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 Researchpertension 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 1(*Pg.no- 252).

Ingredients of *"KAC"*

Fig.no:01 Ingredients of *KAC*

(a).*Maranta arundinacea* (*Koogai neeru*) **(b).*Piper longum* (*Thippili*)**

(c).*Glycyrrhiza glabra* (*Athimadhuram*)

(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*)

Table no.1. Composition of *KAC*

S.NO	NAME OF THE DRUG	BOTANICAL NAME	QUANTITY
1	*Koogai neeru*	*Maranta arundiacea*	1 palam [35gms]
2	*Thippili*	*Piper longum*	1 palam [35gms]
3	*Athimadhuram*	*Glycyrrhiza glabra*	1 palam [35gms]
4	*Seenthil sarkkarai*	*Tinospora cordifolia*	1 palam [35gms]
5	*Sadamanjil*	*Nardostachys grandiflora*	1 palam [35gms]
6	*Vaal milagu*	*Piper cubeba*	1 palam [35gms]
7	*Paereechankai*	*Phonex dactilifera*	1 palam [35gms]
8	*Seeragam*	*Cuminum cyminum*	1 palam [35gms]
9	*Sarkkarai*	*Saccharum officinarum*	Half amount for the total amount of chooranam

Collection of the Drug

KAC contains 9 ingredients, such as *Koogai neeru (Maranta arundinacea), Thippili (Piper longum), Athimadhuram (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 1116-1124/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 *Athimathuram (G.glabra) and Sadamanjil (N.grandiflora),* dry unripe fruit of *Paereechankai (Phonex dactilifera) and* seeds of *Seeragam (C.cyminum)* were powdered in iron motor and sieved. Powder of *T.cordifolia (Seenthil sarkkarai), M.arundinacea (Koogai neeru)* 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.

3. RESULTS

ORGANOLEPTIC CHARACTER OF *KAC*

Figure 2. Prepared form of *KAC*

Table no 2. Organoleptic character

S.No	PARAMETER	RESULT
1	State	Solid
2	Nature	Fine
3	Odour	Characteristic
4	Touch	Soft
5	Flow property	Free flowing
6	Appearance	Brownish
7	Taste	Bitter

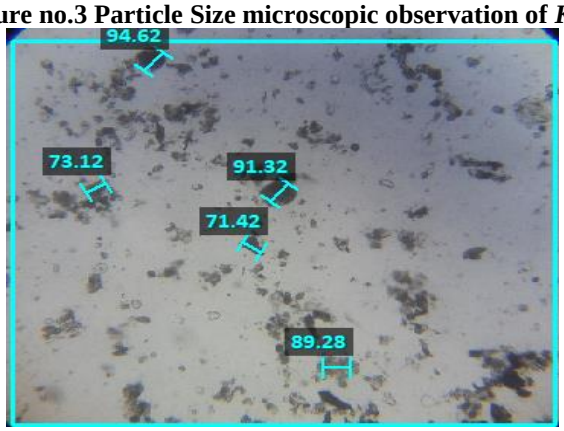
PHYSICOCHEMICAL CHARACTERISTICS

Table no.3 Physico-chemical Analysis – *KAC*

S.No	PARAMETERS	PERCENTAGE
1	Loss on drying	6.98%
2	Total ash value	5.37%
3	Acid insoluble ash	7.60%
4	Water soluble ash	1.25%
5	Water soluble extraction	24.007%
6	Alcohol soluble extraction	7.62%

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*

S.No	Solvent Used	Solubility / Dispersibility

1	Chloroform	In Soluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	In Soluble
5	Hexane	In Soluble
6	DMSO	Soluble

pH DETERMINATION

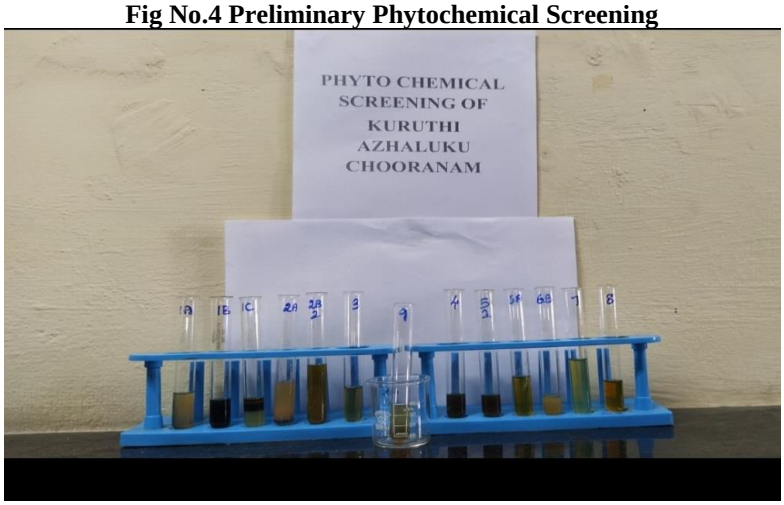
pH of the *KAC* was determined as 5.46 [15,16].

QUALITATIVE PHYTO-CHEMICAL ANALYSIS

Table no. 5 Result of Phytochemical Analysis

S.No	PHYTOCHEMICALS	H_2O EXTRACT
1	Alkaloids	Present
2	Carbohydrates	Present
3	Saponin	Present
4	Phenols	Present
5	Tannins	Present
6	Flavonoids	Present
7	Diterpenes	Present
8	Gum & Mucilage	Present

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.

Fig no 5. TLC Chromatogram of *KAC* at 366 nm

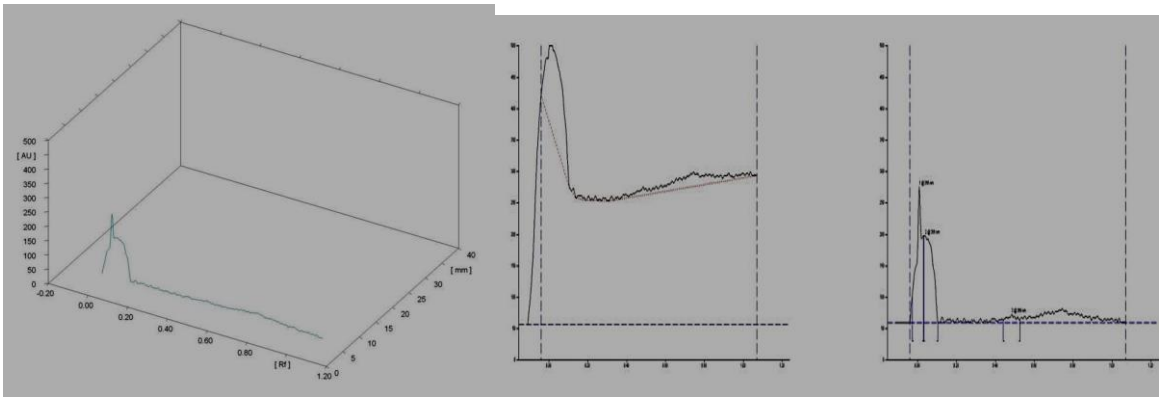


Fig no 6. Finger printing of *KAC*-HPTLC

Peak table No. 6

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.03	42.2	0.01	216.6	58.78	0.03	138.2	3096.1	46.97
2	0.03	138.2	0.04	138.3	37.53	0.10	0.5	3182.9	48.28
3	0.44	4.8	0.49	13.6	3.69	0.53	4.7	313.1	4.75

BIOCHEMICAL ANALYSIS OF *KAC*

Table no. 7 Test for acid radicles

S.No	Test	Inference	Result
1	Sulfates	Presence e of white precipitate	Positive
2	Phosphates	Presence of yellow precipitate	Positive

HEAVY METAL ANALYSIS BY ATOMIC ABSORPTION SPECTROMETRY

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

Table 8: AAS Interpretation - *KAC*

S.No	Name of the heavy metal	Absorption max A Max	Result analysis	Maximum limit
1	Lead	217.0 nm	BDL	10 ppm
2	Arsenic	193.7 nm	BDL	3 ppm
3	Cadmium	228.8 nm	BDL	0.3 ppm
4	Mercury	253.7 nm	BDL	1 ppm

BDL- Below Detection Limit

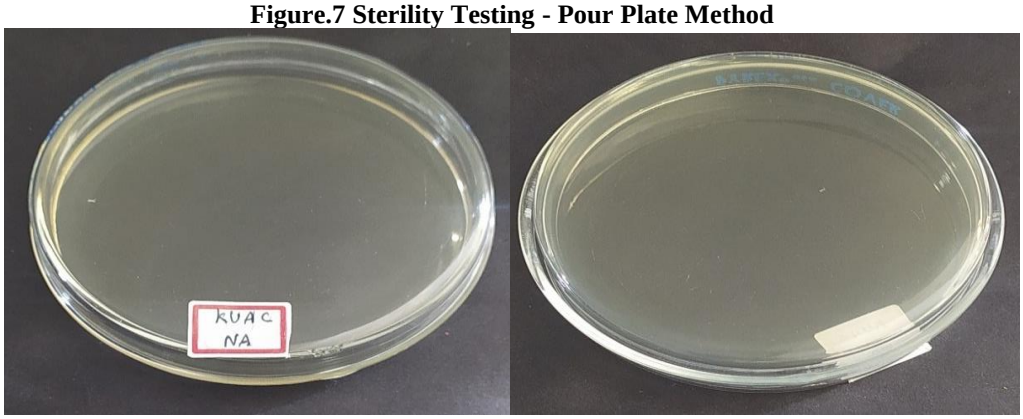
POUR PLATING METHOD FOR STERILITY TEST

Table 9. Pour Plating Method – For Sterility Test

S.No	Test	Result	Specification	As per AYUSH/WHO
1	*Total Bacterial Count*	Absent	NMT 10^5CFU/g	As per AYUSH specification

2	*Total Fungal Count*	Absent	NMT 10^3CFU/g	

Figure.7 Sterility Testing - Pour Plate Method



PATHOGEN (SPECIFIC) TESTING

The sample did not reveal any growth or colonies in the inoculated plates.

Table no. 10 Pathogen (Specific) - *KAC*

S.No	Organism	Specification	Result	Method
1	*E-coli*	Absent	Absent	As per AYUSH specification
2	*Salmonella*	Absent	Absent	
3	*Staphylococcus Aureus*	Absent	Absent	
4	*Pseudomonas Aeruginosa*	Absent	Absent	

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

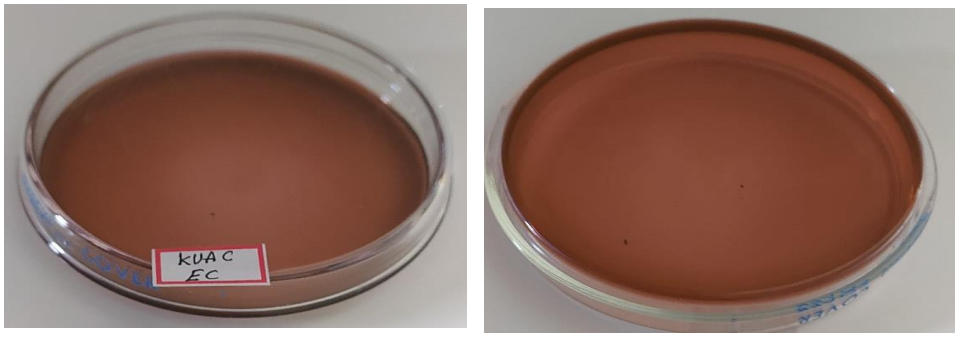


Fig no 9. Salmonella (SA) specific medium

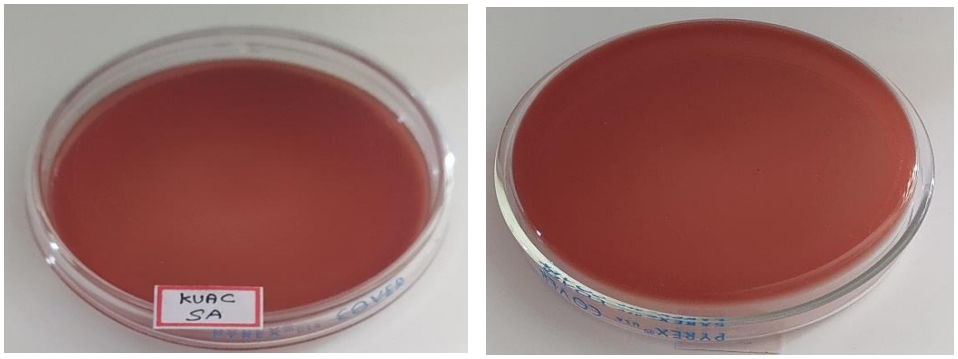


Fig no 10. Staphylococcus Aureus (ST) specific medium

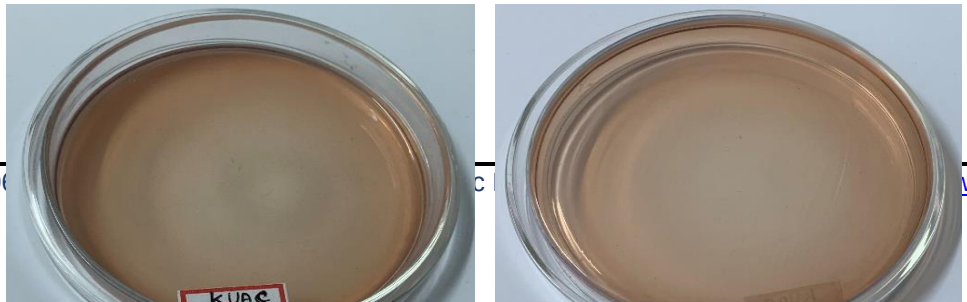
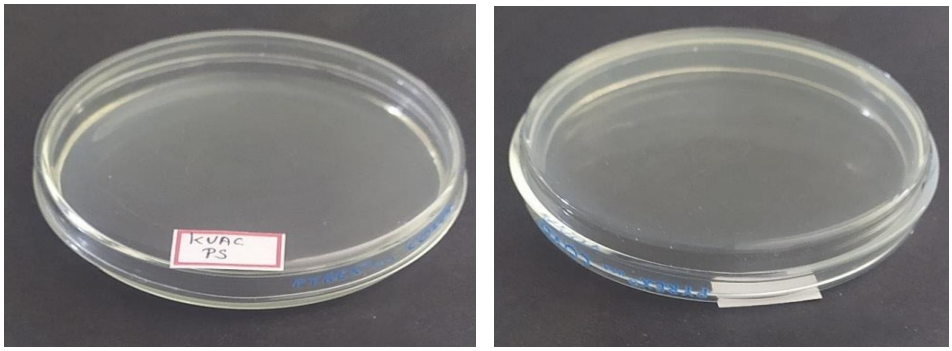


Fig no 11. Pseudomonas Aeruginosa (PS) specific medium



PESTICIDE RESIDUE ANALYSIS OF *KAC*

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

Table no 11. Pesticide residue

Pesticide residue	**Sample *KAC***	**AYUSH Limit (mg/kg)**
I.Organo chlorine pesticides		
Alpha BHC	BQL	0.1 mg/kg
Beta BHC	BQL	0.1 mg/kg
Gamma BHC	BQL	0.1 mg/kg
Delta BHC	BQL	0.1 mg/kg
DDT	BQL	1 mg/kg
Endosulphan	BQL	3 mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1 mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1 mg/kg
III. Organo carbamates		
Carbofuran	BQL	1 mg/kg
IV.Pyrethroid		
Cypermethrin	BQL	1 mg/kg

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, G1and G2.

Table No 12. Aflatoxin

Aflatoxin	**Sample *KAC***	**AYUSH Specification Limit**
B1	Not Detected	0.5 ppm (0.5mg/kg)
B2	Not Detected	0.1 ppm (0.1mg/kg)
G1	Not Detected	0.5 ppm (0.5mg/kg)
G2	Not Detected	0.1 ppm (0.1mg/kg)

4. DICUSSION:

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].

Piperine 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]. Piplartine is an amide alkaloid of *Thippili* (*Piper longum)*. It has potential antithrombotic, anti-atherosclerotic, antihyperlipidemic, anti-inflammatory activities [23]. Diterpenoid of *Seenthil sarkkarai* (*Tinospora cordifolia)* has antihypertensive activity [24]. Alkaloids, flavonoids of *Sadamanjil* (*N.grandiflora)* have shown ACE- inhibitory activity [25]. Flavonoids of *Paereechankai (P.dactylifera)* 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 phytocomponents present in it. The Rf value of the peaks ranges from 0.03 to 0.44. The acid radicle 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 Azhaluku 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 Azhaluku Chooranam,* was harmless to use as internal medicine. Since, further studies should be needed to explore the medicinal value of the test drug.

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Conflicts of interest: No conflicts of interest.

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