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Standardization and Physicochemical Evaluation of Traditional Siddha formulation *Keelvayu Nivarana Chooranam* by Modern Pharmaceutical analytical Techniques

T.Giftillda Selva Elsee *[1], M.D Saravana Devi [2], K.Rajammadevi Sorubarani [3], V.Velpandian[4]

[*1]PG Scholar, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
[2] Professor, Dept of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
[3]Lecturer, Dept of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.
[4]Professor, HOD, Dept of Gunapadam (Pharmacology),Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

Corresponding Author: **Dr. T.Giftillda Selva Elsee**
E-mail: *giftillda@gmail.com*
Corresponding Address: PG Scholar, Department of Gunapadam (Pharmacology), Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

Abstract

Siddha method of treatment offers greater advantages over allopathic medicine with respect to its potential on curing, rejuvenation, immune boosting and stamina development etc. Siddha has versatile formulation like herbal, minerals, metals, poly herbals, herbominerals, distillates and extractives. Herbs play an inevitable role in siddha formulation since several centuries in providing ailment for treating dreadful diseases. Phytochemicals of medicinal plants encompass a diverse chemical space for drug discovery. India is rich with a flora of indigenous medicinal plants that have been used for centuries in traditional Indian medicine to treat human maladies. Data from the WHO show that 25% of modern medicines are made from plants that were first used traditionally. In India venture of adopting good manufacturing practice has been applied to herbal medicine. Standardization of siddha formulation becomes essential to establish the monograph of the particular formulation along with this it encompasses the genunity, purity and safety of the preparations intended for usage in patients. The main objective of the present investigation is to systematically standardize the novel siddha formulation *KeelvayuNivaranaChooranam* (KVNC) by physicochemical evaluation through modern analytical techniques. The organoleptic character of the drug KVNC justifies the purity and its bitter taste and fine powder nature confirms the quality of the formulation. The results obtained from the physicochemical evaluation revels that the total ash value of KVNC was 4.78 % in which the water soluble ash value was 2.75% and acid insoluble ash value was 0.40 %. Loss on drying value of the formulation was found to be 5.20 % in which the water soluble extractive value was 16.65% and alcohol soluble extractive value was 16.10 % respectively. Phytochemical analysis reveals the presence of phytocomponents such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, phenols and proteins. Biochemical investigation of the test drug KVNC reveals the presence of the following radicals such as potassium, calcium, magnesium, sodium, Iron, sulphate and

phosphates. HPTLC analysis of the KVNC reveals the presence of 10 phytocomponents with Rf value ranges from 0.04 to 0.70. Elemental analysis of the sample KVNC reveals the presence of potassium, calcium, magnesium, sodium, zinc and phosphorus. In conclusion the trial drug KVNC confirms the regulatory requirement and also possess significant phytocomponents which contributes to the beneficial effect on the formulation towards several dreadful disorders.

Keywords: Siddha, Standardization,Physicochemical ,*KeelvayuNivaranaChooranam*, Phytochemical, HPTLC.

1. Introduction

Siddha system of Indian medicine is considered to be science and art of healing and provides basic ailment for mankind through its novel medications. Healing by rejuvenation is one of the principle involved in the siddha medicine. Siddha system attains greater popularity due to their versatile preparation's. However most of the siddha formulations are herbal and poly herbal components. However, the health benefits of herb and spice extracts have been discussed for centuries [1]. They have been used in many branches of industry such as medicine, pharmacy, cosmetology, and food production [2,3].

The use of herbal medicines for treatment of diseases was documented several thousand years ago. As seen from journals, studies on herbal medicines have been encompassed under several different names, such as plant medicine, phytomedicine, pharmacognosy, and natural products. "Natural products" usually refer to products processed or derived from living organisms, including plants, animals, insects, microorganisms, and marine organisms.

Standardization of an herb usually refers to the chemical analysis of the characteristic bioactive and main components for identification or comparison of species. At present fingerprints of HPTLC chromatograms are the most popular method. Thin layer chromatography (TLC) is another common one with lower cost.Quality control for an herbal material usually includes not only the quantitative analysis of the main compounds from herbs, but also other analyses related to hygiene or safety examination such as heavy metal, pesticides, and microorganisms. For each individual formulation of the preparation, quality control has different requirements, such as precipitation test of an oral liquid and time for disintegration of a tablet.

Currently, the contamination of heavy metal, pesticides, and microorganisms is still a problem for many raw materials and their products. To a large extent, the efficacy and toxicity of a product depend on quality control. Toxicity or death reports by consumers by herbal products are mostly related to poor quality control during preparation, storage, or transportation [4,5].

Since ancient time, Indian society depends on traditional medicinal systems practiced here. Introduction of allopathic drug during British era and neglecting Indian traditional medicine by British ruler are responsible for significant erosion of Indian traditional medicine. High scientific progress in allopathic medicine and modern facilities also resists the growth of traditional medicine. Still, about 70% rural populations of India are believed in traditional medicine for primary healthcare.As a measure of focusing upon the need of drug standardization the present investigation work undertaken to standardize the traditional siddha formulation *Keelvayu Nivarana Chooranam* (KVNC) which has been used for the treatment of various ailments by phytochemical, physiochemical and elemental evaluations in accordance with AYUSH regulations.

2. Materials and Methods

2.1.Physicochemical Evaluation[6-7]

2.1.1.Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish .The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

2.1.2.Determination of Total Ash

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ^{o}C until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.1.3.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 hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.1.4.Determination of Water Soluble Ash

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15mins at a temperature not exceeding 450ºC. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.1.5.Determination of Alcohol Soluble Extractive

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, 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 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.
Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

2.1.6.Determination of Water Soluble Extractive

About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand and 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 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

2.1.7.Determination of pH

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the subjected to pH evaluation

2.1.8.Action on heat

A small amount of the sample was taken in a dry test tube and heated gently. If strong white fumes evolve indicate the presence of Carbonate.

2.1.9.Flame test

A small amount of the sample was made into a paste with con.Hcl in a watch glass and introduced into non-luminous part of the Bunsen flame. Appearance of bluish green flame indicates the presence of Copper.

2.1.10.Ash Test

A filter paper was soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited. Appearance of yellow colour flame indicates the presence of Sodium.

2.2.Phytochemical Evaluation [8]

The Phytochemical screening of the test drug gives general idea regarding the nature of chemical constituents present in the crude drug.

Test for Alkaloids

A small portion of solvent free extracts were stirred separately with few drops of dilute hydrochloric acid and filtered & tested carefully with various alkaloidal reagents.

Mayer's reagent - Cream precipitate
Dragendroff's reagent - Orange brown precipitate
Hager's reagent - Yellow precipitate
Wagner's reagent - Reddish brown precipitate

Test for Carbohydrates and Reducing Sugars

The minimum amount of extracts were dissolved in 5ml of distilled water & filtered. The filtrate was subjected to test for carbohydrates & glycosides.

a) Molisch's test

The filtrate 1 ml was treated with 2-3 drops of 1% alcoholic alpha naphthol& 2ml concentrated sulphuric acid was added along the sides of test tube. Violet ring was observed at the junction of 2 layers which showed the presence of carbohydrate.

b) Benedict's test

The filtrate 1 ml was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's test

The filtrate 1 ml was treated with equal volume of Fehling's solution A and B and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Test for Glycosides

The extract was hydrolyzed with dil. HCl and subjected to test for glycosides.

a) Modified Borntrager's test

To the hydrolysate extract, 1 ml of Ferric chloride solution was added and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal voulme of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of Anthranol glycosides.

b) Legal's test

The hydrolysate extract was treated with Sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of Cardiac glycosides.

Test for Saponins

The extract 0.5 ml was shaken with 5 ml distilled water. The presence of saponins was indicated by formation of copious lather.

Test for Tannins

Gelatin test

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Test for Phenolic compounds

To 0.5 ml of extract, 1 ml of alcoholic ferric chloride solution was added. Formation of bluish green or bluish black indicates the presence of Phenolic compounds.

Test for Phytosterol

Ferric chloride – acetic acid test

1 ml of extract was treated with 1 ml of chloroform and then, 2 ml of ferric chloride acetic acid reagent was added followed by 1 ml of conc. sulphuric acid. Appearance of reddish pink colour shows the presence of phytosterol.

Test for Diterpenes

Copper acetate test

1 ml of extract was dissolved in water and treated with 3-4 drops of Copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Triterpenes

Salkowski's test

1 ml of extract was treated with 1 ml of chloroform followed by 1 ml of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour shows the presence of triterpenes.

Test for Flavonoids

a) Alkaline reagent test

To 1 ml of extract, 1 ml of 10% sodium hydroxide solution was added. Formation of dark yellow colour indicates the presence of flavonoids.

b) Lead acetate test

To 1 ml of extract, 3-4 drops of 10% lead acetate solution was added. Formation of yellow precipitate indicates the presence of flavonoids.

c) Ferric chloride test

To 1 ml of extract, 3-4 drops of ferric chloride solution was added. Formation of dark green colour indicates the presence of flavonoids.

d) Shinoda test

To 1 ml of extract, few mg of magnesium turnings was added followed by few drops of conc. hydrochloric acid and boiled for five minutes in a boiling water bath. Formation of red colour indicates the presence of flavonoids.

Test for Proteins and Free Amino Acids

a) Xanthoproteic test

To 1 ml of extract, 3-4 drops of conc. nitric acid was added. Formation of yellow precipitate indicates the presence of proteins.

b) Million's test

To 0.5 ml of extract, 2.5 ml of Million's reagent was added. Formation of white precipitate and the precipitate warmed indicates the presence of proteins.

c) Biuret test

To 0.5 ml of extract, 2.5 ml of diluted Biuret reagent was added. Appearance of purple colour or brick red precipitate showed the presence of proteins and free amino acids.

Test for Quinones

Sodium hydroxide test

To 0.5 ml of extract, 1 ml of 10% sodium hydroxide was added. Appearance of blue or green or red colour shows the presence of quinones.

2.3.High Performance Thin Layer Chromatography Analysis [9]

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. In addition it is a reliable method for the quantitation of nano grams level of samples. 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 medicinal plant raw materials. In the present study the formulation KVNC developed with the mobile of Hexane: Ethyl Acetate: (7.5:2.5 v/v) ratio.

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 analysed. After elution, plates were taken out of the chamber and dried.

Scanning

Plates were scanned under UV at 254,366nm and 520 nm. 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 extract and Rf values were tabulated.

2.4.Biochemical analysis of Basic and acidic radicals

Biochemical analysis of the trial drug KVNC was subjected for qualitative analyses of acid and basic radicals as per the procedure described by Asokan and sofowora et al [10-11]..

2.5.Heavy Metal Analysis by ICPOES [12]

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.

3. Results

3.1. Results of Physicochemical evaluation of KVNC

Organoleptic property of the KVNC with respect to its brown color, pleasant odor, bitter taste and fine powder nature justifies the genunity, purity and quality of the finished formulation with respect to its color and fine powdered texture. The results obtained from the physicochemical evaluation revels that the total ash value of KVNC was 4.78 % in which the water soluble ash value was 2.75% and acid insoluble ash value was 0.40 %. Loss on drying value of the formulation was found to be 5.20 % in which the water soluble extractive value was 16.65% and alcohol soluble extractive value was 16.10 % respectively. pH of the formulation KVNC was found to be 5.58. The results were tabulated in Table 1.

Table 1: Results of Physicochemical evaluation of *Keelvayu Nivarana Chooranam*

S.NO	PARAMETER		RESULT
1	pH		5.58
2	Total ash		4.78
3	Water soluble ash		2.75
4	Acid soluble ash		0.40
5	Loss on drying (at 105°C)		5.20
5	Water soluble extractive		16.65
6	Alcohol soluble extractive		16.10
7	Solubility		+ve
	i	Distilled water	Soluble
	ii	Benzene	Soluble
	iii	Chlroform	Soluble
8	Action on heat		- ve
9	Flame test		- ve
10	Ash test		- ve

3.2.Qualitative Phytochemical evaluation of KVNC

The result of the qualitative phytochemical analysis indicates that the formulation KVNC shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, phenols and proteins. The results were tabulated in Table 02.

Table 2: Qualitative chemical and preliminary phytochemical analysis of *Keelvayu Nivarana Chooranam*

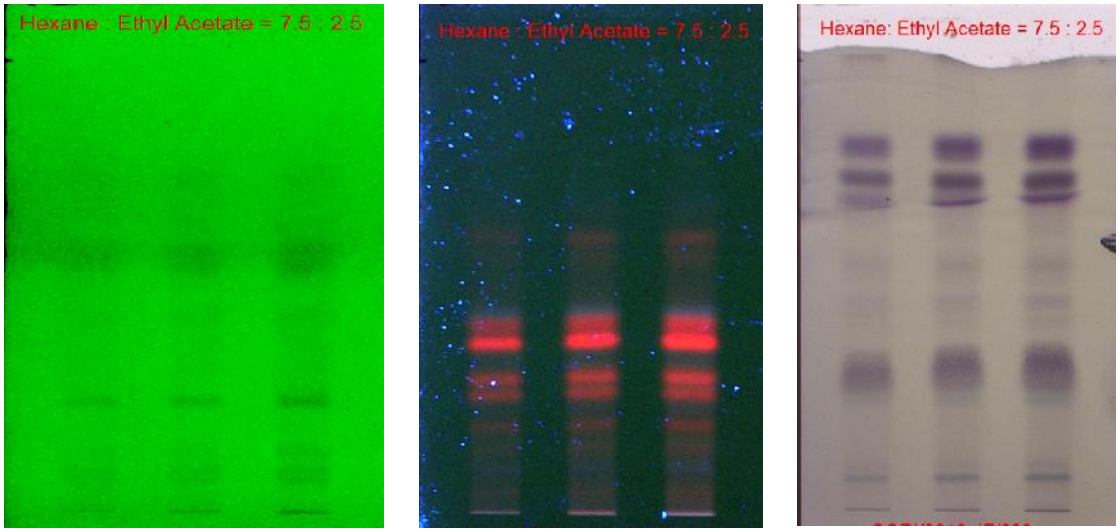
PHYTOCHEMICALS	TEST	RESULT
1. Alkaloids	a. Mayer's test	++
	b. Wagner's test	-
	c. Dragendroff's test	-
	d. Hager's test	-
2. Carbohydrates	a. Molisch's test	+
3. Reducing sugars	a. Benedicts test	-
	b. Fehling's test	-
4.Anthranol Glycosides	Modified Borntrager's test	+
5. Saponins	a. Froth test	+
	b. Foam test	-
6. Tannins	Gelatin test	++
7. Phenols	Alcoholic Ferric chloride test	+
8. Phytosterols	Ferric chloride acetic acid test	-
9. Diterpenes	Copper acetate test	-
10. Triterpenes	Salkowski's test	-
11. Flavanoids	Alkaline reagent test	+++
	b. Lead acetate test	-
	c. Ferric chloride test	-
	d. Shinoda test	+
12. Proteins	a. Xanthoproteic test	-
	b. Biuret's test	+
	c. Million's test	-

(+) → Indicates Presence and (-) → Indicates Absence

3.3 HPTLC analysis of KVNC

The results of HPTLC analysis of the sample KVNC reveals the presence of 10 prominent peaks corresponds to 10 different compound's with Rf value ranging from 0.04 to 0.70 with percentage area of 3.39 to 23.68%. The bands revealed presence of six green, two blue and one fluorescent yellow, bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines. The results were tabulated in Table 03 and illustrated in Figure 1 and 2

Figure 1: TLC Chromatogram of *Keelvayu Nivarana Chooranam*



λ = 254 nm **λ= 366 nm** **λ=520nm**

Figure 2: HPTLC Chromatogram of *Keelvayu Nivarana Chooranam*

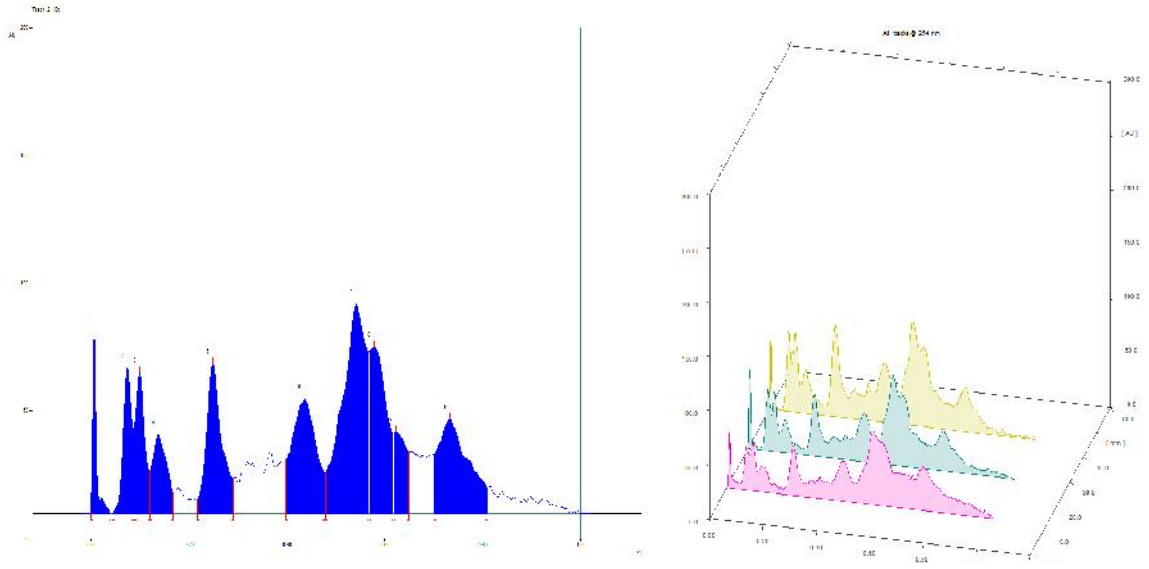


Table 3: Peak Table HPTLC analysis of *Keelvayu Nivarana Chooranam*

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.01 Rf	72.4 AU	13.52 %	0.04 Rf	0.1 AU	535.5 AU	3.39 %
2	0.04 Rf	0.1 AU	0.07 Rf	57.2 AU	10.67 %	0.09 Rf	33.0 AU	939.8 AU	5.94 %
3	0.09 Rf	33.7 AU	0.10 Rf	55.2 AU	10.30 %	0.12 Rf	16.6 AU	969.5 AU	6.13 %
4	0.12 Rf	16.8 AU	0.14 Rf	31.0 AU	5.78 %	0.17 Rf	8.2 AU	845.4 AU	5.34 %
5	0.22 Rf	5.5 AU	0.25 Rf	58.7 AU	10.95 %	0.29 Rf	13.7 AU	1749.1 AU	11.06 %
6	0.40 Rf	21.1 AU	0.44 Rf	45.0 AU	8.40 %	0.48 Rf	16.4 AU	2050.6 AU	12.96 %
7	0.48 Rf	16.4 AU	0.54 Rf	82.1 AU	15.32 %	0.57 Rf	63.2 AU	3746.2 AU	23.68 %
8	0.57 Rf	63.4 AU	0.58 Rf	65.4 AU	12.20 %	0.62 Rf	31.3 AU	2085.9 AU	13.19 %
9	0.62 Rf	31.5 AU	0.62 Rf	32.0 AU	5.97 %	0.65 Rf	24.4 AU	754.4 AU	4.77 %
10	0.70 Rf	23.2 AU	0.73 Rf	36.9 AU	6.89 %	0.81 Rf	9.7 AU	2142.1 AU	13.54 %

3.4. Biochemical Analysis of KVNC

The results of the biochemical analysis of the test sample KVNC reveals the presence of potassium, calcium, magnesium, sodium, Iron, sulphate and phosphates. Most of these radicals are highly involved in enzyme synthesis and their action against specific target, hence for sure the formulation KVNC has tendency to alters the physiological function in diseased patients. The results were tabulated in table 4.

Table 4 : Results of Biochemical Analysis of *Keelvayu Nivarana Chooranam*

TEST FOR BASIC RADICALS			
S.NO	**PARAMETER**	**OBSERVATION**	**RESULT**
1	Test for Potassium	Yellow colour precipitate	+ ve
2	Test for Calcium	White colour precipitate	+ve
3	Test For Magnesium .	White colour precipitate	+ve
4	Test For Sodium	Intense yellow colour	+ ve
5	Test for Iron (Ferrous)	Blood red colour	+ve
TEST FOR ACID RADICALS			
S.NO	**PARAMETER**	**OBSERVATION**	**RESULT**
6	Test for Sulphate	Formation of white precipitate	+ ve
7	Test for Phosphate	Formation of Yellow precipitate	+ ve

3.4. Elemental Analysis of KVNC

From the results of the present investigation it was evident that the heavy metals such as aluminium, cadmium, mercury, and Lead were found below detection level. Further it was notifed the presence of the following the elements such as calcium (221.160 mg/L), potassium (43.114 mg/L), magnesium (01.324 mg/L) sodium (05.310 mg/L),phosphorus (104.341 mg/L) and zinc (01.208 mg/L) in the formulation at the desired level. The results were tabulated in table 5.

Table 5 : Results of Elemental analysis of *Keelvayu Nivarana Chooranam*

S. NO	ELEMENTS	DETECTED LEVELS
1	Al 396.152	BDL
2	As 188.979	BDL
3	Ca 315.807	221.160 mg/L
4	Cd 228.802	BDL
5	Cu 327.393	BDL
6	Hg 253.652	BDL
7	K 766.491	43.114 mg/L
8	Mg 285.213	01.324 mg/L
9	Na 589.592	05.310 mg/L
10	Ni 231.604	BDL
11	Pb 220.353	BDL
12	P 213.617	104.341 mg/L
13	Zn 206.200	01.208 mg/L

BDL- Below Detective Level

4. Discussion

Medicine from Siddha system is used to cure diverse diseases like skin problems (psoriasis), sexual transmitted diseases, urinary tract infections, liver and gastro-intestinal diseases, diabetes, general debility, postpartum anaemia, diarrhoea, rheumatic diseases, prostate enlargement, bleeding piles, peptic ulcer, venereal diseases, fever, allergic disorders and general fevers other than emergency cases [13].

Standardization becomes highly mandatory as it evident the physiochemical, phytochemical as well as the bioactive component profile of the siddha preparations.Organoleptic property of the KVNC with respect to its brown color, pleasant odor, bitter taste and fine powder nature justifies the genunity, purity and quality of the finished formulation with respect to its color and fine powdered texture. The results obtained from the physicochemical evaluation revels that the total ash value of KVNC was 4.78 % in which the water soluble ash value was 2.75% and acid insoluble ash value was 0.40 %. Loss on drying value of the formulation was found to be 5.20 % in which the water soluble extractive value was 16.65% and alcohol soluble extractive value was 16.10 % respectively. pH of the formulation KVNC was found to be 5.58.

Approximately 25,000 effective plant-based formulations are available in indian system of medicine which is commonly used by rural and ethnic people in India and the popularity of such medicine is also increasing among the common people. It was also estimated that >2000 tons of medicinal plant raw material is required annually. The result of the qualitative phytochemical analysis indicates that the formulation KVNC shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, phenols and proteins. The results of HPTLC analysis of the sample KVNC reveals the presence of 10 prominent peaks corresponds to 10 different compound's with Rf value ranging from 0.04 to 0.70 with percentage area of 3.39 to 23.68%. The bands revealed presence of six green, two blue and one fluorescent yellow, bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.

Heavy metals are often being present in the siddha preparation as an additive and trace by knowing or unknowingly. But prolong exposure towards certain heavy metals greatly affects the respiratory, cardiovascular, excretory and nervous system by induction of inflammation and further leads to loss of physiology. From the results of the present investigation it was evident that the heavy metals such as aluminium, cadmium, mercury, and Lead were found below detection level. Further it was notifed the presence of the following the elements such as calcium (221.160 mg/L), potassium (43.114 mg/L), magnesium (01.324 mg/L) sodium (05.310 mg/L),phosphorus (104.341 mg/L) and zinc (01.208 mg/L).

More than 1500 herbals are also sold as dietary supplements or ethnic traditional medicines. It was also estimated that nearly 960 species of medicinal plants are in trade, among them 178 species have annual consumption levels more than 100 metric tones. Domestic trade of AYUSH industry is approximately INR. 80–90 billion, and export value of medicinal plants and related products from India is approximately l10 billion. In 2012–2013, the export of AYUSH products was INR. 24,741.2 crores, though in next financial year (2013–2014) it was reduced slightly. The percentage share of AYUSH products in the total trade of India in 2013–2014 was 0.36%. The global market for herbal drugs is increasing in steady manner and the global herbal trade will reach USD 7 trillion by 2050 [14-15].

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5388083/ - bib58

All the substances in the universe, including plants, are composed of chemical com-pounds. To study herbal medicine, the major bioactive chemical components should be first known. Only after the biological compounds in herbs are correctly extracted, isolated, and identified can biochemical, biological, or pharmacological studies be performed scientifically.

Medicine from Siddha system is used to cure diverse diseases like skin problems (psoriasis), sexual transmitted diseases, urinary tract infections, liver and gastro-intestinal diseases, diabetes, general debility, postpartum anaemia, diarrhoea, rheumatic diseases, prostate enlargement, bleeding piles, peptic ulcer, venereal diseases, fever, allergic disorders and general fevers other than emergency cases [13].

Standardization becomes highly mandatory as it evident the physiochemical, phytochemical as well as the bioactive component profile of the siddha preparations.Organoleptic property of the KVNC with respect to its brown color, pleasant odor, bitter taste and fine powder nature justifies the genunity, purity and quality of the finished formulation with respect to its color and fine powdered texture. The results obtained from the physicochemical evaluation revels that the total ash value of KVNC was 4.78 % in which the water soluble ash value was 2.75% and acid insoluble ash value was 0.40 %. Loss on drying value of the formulation was found to be 5.20 % in which the water soluble extractive value was 16.65% and alcohol soluble extractive value was 16.10 % respectively. pH of the formulation KVNC was found to be 5.58.

Approximately 25,000 effective plant-based formulations are available in indian system of medicine which is commonly used by rural and ethnic people in India and the popularity of such medicine is also increasing among the common people. It was also estimated that >2000 tons of medicinal plant raw material is required annually. The result of the qualitative phytochemical analysis indicates that the formulation KVNC shows the presence of biologically significant phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, phenols and proteins. The results of HPTLC analysis of the sample KVNC reveals the presence of 10 prominent peaks corresponds to 10 different compound's with Rf value ranging from 0.04 to 0.70 with percentage area of 3.39 to 23.68%. The bands revealed presence of six green, two blue and one fluorescent yellow, bands showing the presence of alkaloids, glycosides, phenols, triterpenes, flavonoids and quinines.

Heavy metals are often being present in the siddha preparation as an additive and trace by knowing or unknowingly. But prolong exposure towards certain heavy metals greatly affects the respiratory, cardiovascular, excretory and nervous system by induction of inflammation and further leads to loss of physiology. From the results of the present investigation it was evident that the heavy metals such as aluminium, cadmium, mercury, and Lead were found below detection level. Further it was notifed the presence of the following the elements such as calcium (221.160 mg/L), potassium (43.114 mg/L), magnesium (01.324 mg/L) sodium (05.310 mg/L),phosphorus (104.341 mg/L) and zinc (01.208 mg/L).

More than 1500 herbals are also sold as dietary supplements or ethnic traditional medicines. It was also estimated that nearly 960 species of medicinal plants are in trade, among them 178 species have annual consumption levels more than 100 metric tones. Domestic trade of AYUSH industry is approximately INR. 80–90 billion, and export value of medicinal plants and related products from India is approximately l10 billion. In 2012–2013, the export of AYUSH products was INR. 24,741.2 crores, though in next financial year (2013–2014) it was reduced slightly. The percentage share of AYUSH products in the total trade of India in 2013–2014 was 0.36%.

The global market for herbal drugs is increasing in steady manner and the global herbal trade will reach USD 7 trillion by 2050 [14-15].

All the substances in the universe, including plants, are composed of chemical com-pounds. To study herbal medicine, the major bioactive chemical components should be first known. Only after the biological compounds in herbs are correctly extracted, isolated, and identified can biochemical, biological, or pharmacological studies be performed scientifically.

5. Conclusion

From the result obtained from the present investigation it was concluded that the formulation *Keelvayu Nivarana Chooranam* (KVNC) possess significant biologically active phyto therapeutics and may act therapeutically in treating several disorder's. Further present investigation had generated an evidence based data with respect to purity, standards and phytochemical nature of the formulation KVNC.

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