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# (Research Article)

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# Standardization of Kandankathiriyaadhy Kyazham: A Classical Siddha Drug

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# Abstract

*Kandankathiriyaadhy Kyazham* is one of the polyherbal formulation mentioned in siddha system of medicine. The main objective of the study was to evaluate the quality of formulation *Kandankathiriyaadhy Kyazham* by conducting Physicochemical, Phytochemical evaluation through advanced analytical techniques. The organoleptic nature of the drug provides the purity and quality of the formulation. The results obtained from physicochemical evaluation shows that the total ash value of *Kandankathiriyaadhy Kyazham* was "2.8±0.3%", in which the acid insoluble ash was "0.041±0.008%", loss on drying at 105°C of the formulation *Kandankathiriyaadhy Kyazham* was noted to be "7.4±0.88" in which water soluble extract value and alcohol soluble extract value was "20.2±1.53 and 8.333=1.15% "respectively. High performance Thin Layer Chromatography analysis of reveals the appearance *Kandankathiriyaadhy Kyazham* of ten prominent peaks corresponds to presence of ten versatile phyto components present with in it.Rf value of the peaks ranges from "0.13 to 0.91". Phyto chemical analysis of *Kandankathiriyaadhy Kyazham* reveals the emergence of phytocomponents like Alkaloids, Flavonoids, Steroids, Triterpenoids, Coumarin, Phenol, Tannin, Protein, Saponins, Sugar, Betacyanin. In conclusion, the polyherbal formulation of *Kandankathiriyaadhy Kyazham* possess significant phytocomponents and have beneficial effects towards treating various disorders.

Keywords: Standardization; Decoction; Physicochemical; Kandankathiriyaadhy Kyazham; Polyherbal.

# 1. Introduction

In favour of the use of medicinal plants, they are the only resource available in nature which have comparatively fewside effects. Synthetic drug in general has potent pharmacodynamic effects, but many also have strong and possibly dangerous harmful side effects. Nowadays Siddha treatment towards common people were well established and seek more attention indicated for the management of various ailments. There are 32 internal medicines available in Siddha literature. In that decoction is considered to be one of the most effective dosage forms in Siddha system of medicine. Its life span was only 3 hours and have to be used in fresh state due to loss of its therapeutic action.

Siddha medicine-a traditional system with its own scientific pedagogy and standardization of its applied pharmacology through which a lot of traditional practitioners and skilled medical professionals from renowned institutions who have been successfully practising all over the region of south India especially Tamilnadu. "*Kandankathiriyaadhy kyazham*" is one of the classical polyherbal siddha preparation for *Suvasa Kasam* which was mentioned in the textbook of "*Sarabendirar vaithiya muraigal*".

Asthma is a disease of airways that is characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli resulting in widespread spasmodic narrowing of the air passages which may be relieved spontaneously or by therapy.

Asthma is an episodic disease manifested clinically by paroxysms of dyspnoea, cough and wheezing.

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Based on the stimuli initiating bronchial asthma, two broad etiologic types are traditionally described:

- Extrinsic (allergic, atopic)
- Intrinsic (idiosyncratic, on atopic) asthma.

Therefore identification and scientific validation of an effective siddha drug to treat Bronchial Asthma is very essential in the current world.

Decoctions are made by pouring water to dry herbal/plant parts or fresh ones and then boiled so that the water content is greatly reduced to 1/16th or 1/8th or 1/4th or 1/2th of its initial volume. Decoctions are water based extracts of herbal drugs which are easily absorbed into the body and enter into the blood stream quickly which gives faster action than other forms of medications. In order to prepare decoctions without difficulty in sourcing raw material premixed coarse powder of the *kudineer* formulations are available as *kyazha chooranam.(2)Kandankathiriyaadhy Kyazham* a polyherbal Siddha medication, has shown great potential in treating Iraipu (Bronchial Asthma) and its related symptoms. But scientific evidences for *Iraipu*(Bronchial Asthma) have not been reported. So there is a need to develop a standardization technique by using preliminary guidelines. Therefore, the current investigation was done to detect physicochemical screening – organoleptic nature, loss on drying, Total ash, Acid insoluble Ash, Alcohol soluble extractive, water soluble extractive, High performance Thin Layer Chromatography (HPTLC), Heavy metal analysis, Sterility testing, Specific pathogen, Pesticide residue, and phytochemical analysis of siddha formulation KKYK according to PLIM guidelines.

# 2. Material and methods

# 2.1. Selection of the drug

The trial drug *Kandankathiriyaadhy Kyazham was* taken from "*Sarabendirar vaithiya muraigal*".for the treatment and management of *Iraipu( Bronchial Asthma)* and its related symptoms *Kandankathiriyaadhy Kyazham* comprises of the following ingredients.

S.no	Tamilname	Botanical name
1	Kandankathiri ver	Solanum virginianum
2	Thandrikkai Thol	Terminalia bellerica
3	Nellimulli	Phyllanthus emblica
4	Chukku	Zingiber officinale
5	Peipudal	Trichosanthus cucumerina
6	Kadukkai Thol	Terminalia chebula
7	Musumusukai	Cucumis madersapatanus

# **Table 1:** Ingredients of Kandankathiriyaadhy Kyazham

#### 2.1.1. Collection of the Raw Materials

The raw drugs *Kandankathiri ver* (Root of *Kandankathiri*)-Solanum virginianum, *Thandrikkai Thol* (Terminalia bellerica), *Nellimulli* (Phyllanthus emblica)-Dry gooseberry without seed, *Chukku* (Zingiber officinale), *Peipudal*(Trichosanthus cucumerina), *Kadukkai Thol*(Terminalia chebula), *Musumusukai* (Cucumis madersapatanus) was bought from Thakkalai, kanyakumari district, Tamilnadu.

# 2.1.2. Identification and Authentication of The Drug

All drugs were recognized and authenticated by Botanist in Government Siddha Medical College, Palayamkottai, Tirunelveli.

# 2.1.3. Purification of the Drug

Purification process were made according to the procedures mentioned in the classical Siddha literature

# 2.1.4. Preparation of Kandankathiriyaadhy Kyazham

The above given ingredients were taken in an equal quantity, then pounded into coarse powder (sieve no: 10). The obtained decoction powder was then stored in clean air-tight container and named as KKYK.



Figure 1 Ingredients of Kandankathiriyaadhy Kyazham



Figure 2 Storage of decoction powder

All the above investigations were performed at Noble Research Solution, Perambur at Chennai

# 2.2. Physico-chemical evaluation

# 2.2.1. Percentage Loss on Drying

Test drug was accurately weighed in evaporating dish. The sample was dried at 105 °C for 5 hours and then weighed.

# Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 <sup>o</sup>C until it turns white in colour which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

# Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hotwater and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

# Determination of Alcohol Soluble Extractive

Test sample was macerated with 100 ml of Alcohol in a closed flask for twenty- four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred 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.

# Determination of Water Soluble Extractive

Test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to standand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

### 2.2.2. pH determination

Required quantity of test sample was mixed with distilled water and the subjected to screening using pH meter.

### 2.3. Particle size determination

### 2.3.1. Methodology

Particle size determination was carried out by optical microscopic method. In which the sample were dissolved in the sterile distilled water (app 1/100th dilution). Diluted sample were mounted on the slide and fixed with stage of appropriate location. Light microscopic image was drawn with scale micrometer to arrive at the average particle size. Minimum 30 observations were made to ascertain the mean average particle size of the sample.

### 2.3.2. Thin Layer Chromatography (TLC) Analysis

Test sample was subjected to Thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-microliter by using pipette at distanceof1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and was observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

### 2.3.3. High performance thin layer chromatography (HPTLC) analysis

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

#### Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

#### Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective Rf values were tabulated.

#### 2.4. Heavy metal analysis by atomic absorption spectrometry

#### 2.4.1. Standard: Hg, As, PbandCd–Sigma Methodology

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

#### 2.4.2. Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L ofHNO3.

# 2.4.3. Standard preparation

As & Hg- 100 ppm sample in1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO3

# 2.5. Sterility test by pourplate method

### 2.5.1. Methodology

Test sample was inoculated in sterile Petri dish to which about 15 ml of molten agar 45 °C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it. (about 10 minutes). Plates were then inverted and incubated at 37 °C for 24-48 hours and further extended for 72 hrs for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

### 2.6. Test for specific pathogen

### 2.6.1. Methodology for Specific Pathogen

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37 °C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic colour with respect to pattern of colony formation in each differential media.

OrganismAbbreviationMediumE-coliECEMB AgarSalmonellaSADeoxycholateagarStaphylococcus AureusSTMannitolsaltagarPseudomonas AeruginosaPSCetrimideAgar

**Table 2** Detail of Specific Medium and their abbreviation

#### 2.7. Pesticide residue

#### 2.7.1. Extraction

Test sample were extracted with acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40°C until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter

# 2.8. Phytochemical evalution

Test drug KKYK was subjected to Preliminary phytochemical screening of the following components.

#### 2.8.1. Test for alkaloids

Mayer's Test: To the test sample, 2ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

# 2.8.2. Test for coumarins

To the test sample, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

#### 2.8.3. Test for saponins

To the test sample, 5 ml of waterwas added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

### 2.8.4. Test for tannins

To the test sample, ferric chloride wasadded, formationofadarkblueor greenish black colour showed the presence of tannins.

### 2.8.5. Test for glycosides-Borntrager's Test

Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of chloroformis added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates presenceof glycosides.

#### 2.8.6. Test for flavonoids

To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow colour indicates the presence of Flavonoids.

### 2.8.7. Test for phenols

#### Leadacetate test

To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

#### 2.8.8. Test for steroids

To the test sample, 2ml of chloroform was added with few drops of conc.Sulphuric acid(3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

### Triterpenoids

Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids.

#### 2.8.9. Test for Cyanins

#### Aanthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 10022C. Formation of bluish green colour indicates the presence of anthocyanin.

#### Betacyanin

To the test sample, 2ml of HCl was addedandheatedfor5minsat100°C.

Formation of pink colour indicates the presence of betacyanin

#### 2.8.10. Test for Carbohydrates-Benedict's test

To the test sample about 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2minutes. A characteristic coloured precipitate indicates the presence of sugar.

# Proteins(Biuret Test)

To extracts 1% solution of copper sulphatewasaddedfollowedby5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

# 3. Results

# 3.1. Physico-chemical evaluation of KKYK

#### 3.1.1. Organoleptic characters

The drug KKYK was coarsely powdered and the results were mentioned in Table: 3

 Table 3 Organoleptic characters of KKYK

Description	Kandankathiriyaadhy kyazha chooranam	Kandankathiriyaadhy kyazham
State	Solid	Liquid
Nature	Coarse Fibrous	NonViscous
Odour	Strong Characteristic	Aromatic
Touch	Hard Texture	Nongreasy
Flow Property	Non free flowing	Free Flowing
Appearance	Brownish	Dark Brownish

# 3.1.2. Solubility Profile

The drug KKYK for solubility profile was given in Table 4

Table 4 Solubility profile of KKYK

S.No	Solvent Used	Solubility/Dispersibility
1.	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethylacetate	Insoluble
5	DMSO	Soluble

The results for physicochemical analysis were tabulated in Table 5

Table 5 Results of physicochemical evaluation of KKYK

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	7.4 ± 0.88
2.	Total Ash (%)	2.8 ± 0.3
3.	Acid insoluble Ash (%)	0.041 ± 0.008
4.	Water soluble Extractive (%)	20.2 ± 1.53
5.	Alcohol Soluble Extractive (%)	8.333 ± 1.15
6.	рН	7.2

# 3.2. Particle size determination

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be " $10.4\pm4.41\mu$ m" further the sample has particle with the size range of lowest 5  $\mu$ m to highest " $15 \mu$ m"

3.2.1. Microscopic Observation of Particle Size for the sample KKYK



**Figure 3** Microscopic Observation of Particle Size for the sample KKYK

# 3.2.2. HPTLC Analysis Of KKYK

HPTLC finger printing analysis of the sample reveals the presence of two prominent peaks corresponds to presence of two versatile phytocomponents present within it.Rf value of the peaks ranges from "0.13 to 0.19"



TLC Visualization of KKYK at 366 nm

Figures 4 3D – Chromatogram



Figure 5 HPTLC finger printing of KKYK

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	0.1	0.07	85.8	20.81	0.10	0.6	1885.0	24.68
2	0.13	5.6	0.17	29.1	7.07	0.20	4.8	592.3	7.75
3	0.29	31.9	0.32	97.2	23.57	0.38	26.1	2514.3	32.91
4	0.43	18.9	0.45	29.4	7.13	0.46	11.3	340.2	4.45
5	0.46	12.4	0.47	25.7	6.25	0.52	5.4	450.5	5.90
6	0.58	7.8	0.63	30.0	7.27	0.67	10.5	748.1	9.79
7	0.72	3.5	0.74	24.1	5.85	0.76	0.8	179.0	2.34
8	0.76	5.5	0.80	19.3	4.68	0.82	14.2	321.5	4.21
9	0.82	12.9	0.86	46.2	11.21	0.86	4.0	434.6	5.69
10	0.91	11.1	0.92	25.4	6.15	0.94	1.2	173.4	2.27

# Table 6 Peak Table

# 3.3. Heavy metal analysis by atomic absorption spectrometry

The result for Heavy metal analysis of the trial drug the was tabulated in table 7.

Table 7 Heavy metal analysis

Name of the Heavy Metal	Absorption Max $\Lambda$ max	<b>Result Analysis</b>	MaximumLimit
Lead	217.0 nm	4.989	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.346	1 ppm

BDL-Below Detection Limit

# 3.4. Result for specific pathogen

Observation of the trial sample and the results were mentioned in table 8.

Table 8 Results for specific pathogen

Organism	Specification	Result	Method
E-coli	Absent	Absent	
Salmonella	Absent	Absent	As per AYUSH specification
Staphylococcus Aureus	Absent	Absent	
Pseudomonas Aeruginosa	Absent	Absent	



Figure 6 Culture plate with E-coli (EC) specific medium



Figure 7 Culture plate with Salmonella (SA) specific medium



Figures 8 Culture plate with Staphylococcus Aureus (ST) specific medium



Figure 9 Culture plate with Pseudomonas Aeruginosa (PS) specific medium

# 3.5. Result for microbial contamination



Figure 10 Microbial contamination by pour plate method

Test for microbial contamination of the given sample was done and the results were given in Table 9.

**Table 9** Results for microbial contamination

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 <sup>5</sup> CFU/g	As non AVUCU specification
Total Fungal Count	Absent	NMT 10 <sup>3</sup> CFU/g	As per Arosh specification

# 3.6. Result for pesticide residues

The results showed that there were no traces of pesticides residues in the given sample and the result was tabulated in table 10.

Table 10 Results for pesticide residues

Pesticide Residue		
I.Organo Chlorine Pesticides	Sample KKYK	AYUSH Limit ( mg/kg)
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	"0.1 mg/kg"
III.Pyrethroid		
Cypermethrin	BQL	"1 mg/kg"

# 3.7. Qualitative phytochemical evaluation of KKYK

The Results for the Analysis of phytochemical present in the given sample was mentioned and the outcome were tabulated in table 11.



Figure 11 Phytochemical Analysis of Kandankathiriyaadhy Kyazham

 Table 11 Qualitative Phytochemical Analysis of Kandankathiriyaadhy Kyazham

S.no	Test	Observation
1	Alkaloids	+
2	Flavanoids	+
3	Glycosides	-
4	Steroids	+
5	Triterpenoids	+
6	Coumarin	+
7	Phenol	+
8	Tanin	+
9	Protein	-
10	Saponins	+
11	Sugar	+
12	Anthocyanin	-
13	Betacyanin	+

(+)>IndicatesPositiveand(-)->Indicates Negative

# 4. Discussion

The drug KKYK was coarsely powdered with hard texture and brownish colour. Fresh preparation of its extract shows non greasy, dark brownish with aromatic odour. Oral bio-availability depends on several factors including Aqueous solubility, drug permeability etc., The drug KKYK soluble in specific solvent like Ethanol, Water and Dimethyl sulfoxide thereby it proves its efficiency of solubility increasing in bio-availability in the stomach indirectly. The loss on drying was found to be "7.4±0.88%" which indicates the moisture content of the drug. Total ash value was found to be "2.8±0.3% "which notes the presence of inorganic components. Acid insoluble ash was "0.041±0.008" which indicates that the drug contains minimum amount of siliceous matter. The water and alcohol soluble extractive values were found to be "20.2±1.53%" and "8.333±1.15%". The PH value was found to be "7.2" which proof that the secondary metabolites are extractable with above solvents and it shows the high polar secondary metabolites such as tannins, proteins, triterpenoids, flavonoids, coumarin, phenol etc., HPTLC finger printing analysis of the sample reveals the presence of ten prominent peaks that corresponds to the presence of ten components. Rf value of the peaks ranges from "0.13 to 0.91". Heavy metal analysis clearly shows that the sample has no traces of heavy metal such as Arsenic, and Cadmium. The sample evidently shows the presence of lead and mercury at "4.989 and 0.3460ppm". These results indicate that the trial drug is extremely safe as it contains heavymetals within specified limits which reveals the safety of the drug. Results obtained from the test for specific pathogen denotes that No growth /colonies were seen in any of the plates inoculated with the test sample KKYK which confirms the absence of E-coli, Salmonella, Staphylococcus Aureus and Pseudomonas aeruginosa in the sample. Analysis of Pesticide residue is an important parameter for quality control of drug and the results obtained further confirms that there were no traces of pesticides residues such as Organochlorine, Organophosphorus and Pyrethroids in the KKYK. Phytochemical analysis indicates that the medication KKYK indicates the presence of Alkaloids which possess anti-inflammatory, anti-cancer, and used as a local anesthetic and pain relief. The trial drug contains Flavonoids which exhibits anti- viral, anti-oxidant, anti-inflammatory and anticarcinogenic Activity. Thus presence of Flavonoids in the test sample it may protect cells from oxidative damage and also have ameliorative effects on symptoms related to Asthma. Presence of Steroids the drug may deliver antiinflammatory and immune modulator activities. Presence of Triterpenoids exhibit Anti-oxidant and Anti-inflammatory activities. It is also useful in treating Bronchial Asthma, metabolic syndrome and obesity. Coumarin has pharmacological properties like anti-inflammatory, anti hyperglycaemic and antioxidant and the presence of coumarin can be good in treating Inflammatory disorders and in inducing relaxant effects in the airway muscles . Presence of Phenols exhibits anti-oxidant activity which is effective in protecting cells from oxidative damage. Presence of Tannin helps to reduce inflammation of mucous membrane and inhibition of carcinogenesis.. Presence of Saponins acts as an immunological adjuvant by increasing the immune response. Presence of Sugar in the test sample acts as an energy source for obvious functions of the body thereby regulating hormonal dysfunction and functions of various tissues and organs. Betacyanin has potential therapeutic efficacy in the treatment of dyslipidemia, cancer and cardiovascular diseases. Due to the presence of betacyanin in the trial is given for reducing abnormally elevated cholesterol and for Allergic Asthma. phytoconstituents can be used as a major tool for obtaining a quality control profile of drug. However the presence of these phytoconstituents hence proved that the trial drug will be effective in treating various disorders.

# 5. Conclusion

Results obtained from the above discussion; this was finally concluded that the Siddha formulation *KKYK* possess potent biologically active components which may helps in treating various disorders. Investigation of those specifications with the help of modern analytical tools helps in standardization of *KKYK*. Hence this present investigation had generated an evidence-based data with respect to purity, standards, physico-chemical, phytochemical nature of the formulation *KKYK*.

# **Compliance with ethical standards**

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# Disclosure of conflict of interest

No conflict of interest.

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