
1. Jathilingam (Red sulphide of mercury)
2. Manosilai (Arsenic disulphide)
3. Thrikadugu
   a) Sukku (Zingiber officinale)
   b) Milagu (Piper nigrum)
   c) Thippili (Piper longum)
4. Haridharam (Arsenic trisulphate)
5. Vengaram (Borax)
6. Karunabi (Aconitum ferox)
7. Kuppaimeni juice (Acalypha indica)

Purification of the Ingredients
1. Thalagam
Take 1 Balam (35gm) of Thalagam, put in between the heap of limestone and, treated with the urine of donkey for ten times. Take that Thalgam wash in water and dried.

2. Manosilai
Manosilai is ground with for three hrs in any one of the following viz. Ginger juice, lemon juice, cow’s milk. Then it is dried.

3. Venkaram
venkaram fried until it lost its humidity.
4. Jathi Lingam
Equal quantity of Lemon juice, Cow’s milk and juice of Acalypha Indica taken and heated with Jathilingam until the mixture dried, then washed with water and dried.

5. Karunabi
Karunabi soaked in the cow’s urine for 24 hrs.

6. Thirikadugu
1. Sukku - removed the outer hard layer.
2. Milagu - Roasted it until pungent smell comes.
3. Thippili - Roasted it until pungent smell comes.

7. Kuppaimeni leaf
Cleaned with sterile dry cloth, removed waste parts in the leaf.

Preparation Process
The purified drug has to be made into powder and rubbed with kuppaimeni juice for 3hrs, make it a pepper sized pills(65mg) and dried in the shade then kept in airtight container and labelled.

Physico Chemical Analysis
Testing Physical characterization of Sample

Colour Examination
Ten tablets were taken into watch glasses and positioned against white back ground in white tube light. Its colour was observed by naked eye and wrote in results.

Odour examination
Ten numbers of tablets were smelled individually. The time interval among two smelling was kept two minutes to overturn the effect of previous smelling. Odour of KSK tablet was noted in results table.

Size examination
The diameter of ten tablets was measured by Vernier caliper. The mean value of diameter was noted. (Lohar DR-Protocol for testing ASU drugs).
Weight Variation Test

It was carried out to make sure that, each number of tablets contains the proper amount of drug. The test was carried out by weighing the 20 tablets individually using analytical balance, then the average weight was calculated, and comparing the individual tablet weights to the average. (Sukalyan Sengupta 1988). The percentage of weight variation is calculated by using this formula.

\[
\% \text{ of wt. variation} = \frac{\text{Individual wt.} - \text{Average wt.}}{\text{Average wt.}} \times 100
\]

Table 1: Weight Variation limits of Tablets (IP)

<table>
<thead>
<tr>
<th>Average weight of tablets</th>
<th>Maximum percentage of weight difference allowed</th>
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<tbody>
<tr>
<td>80 mg or less</td>
<td>±10.0</td>
</tr>
<tr>
<td>Between 80 mg and 250 mg</td>
<td>±7.5</td>
</tr>
<tr>
<td>250 mg and more</td>
<td>±5.0</td>
</tr>
</tbody>
</table>

Accepted tablet

Weight Variation limits of the sample not more than two tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the above table.

Suspected tablet

Suspected tablet variation was not more than six tablets are outside the percentage limit and no tablet differs by more than two times the percentage limit according to the table.

Rejected tablets

When a tablet weight variation test results showed rejected tablets mean in that test sample one tablet differs by more than two times the percentage limit according to the table or More than six tablets are outside the percentage limit. (Sukalyan Sengupta, 1988).

Solubility

A pinch of the sample was taken in a dry test tube and shaken well with distilled water. A little amount of the sample is shaken well with con HCl and then Con.H2SO4. Test sample Solubility was observed.

PH Value

Potentiometrically pH value was determined by a glass electrode and a suitable pH meter. The pH of the KSK tablet was written in results column.
Total Ash
Two grams of grounded air dried powder of *Kasaswa kshaya kulanthaga mathirai* was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450°C until it was white. The sample was cooled in a desiccators and weighed. The percentage of total ash was calculated with reference to air-dried drug.

Acid Insoluble Ash
The ash was boiled with 25ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited cooled in a desiccators, and weighed. The percentage of acid insoluble ash calculated with reference to the air-dried drug.

Loss of drying (Indian Pharmacopoiea, 1996)
Loss on drying is the loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. A glass stopper, shallow weighing bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in a desiccators before weighing.

Alcohol Soluble Extractive
Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Water Soluble Extractive
Proceed as directed for the determination of Alcohol-soluble extractive, using chloroform water instead of ethanol.
Tablet Disintegration Test
Each KSK tablet was placed in each of the six tubes of the basket present in the disintegration apparatus. The apparatus was operated by using water as the immersion fluid maintained at 35-39 °C. At the end of the 30 min, the basket is lifted from the fluid and the state of the tablet is observed. The disintegration time of KSKM was recorded (Loher.Dr).

Determination of Total Aerobic Microbial Count
Dissolve 10g or dilute 10ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution pH 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml the same medium. If necessary, adjust the pH to about 7.

Membrane filtration
Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Sterilise and assemble the filtration apparatus described under tests for sterility.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. For fatty substances add to the liquid polysorbate 20 or polysorbate 80. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, to the surface of a plate of Sabouraud dextrose agar with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30° to 35° in the test for bacteria and 20° to 25° in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

Plate Count: For Bacteria
Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45°.
Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30° to 35° for 5 days, unless a more reliable count is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For Fungi
Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 20° to 25° for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

Thin Layer Chromatography
Thin-layer chromatography (TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products. TLC is a simple, quick and inexpensive procedure that gives how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound (preferably both run on the same TLC plate). Chromatography works on the principle that different compounds will have different solubilities and adsorption to the two phases between which they are to be partitioned. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed on to the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

High Performance Thin Layer Chromatography
HPTLC is a major advancement of TLC principle requiring shorter time and better resolution. The basic difference between conventional TLC and HPTLC is only in particle and pore size of the sorbents.
The plates are similar to conventional TLC plates. Layers of HPTLC are available in the form of precoats. Silica gel of very fine particle size is widely used as sorbent in HPTLC. The use of smaller particle size helps in greater resolution and sensitivity. About 3-6 cm solvent front migration is sufficient to effect proper separation.

**PROCEDURE**

**Sample preparation**

About 1gm of sample was macerated with 0.1N Sulphuric acid and shaken vigorously for 15 minutes. Then it was filtered, to the filtrate add ammonia solution to pH 10, then the filtrate was extracted with chloroform. The chloroform layer was evaporated to dryness. The dried residue was made up to known volume and is used for TLC analysis.

**Stationary phase**: Silica Gel 60 F<sub>254</sub>

**Mobile phase**: Toluene: Ethyl acetate: Diethylamine(70:20:10)

**Procedure**: Applied 10µl, 20 µl of test solution on a precoated Silica gel 60 F<sub>254</sub> HPTLC plate (E.Merck) of uniform thickness 0.2mm using Linomat5 sample applicator. Developed the plate in the solvent system to a distance of 8 cm. Observed the plate under UV light at 254nm & 366nm using CAMAG REPROSTAR 3.

**Wave length**: 254nm & 366nm

**Evaluation**: Test related to Alkaloids.

**RESULTS AND DISCUSSION**

**Physico Chemical Analysis of Kasa swasa kshaya kulanthaga mathirai**

**Table No. 1**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Organoletic characters</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Colour</td>
<td>Dull Brown</td>
</tr>
<tr>
<td></td>
<td>b. Odour</td>
<td>Pleasant odour</td>
</tr>
<tr>
<td></td>
<td>c. Sense of touch</td>
<td>Hard</td>
</tr>
<tr>
<td></td>
<td>d. Appearance</td>
<td>Rounded</td>
</tr>
<tr>
<td></td>
<td>e. Taste</td>
<td>Light sour, Bitter</td>
</tr>
<tr>
<td>2</td>
<td>Average Net Weight</td>
<td>0.1843g</td>
</tr>
<tr>
<td>3</td>
<td>Uniformity of weight</td>
<td>72.49% to 124.74%</td>
</tr>
<tr>
<td>4</td>
<td>pH</td>
<td>7.1-7.5</td>
</tr>
<tr>
<td>5</td>
<td>Disintegration Time</td>
<td>25 min 39 sec</td>
</tr>
<tr>
<td>6</td>
<td>Loss on Drying@105°C</td>
<td>6.0714% w/w</td>
</tr>
<tr>
<td>7</td>
<td>Total Ash</td>
<td>41.0188% w/w</td>
</tr>
</tbody>
</table>
Kasa swasa kshaya kulanthaga mathirai passed the Uniformity weight test for tablets. The uniformity test resembles uniformed distribution of this tablet helps good absorption and distribution. According to AYUSH guidelines the disintegration time of Kasa swasa kshaya kulanthaga mathirai was comes under than the guided value. (with in 30 min). This implies a reasonable disintegration time, thereby a better absorbability and solubility is achieved. Solubility is one of the important parameters to attain desired concentration of drug in systemic circulation the required pharmacological response. The oral bioavailability depends on several factors including aqueous solubility, drug permeability etc. Solubility of the drug is highly soluble in water medium. So it is easily absorbable in the gut. Alkaline balance is very important for our body’s health. pH of the drug represents alkaline. Alkaline nature of this drug increases its chemotherapeutic power. (Gerry K et al., 2011).

### Determination of loss on drying

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 6.071% 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.

### 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 KSKM were 41.0188%.The value of total ash in the formulation is comparatively high. The value of total ash indicates that the inorganic contents

<table>
<thead>
<tr>
<th>8</th>
<th>Acid Insoluble ash</th>
<th>5.4155%w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Water soluble Extractive</td>
<td>43.2154%w/w</td>
</tr>
<tr>
<td>10</td>
<td>Alcohol Soluble Extractive</td>
<td>11.3785% w/w</td>
</tr>
<tr>
<td>11</td>
<td>Total Viable aerobic count</td>
<td>1.1*10^4col/g</td>
</tr>
<tr>
<td>12</td>
<td>Total Enterobacteriaceae</td>
<td>3*10^1 col/g</td>
</tr>
<tr>
<td>13</td>
<td>Total fungal count</td>
<td>1.4*10^1 col/g</td>
</tr>
<tr>
<td>14</td>
<td>Test for specific pathogens</td>
<td></td>
</tr>
</tbody>
</table>
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 (5.4155%) 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 which shows 11.3785% alcohol-soluble extractive value and 43.2157% 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. Total ash, acid-insoluble ash, and water-soluble ash were found to be 41.0188%, 5.4155%, 43.2154% 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 43.2154% of the formulation depicts that water is a better solvent of extraction for the formulation than alcohol.

Total viable aerobic counts within the normal level. (Normal-1x10^5 col./g.)

Total Enterobacteriaceae counts within the normal level. (Normal-1x10^3 col./g)

Total fungal count within the normal level. (Normal-1x10^4 col./g)

Specific pathogens Salmonella sp., Staphylococcus aureus, E.coli, Pseudomonas aeruginosa are Nil.
HPTLC and TLC Result

![TLC Result Image]

**Fig No. 1: TLC Result**

**HPTLC FINGERPRINTING PROFILE OF KSK (Based on Alkaloids)**

**PHOTO DOCUMENTATION UNDER UV**

**AT 254nm**

**AT 366nm**

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**TLC DETAILS**

Track 1-10µl of Sample

Track 2-20µl of Sample

Under UV 254 nm and 366 nm test related to alkaloids, it shows major spots short at Rf 0.67 (Greenish violet), 0.71(Green), 0.81 (Light green) and long at Rf 0.67(Orangish blue), 0.71(Yellow), 0.81(Green).3 major compounds are found. TLC of KSK tablet various Rf values was observed. The variation of Rf values indicated the presence of alkaloids, phenols, tannin and some unknown compounds in this drug.
HPTLC analysis the solvent front was standardized as chloroform:Ethyl acetate 70:20:10. The extract was fractionized. There were 6 compounds clearly visible at 254nm and 366nm of the test drug KSK. HPTLC plate showed the colour bands, it indicates the presence of alkaloids. Even though KSK mathirai is a herbo-mineral medicine, during the pharmaceutical processing KSKM processed with herbal juices. So that the HPTLC analysis showed the presence of alkaloids.

ALKALOIDS
Alkaloids possess antispasmodic, analgesic, bactericidal effects. Alkaloids are the active principles producing many essential effects in protecting the body.

CONCLUSION
In Physico-chemical analysis shows, all the parameters are within acceptable range. So it can be stored for a long period and would not easily be attacked by microbes. Water soluble extract shows, water is a better solvent of extraction for the formulation. Disintegration time shows, quickly disintegrate with water. Water can be used as adjuvant for KSK mathirai. TLC&HPTLC result shows variation of Rf values indicated the presence of alkaloids, phenols, tannin, and some unknown compounds in this drug. Alkaloids possess antispasmodic, analgesic, bactericidal effects.

REFERENCES
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