Pharmaceutical Standardization of Siddha Poly herbal formulation

“Kuruthi Azhaluku Chooranam”

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Abstract- Hyper tension is a common disease nowadays and it is related in siddha system of medicine as Kuruthi Azhal Noi. Various modern drugs are recommended for hypertension, but long-term use of these drugs produce untoward effects like cardiac failure. Kuruthi Azhaluku Chooranam is a poly-herbal Siddha formulation that is indicated for hypertension. The ingredients present in this drug are known to have good effect in controlling kuruthi azhal noi. The standardization of this poly herbal formulation is undertaken and the drug is analyzed for its phytochemical, physico-chemical and biochemical analysis. Moreover, this formulation is investigated for presence of pesticide residue, aflatoxin, and heavy metal and microbial load analysis as per PLIM guidelines. The existing results were within normal limits in this study, which is precisely expressed in this paper.

Key words: Hypertension, Kuruthi azhal noi, Kuruthi azhaluku chooranam, Standardization, Phytochemical analysis.

1. INTRODUCTION

International Journal of Advanced Medical and Health Research hypertension is termed as Kuruthyazhal in the Siddha system of medicine. Hypertension is a condition in which the blood vessels have constantly increased pressure, placing them under increased stress. The increased pressure makes the heart pump hard. Normal adult blood pressure is 120/90 mmHg and ≥140/90 mmHg is considered to be hypertension. Many hypertension patients experienced no symptoms at all. This is why it is called as the “Silent killer” [1]. Many formulations were mentioned in various Siddha literatures. One of those medicine is Kuruthi Azhaluku Chooranam (KAC) reported in Yugi vaithiya sinthamani perunool 800 part 1,pg.no-252 indicated for Kuruthiyazhal [2]. It has 9 ingredients, which includes Koogai Neeru (Maranta arundinacea), Thippili (Piper longum), Athimathuram (Glycyrrhiza glabra), Seenthil sarkkarai (Tinospora cordifolia), Sadamanjil (Nardostachys grandiflora), Vaalmilagu (Piper cubeba), Paerichankaai (Phonex dactilifera), Seeragam (Cuminum cyminum), Sarkkarai (Saccharum officinarum). Any drug before human use should be standardized scientifically. The aim of this study is to validate the standardization of KAC through qualitative analysis as per PLIM guidelines.

2. MATERIALS AND METHODS:

Selection of drug
KAC was mentioned to treat hypertension in Yugi munivar Vaithiya Sinthamani Perunool 800- Part I(Pg.no- 252).

Ingredients of “KAC”

![Fig.no:01 Ingredients of KAC](image)

(a).Maranta arundinacea
(Koogai neeru)  
(b).Piper longum
(Thippili)
Table no.1. Composition of KAC

(c). *Glycyrrhiza glabra* (Athimadhum)

(d). *Tinospora cordifolia* (Seenthil sarkkarai)

(e). *Nardostachys grandiflora* (Sadaamanjil)

(f). *Piper cubeba* (Vaal milagu)

(g). *Phoenix dactylifera* (Paereechankai)

(h). *Cuminum cyminum* (Seerakam)

(i). *Saccharum officinarum* (Sarkkarai)
Collection of the Drug
KAC contains 9 ingredients, such as Koogai neeru (Maranta arundinacea), Thippili (Piper longum), Athimadharum (Glycyrrhiza glabra), Seenthil sarkkarai (Tinospora cordifolia), Sadamanjil (Nardostachys grandiflora), Vaal milagu (Piper cubeba), Paereechankai (Phonex dactilifera), Seeragam (Cuminum cyminum), Sarkkarai (Saccharum officinarum). They were acquired from undisputed stores.

Recognition and verification of Drugs
Each raw drug was identified and authenticated by the Botany department in Government Siddha Medical college Chennai. Specimen sample of individual raw materials were stored in the Gunapadam Department, Government Siddha Medical College, Chennai and labeled as 1116-[PGG/320220100510/GSMC-CH/2020-2023.}

Purification Process
Each drug of the KAC was purified as mentioned in Sikicha raththana dheepam [3].

Method of Preparation
Equal amount of fruit of Thippili (P. longum) and Vaal milagu (P. cubeba), roots of Athimadhuram (G. glabra) and Sadamanjil (N. grandiflora), dry unripe fruit of Paereechankai (Phonex dactilifera) and seeds of Seeragam (C. cyminum), Sarkkarai (S. officinarum) were added, and the total amount of powder was calculated. Half amount of ground sugar (S. officinarum) was added to the total amount of Chooranam, filtered by mesh size 85 as a fine grain. The above powders were blended well together. Finally, Chooranam was stored in an air-tight container for further analysis.

Dose: Mooviral alavu (800-1000 mg), 2-4 times a day
Indication: Kuruthi azhal (Hypertension)

Organoleptic nature
The Organoleptic characters such as nature, state, odour, feel, physical appearance, flow, property, and taste were recognized.

Qualitative Analysis Investigation
As per PLIM guidelines, Qualitative analysis was executed. Physicochemical and Phytochemical analysis were completed at, The Tamil Nadu Dr. M.G.R Medical University, Guindy, Chennai. Biochemical analysis, heavy metal analysis, sterility testing, high performance thin layer chromatography, Pesticide residue, specific pathogen testing, Aflatoxin were performed at, Noble research institute, Perambur, Chennai.

Physicochemical Evaluation [4,5]
Loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, and water-soluble extractive, pH were discovered.

Phytochemical Evaluation [6]
In a Phytochemical evaluation, tests for alkaloids, saponins, tannins, glycosides, flavonoids, phenols, steroids, Diterpenoids, cyanins and carbohydrates were done.

Biochemical Analysis of Basic and Acidic Radicals [7]
Biochemical analysis was done for detection of sulfates, phosphates, carbonates, chlorides, sulphides, fluride and oxalate, borates, nitrates.

Heavy Metal Analysis by [AAS] Atomic Absorption Spectroscopy [8]
Heavy metals such as Cadmium, Lead, Mercury, Arsenic were tested.

Sterility Test [9]
Identification of the organism was done by the pour plate method. The Colony Forming Unit was counted.

Individual Pathogen Testing [10]
A specific medium such as EMB agar, Deoxycholate agar, Mannitol salt agar, and cetrimide agar were used for precise identification of individual pathogen.

Pesticide Residue Analysis [11,12]
Pesticide residues such as glyphosate, inorganic aluminium phosphide, calcium arsenate were tested.

Aflatoxin Assay [13]
Tests were done for Aflatoxin B1, B2, G1 and G2.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>NAME OF THE DRUG</th>
<th>BOTANICAL NAME</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Koogai neeru</td>
<td>Maranta arundinacea</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>2</td>
<td>Thippili</td>
<td>Piper longum</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>3</td>
<td>Athimadharum</td>
<td>Glycyrrhiza glabra</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>4</td>
<td>Seenthil sarkkarai</td>
<td>Tinospora cordifolia</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>5</td>
<td>Sadamanjil</td>
<td>Nardostachys grandiflora</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>6</td>
<td>Vaal milagu</td>
<td>Piper cubeba</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>7</td>
<td>Paereechankai</td>
<td>Phonex dactilifera</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>8</td>
<td>Seeragam</td>
<td>Cuminum cyminum</td>
<td>1 palam [35gms]</td>
</tr>
<tr>
<td>9</td>
<td>Sarkkarai</td>
<td>Saccharum officinarum</td>
<td>Half amount for the total amount of chooranam</td>
</tr>
</tbody>
</table>
3. RESULTS

ORGANOLEPTIC CHARACTER OF KAC

Table no 2. Organoleptic character

<table>
<thead>
<tr>
<th>S.No</th>
<th>PARAMETER</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>State</td>
<td>Solid</td>
</tr>
<tr>
<td>2</td>
<td>Nature</td>
<td>Fine</td>
</tr>
<tr>
<td>3</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>4</td>
<td>Touch</td>
<td>Soft</td>
</tr>
<tr>
<td>5</td>
<td>Flow property</td>
<td>Free flowing</td>
</tr>
<tr>
<td>6</td>
<td>Appearance</td>
<td>Brownish</td>
</tr>
<tr>
<td>7</td>
<td>Taste</td>
<td>Bitter</td>
</tr>
</tbody>
</table>

PHYSICOCHEMICAL CHARACTERISTICS

Table no.3 Physico-chemical Analysis – KAC

<table>
<thead>
<tr>
<th>S.No</th>
<th>PARAMETERS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Loss on drying</td>
<td>6.98%</td>
</tr>
<tr>
<td>2</td>
<td>Total ash value</td>
<td>5.37%</td>
</tr>
<tr>
<td>3</td>
<td>Acid insoluble ash</td>
<td>7.60%</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble ash</td>
<td>1.25%</td>
</tr>
<tr>
<td>5</td>
<td>Water soluble extraction</td>
<td>24.007%</td>
</tr>
<tr>
<td>6</td>
<td>Alcohol soluble extraction</td>
<td>7.62%</td>
</tr>
</tbody>
</table>

PARTICLE SIZE DETERMINATION

Figure no.3 Particle Size microscopic observation of KAC

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 82.17± 17.23µm [14].

RESULTS OF SOLUBILITY PROFILE

Table no.4 Solubility Profile – KAC

<table>
<thead>
<tr>
<th>S.No</th>
<th>Solvent Used</th>
<th>Solubility / Dispersibility</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>S.No</th>
<th>PHYTOCHEMICALS</th>
<th>H₂O EXTRACT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Diterpenes</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Gum &amp; Mucilage</td>
<td>Present</td>
</tr>
</tbody>
</table>

Fig No.4 Preliminary Phytochemical Screening

HPTLC ANALYSIS OF KAC
High performance thin layer chromatography analysis reveals three major peaks correlating with four variable phytocomponents present. The Retention frequency value of the peaks were from 0.00 to 0.45.
BIOCHEMICAL ANALYSIS OF KAC

Table no. 7 Test for acid radicles

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Inference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sulfates</td>
<td>Presence e of white precipitate</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Phosphates</td>
<td>Presence of yellow precipitate</td>
<td>Positive</td>
</tr>
</tbody>
</table>

HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROMETRY

There were absences of heavy metals such as lead, arsenic, cadmium, mercury.

Table 8: AAS Interpretation - KAC

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the heavy metal</th>
<th>Absorption max A Max</th>
<th>Result analysis</th>
<th>Maximum limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lead</td>
<td>217.0 nm</td>
<td>BDL</td>
<td>10 ppm</td>
</tr>
<tr>
<td>2</td>
<td>Arsenic</td>
<td>193.7 nm</td>
<td>BDL</td>
<td>3 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Cadmium</td>
<td>228.8 nm</td>
<td>BDL</td>
<td>0.3 ppm</td>
</tr>
<tr>
<td>4</td>
<td>Mercury</td>
<td>253.7 nm</td>
<td>BDL</td>
<td>1 ppm</td>
</tr>
</tbody>
</table>

BDL - Below Detection Limit

POUR PLATING METHOD FOR STERILITY TEST

Table 9. Pour Plating Method – For Sterility Test

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Result</th>
<th>Specification</th>
<th>As per AYUSH/WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacterial Count</td>
<td>Absent</td>
<td>NMT 10^6 CFU/g</td>
<td>As per AYUSH specification</td>
</tr>
</tbody>
</table>
PATHOGEN (SPECIFIC) TESTING
The sample did not reveal any growth or colonies in the inoculated plates.

Table no. 10 Pathogen (Specific) - KAC

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism</th>
<th>Specification</th>
<th>Result</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>Absent</td>
<td>Absent</td>
<td>As per AYUSH specification</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Staphylococcus Aureus</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas Aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
<td></td>
</tr>
</tbody>
</table>

Fig no 8. E-coli (EC) specific medium

Fig no 9. Salmonella (SA) specific medium

Fig no 10. Staphylococcus Aureus (ST) specific medium
Fig no 11. Pseudomonas Aeruginosa (PS) specific medium

PESTICIDE RESIDUE ANALYSIS OF KAC

Pesticide residaues like Organochlorine, Organophosphorus, Organocarbamates and Pyrethroids were present at below the quantification limit.

Table no 11. Pesticide residue

<table>
<thead>
<tr>
<th>Pesticide residue</th>
<th>Sample KAC</th>
<th>AYUSH Limit (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Organochlorine pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha BHC</td>
<td>BQL</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Beta BHC</td>
<td>BQL</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Gamma BHC</td>
<td>BQL</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Delta BHC</td>
<td>BQL</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>DDT</td>
<td>BQL</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Endosulphan</td>
<td>BQL</td>
<td>3 mg/kg</td>
</tr>
<tr>
<td>II. Organophosphorus Pesticides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malathion</td>
<td>BQL</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>Chlorpyriphos</td>
<td>BQL</td>
<td>0.2 mg/kg</td>
</tr>
<tr>
<td>Dichlorovos</td>
<td>BQL</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>III. Organocarbamates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbofuran</td>
<td>BQL</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>IV. Pyrethroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>BQL</td>
<td>1 mg/kg</td>
</tr>
</tbody>
</table>

BQL - Below Quantification Limit

AFLATOXIN:
The results revealed that there were no spots were identified in the test sample loaded on TLC plates, which denotes that the sample KAC were free from Aflatoxin B1, B2, G1 and G2.

Table No 12. Aflatoxin

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Sample KAC</th>
<th>AYUSH Specification Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Not Detected</td>
<td>0.5 ppm (0.5mg/kg)</td>
</tr>
<tr>
<td>B2</td>
<td>Not Detected</td>
<td>0.1 ppm (0.1mg/kg)</td>
</tr>
<tr>
<td>G1</td>
<td>Not Detected</td>
<td>0.5 ppm (0.5mg/kg)</td>
</tr>
<tr>
<td>G2</td>
<td>Not Detected</td>
<td>0.1 ppm (0.1mg/kg)</td>
</tr>
</tbody>
</table>

4. DISCUSSION:

KAC is a fine powder, aromatic brownish in color and free flowing in nature. Loss on drying was 6.98%, representing a good shelf life and stability of the drug. 5.37%, was the total ash value, illustrating the presentation of minerals. Acid insoluble ash was 7.60%, which is representing the amount of siliceous matter in the drug. The alcohol soluble extractive value and the water soluble extractive value were expressed for the same purpose. Less extractive values denote addition of exhausted material, contamination or inaccurate processing during drying, or storage or formulating [17].
Solubility is one of the important parameters for achieving the required pharmacological response [18]. KAC was soluble in ethanol, water and DMSO (Dimethyl sulfoxide). The test drug was insoluble in chloroform, and ethyl acetate. Soluble in water and ethanol is directly related to enhancing the bioavailability of the test drug. The existence phytochemicals of KAC are alkaloids, carbohydrates, saponin, phenols, tannins, flavonoids, diterpenes, gums & mucilage.

Alkaloids show Anti-inflammatory, antimicrobial activity. Tannins precipitate the microbial proteins, thus making nutritional protein unavailable for them. They exhibit antibacterial and antiviral activity [19]. Antioxidant activity of phenolic compounds is due to their high tendency to chelate metals [20]. Flavonoids decrease the risk for CVD, through improved endothelial function, and a reduction in platelet activity, LDL and blood pressure [21].

Pipeperine caused a decrease in mean arterial pressure (MAP) in normotensive anesthetized rats and caused partial inhibition of force and rate of ventricular contractions and coronary flow. In rat aorta, piperine demonstrated endothelium independent vasodilator effect [22]. Pipiplartine is an amide alkaloid of Thippili (Piper longum). It has potential antiarthrombic, anti-atherosclerotic, antihyperlipidemic, anti-inflammatory activities [23]. Diterpenoid of Seenthil sarkkari (Tinospora cordifolia) has anti hypertensive activity [24]. Alkaloids, flavonoids of Sadamanjil (N.grandiflora) have shown ACE- inhibitory activity [25]. Flavonoids of Paereechankai (P. dactylyfera) have the potential to attenuate vascular disease in humans, particularly plasma lipid levels including triglycerides & cholesterol indices of oxidative stress and inflammation [21]. Flavonoids of Cumin inhibit alcohol and thermally oxidized oil induced hyperlipidemia. It decreased aspartate transaminase (AST), Alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activities, decreased the tissue (liver and kidney) levels of cholesterol, triglycerides and phospholipids and prevented the changes in the composition of fatty acids in the plasma [26].

HPTLC finger printing analysis of the KAC sample reveals the presence of two prominent peaks corresponds to the presence of two versatile phyto components present in it. The Rf value of the peaks ranges from 0.03 to 0.44. The acid radix test reveals the presence of phosphates and sulfates. Development of hypertension is associated with low serum phosphate because of increased sympathoadrenal activity. Serum phosphates are inversely proportional in normotensive individuals to BP [27]. Potassium normalizes heart rhythms and regulates the body’s waste balance. It maintains normal alkalinity of body fluids and supports reducing high blood pressure [28]. Heavy metals, such as lead, arsenic, cadmium and mercury were BDL in KAC. The KAC was unaffected by specific pathogens and the safety limit of the total bacterial and total fungal count were within the limit for internal medicine. The pesticide residues were also discovered to be below the limit of quantification. It indicates the collection of wild plants as per good collection practices. Since aflatoxin B1, G1 (less than 0.5 ppm), B2 and G2 (less than 0.1 ppm), were realized. It could be given internally.

5. CONCLUSION
The obtained results of phytochemical, physicochemical, biochemical screening, sterility test, test for specific pathogen, aflatoxin and pesticide residue of KAC was very useful tool for the assessment of standardization, quality control of polyherbal Siddha formulation Kuruthi Azhalukku chooranam. The observed pH value was 5.46, indicating the acidic nature of the drug. The phytochemical analysis discovered the presence of alkaloids, carbohydrates, saponin, phenols, tannins, flavonoids, diterpenes, gums & mucilage. They were established that the above trial drug, Kuruthi Azhalukku Chooranam, was harmless to use as internal medicine. Since, further studies should be needed to explore the medicinal value of the test drug.

Acknowledgement
My hearty thanks to Dr M.G.R. Medical University, Chennai and Principal, Head of the Department and staff of PG Gunapadam Department, GSMC, Chennai for their valuable suggestions and Noble research solutions, Chennai for their technical support.

Ethical approval: Approved
Source of Funding: None
Conflicts of interest: No conflicts of interest.

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