INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 9, Issue 3 - 2022

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2022.09.03.001

Formulation and Standardization of Siddha drug - Paandu Kudineer (PDK)

Vijayalakshmi V[1], Victoria S[2], Shanmugapriya C[3], Manju Hemamalini N[4]
[1]Post Graduate, Department of KuzhanthaiMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[2]HOD, Department of KuzhanthaiMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[3]Lecturer, Department of KuzhanthaiMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.
[4]Lecturer, Department of KuzhanthaiMaruthuvam, Govt. Siddha Medical College, Chennai – 600106, Tamil Nadu, India.

Abstract

Background: A Siddha system of medicine is the oldest holistic management system with meticulously documented medicines and being practiced by a large population in south India particularly in Tamil Nadu. The system of medicine also deals with paediatrics disease in a separate chapter “balavakadam”. Among the disease of the children nutritional disorders play a major role. Paandunoi (Iron deficiency anemia) is one among the nutritional disorders which hinders the growth and development of the children. It is caused by the deficiency of iron in children. Accroding to 4th National Family Health Survey (NFHS-4) determine 79% of Indian children have anemia. One of the important medicine of Paandu Kudineer (PDK) is mentioned in Sigicha Rathna Deepam, part II - used to treat Paand unoi. PDK is consist of five major herbs. The objective of drug standardization is to ensure the quality and efficacy of medicinal products in terms of their chemical and biological properties.

Aim: The goal of this study was to standardize this traditional polyherbal preparation on the basis of qualitative and quantitative methods.

Methods: The drug was screened for Physiochemical, Phytochemical analysis and HPTLC to estimate the quality of study drug.

Result: The Paandu Kudineer is in liquid form which is dark brown in colour. It is non viscous and non greasy with aromatic in nature. The result of analysis for pesticide residue show no traces of pesticides residues in the kudineer.

© 2022, IJCRCPS. All Rights Reserved

The aflatoxin assay revealed that the PDK is free of aflatoxins. The formulation is free of microbial contamination and shows positive for the presence of alkaloids, flavonoids, steroids, coumarins, phenols, tanins, saponins, sugar, etc. The physiochemical analysis of the PDK estimates about 5.867 ± 0.7506%total ash,0.83 ± 0.06245% acid insoluble ash,27.2 ± 2.516% water soluble extractive, 22.23 ± 2.003% alcohol soluble extraction. The heavy metals such as arsenic, lead, mercury, cadmium as below detectable limits. PDK is free from specific pathogen like *E.coli*, Salmonella, Pseudomonus and *Staphylococcus aureus.*

Conclusion: The study result ensures the safety profile of the PDK – siddha polyherbal kudineer intended for paediatric usage and indicative of presence active phytocontituents that are responsible for efficacy in treating Paandu noi (IDA) in children.

Keywords: Paandu kudineer, Paandu noi, Iron Deficiency Anaemia, Physiochemical analysis, phytochemical analysis and HPTLC.

Introduction

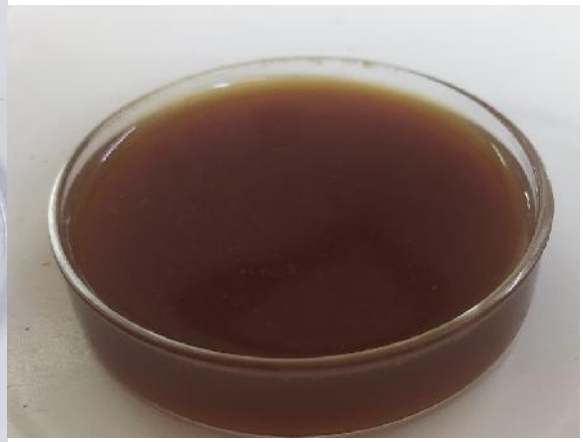
Paandu noi (Anemia) which is called as Veluppu noi in siddha medicine is a blood disorder commonly affecting children, adolescent girls, pregnant women, and menstruating women. Iron deficiency is the most common etiological factor in anaemias. In siddha system, many numbers of formulations are mentioned in various literature to treat anemia. Most of the formulations are using metals and minerals as the main ingredient of the medicine. But there are formulations that are based on only with herbal raw drugs as their ingredients. One of such herbal formulation is “Paandu Kudineer” mentioned in the text “Sigicha Rathna Deepam”. This formulation is supposed to have promising results in the disorder of anemia by improving the haemoglobin level in the blood. This results in better cognitive improvement, mental development and immune boosting mechanism of the child to have a better citizen of the country.

Materials and Methods

Drug Authentication and Preparation

PDK is a polyherbal formulation comprising of 5 types of herbs that is Keezhanelli (*Phyllanthus amarus*), Karisalanganni (*Eclipta prostrata*), Paeipudal (*Trichosanthus lobate*), Venmilagu (*Piper nigrum*), Vilvaver (*Pimpinella anisum*). The drugs were authenticated by medicinal botany department on Government siddha medical college, Arumbakkam, Chennai. The raw drugs purifications are followed by mentioned classical siddha literature. The purified raw drugs are taken in equal quantity. All the raw drugs are crushed into coarse powder, the coarse powder is taken in mod pot, 120ml of water is added and heated till it is reduced into 30ml.

Physio-chemical analysis:



© 2022, IJCRCPS. All Rights Reserved

Solubility Profile

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

Percentage Loss on Drying

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

State	Solid	Liquid
Nature	Coarse woody material	Non Viscous
Odor	Characteristic	Aromatic
Touch	Hard Texture	Non greasy
Flow Property	Non free flowing	Free Flowing
Appearance	Moderately brown	Dark Brownish

Determination of Total Ash

Test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400 ºC 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.

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.

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 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.

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 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.

© 2022, IJCRCPS. All Rights Reserved

Final Test report

S.No	Parameter	Mean (n=3) SD
1.	Loss on Drying at 105 °C (%)	2.3 ± 0.2646
2.	Total Ash (%)	5.867 ± 0.7506
3.	Acid insoluble Ash (%)	0.83 ± 0.06245
4.	Water soluble Extractive (%)	27.2 ± 2.516
5.	Alcohol Soluble Extractive (%)	22.23 ± 2.003

Phytochemical analysis

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.

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 color.

Test for saponins:

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

Test for tannins:

To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

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 choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

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 color indicates the presence of Flavonoids.

Test for phenols:

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

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.

Test for Cyanins

A.Aanthocyanin

To the test sample, 1 ml of 2N sodium hydroxide was added and heated for 5 min at 100°C. Formation of bluish green colour indicates the presence of anthocyanin.

© 2022, IJCRCPS. All Rights Reserved

Test for Carbohydrates - Benedict's test

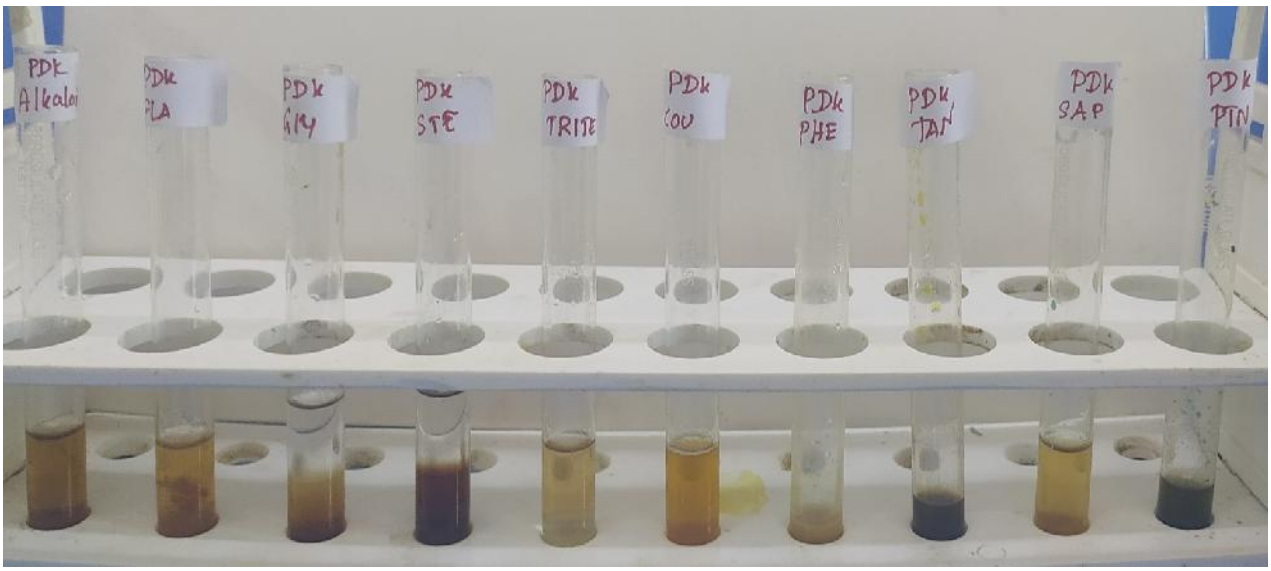
To the test sample about 0.5 ml of Benedic's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugar.

Proteins (Biuret Test)

To extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Results

Qualitative Phytochemical Investigation



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 were used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 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

High Performance Thin Layer Chromatography 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.

© 2022, IJCRCPS. All Rights Reserved

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.

Methodology for heavy metal analysis

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.

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 of HNO_3.

Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl
Cd &Pb- 100 ppm sample in 1mol/L HNO_3

Test Report

Name of the heavy metal	Absorption max	Result analysis	Maximum limit
Lead	217.0 nm	7.11 PPM	10 ppm
Arsenic	193.7 nm	0.22 PPM	3 ppm
Cadmium	228.8 nm	0.28 PPM	0.3 ppm
Mercury	253.7 nm	0.12 PPM	1 ppm

Report and Inference

Results of the present investigation have clearly shows the presence of Lead at 7.11 ppm, Arsenic at 0.22 ppm, cadmium at 0.28 ppm and Mercury at 0.12 ppm.

Methodology for Pesticide

Test sample were extracted with 100 ml of 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 40ºC until the solvent has almost completely evaporated. To the residue add a few milliliters of toluene and heat again until the acetone is completely removed. Resultant residue will be dissolved using toluene and filtered through membrane filter.

Result:

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organophosphorus, Organocarbamates and pyrethroids in the sample provided for analysis.

© 2022, IJCRCPS. All Rights Reserved

Methodology for aflatoxin:

Standard aflatoxin was applied on to the surface to pre coated TLC plate in the volume of 2.5 µL, 5µL, 7.5 µL and 10 µL. Similarly, the test sample was placed and Allow the spots to dry and develop thechromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of chloroform, acetone and isopropyl alcohol (85: 10: 5) until the solvent front has moved not less than 15 cm from the origin. Remove the plate from the developing chamber, mark the solvent from and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 365 nm.

Aflatoxin	Sample PDK	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm
B2	Not Detected - Absent	0 .1 ppm
G1	Not Detected - Absent	0.5 ppm
G2	Not Detected - Absent	0.1 ppm

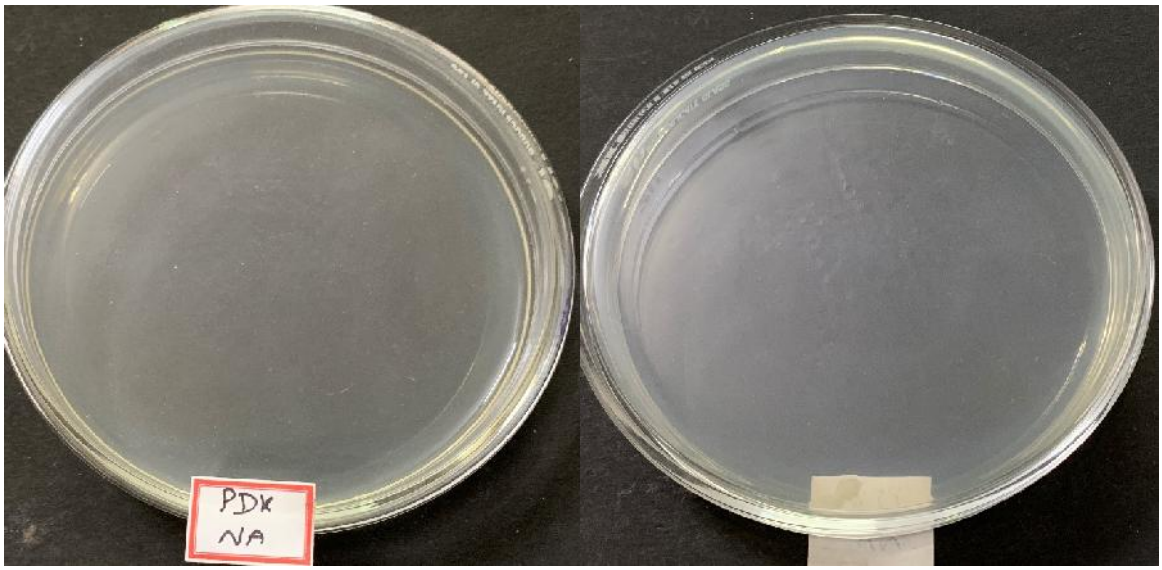
Result:

The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compare to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2.

Test for Sterility

Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45^{o}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^{o} 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.



© 2022, IJCRCPS. All Rights Reserved

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

No growth / colonies was observed in any of the plates inoculates with the test sample.

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10^5CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10^3CFU/g	

Methodology for Specific Pathogen

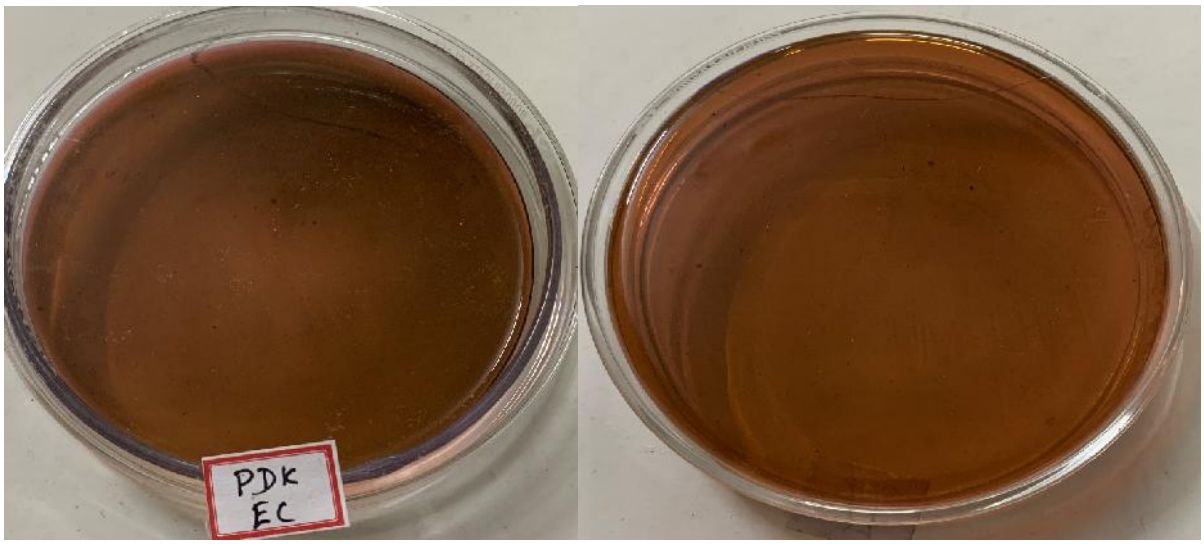
Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37^{o}C for 24 - 72h for observation. Presence of specific pathogen identified by their characteristic color with respect to pattern of colony formation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

Result

No growth / colonies were observed in any of the plates inoculated with the test sample.

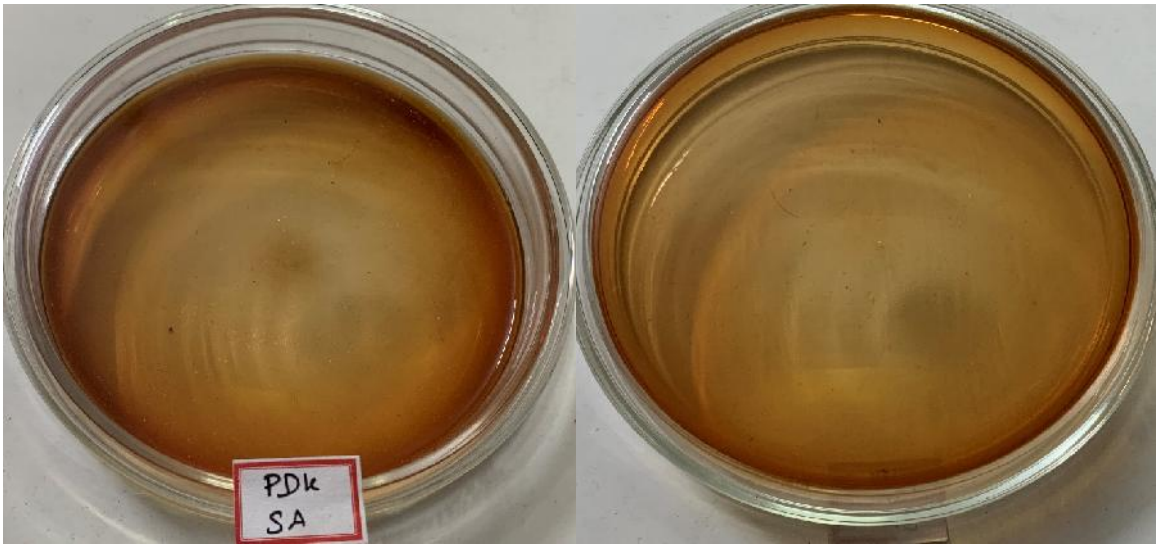
Organism	Specification	Result	Method
E-coli	Absent	Absent	As per AYUSH specification
Salmonella	Absent	Absent	
Staphylococcus aureus	Absent	Absent	
Pseudomonas aeruginosa	Absent	Absent	

Culture plate with E-coli (EC) specific medium

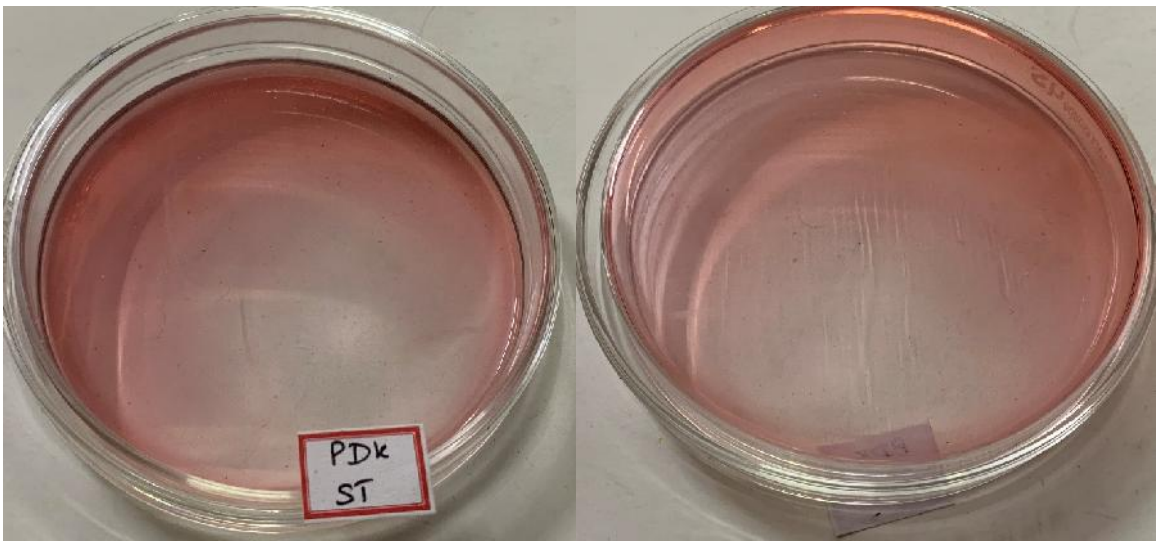


© 2022, IJCRCPS. All Rights Reserved

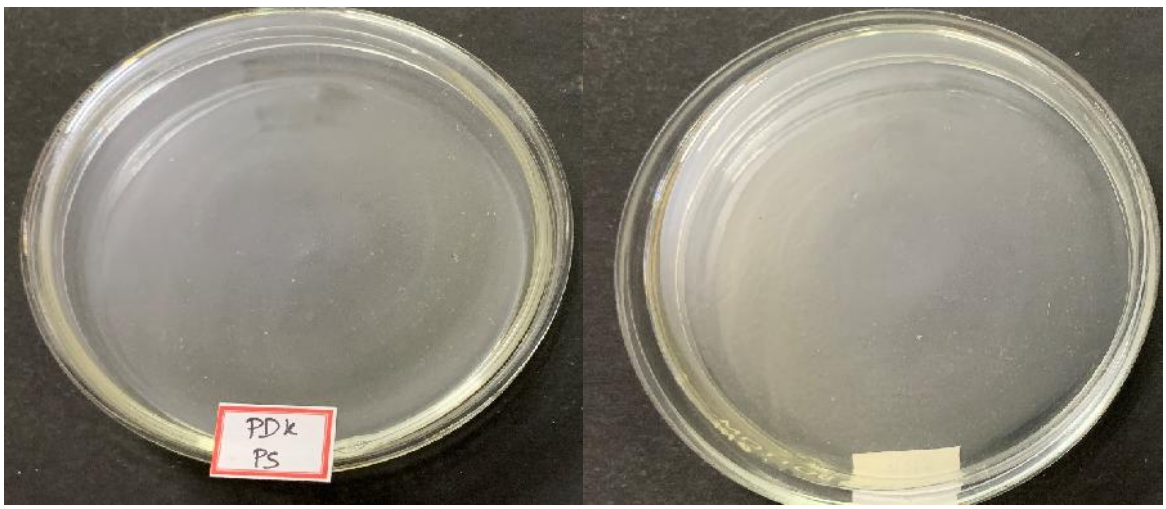
Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus aureus (ST) specific medium



Culture plate with Pseudomonas aeruginosa (PS) specific medium



© 2022, IJCRCPS. All Rights Reserved

Discussion

For the siddha formulation PDK, reports of Organoleptic, preliminary Physiochemicals, Phytochemnicals, Microbial load, Specific Pathogen screening tests are not available. Therefore discussion is made with study related to single drugs, i.e. ingredients of PDK. Preliminary phytochemical screening of extract of *Phyllanthus amarus*, it should the presence of Saponins, flavonoids, phenols, couramins, proanthocyanidins (Sowmiyamaity et, al 2013). Preliminary phytochemical screening extract of PDK is similar to above results except for couramins and proanthocyanidins. Phytochemical Screening extract of *Eclipta prostrata*, it should the presence of phenols, flavonoids, steroids, tanins, triterpenoids, diterpenes. (Preethy Jhon et, al 2018). Preliminary phytochemical screening extract of PDK is similar to above results except diterpenes and triterpenoids. Morpho-Anatomy and Phytochemical Screening extract of *Trichosanthus lobata*, it should the presence of alkaloids, flavonoids, phenols, terpenoids, (PatilKalpana et, al 2017). Preliminary phytochemical screening extract of PDK is similar to above results except triterphenoids. The petrolium either extract of *Piper nigrum* contain alkaloids, tanins, flavonoids, glycosides, phenols (Dhirender Kaushik et, al 2012). Survey of literature revealed the presence of alkaloids, tanins, phenols in the different part of piper nigrum. Screening of extract *Pimpinella anisum*, it should the presence of falvanoids, alkaloids, cardiac glycosides, tanins, phenols, terpenoids (Zubaidamarufee, et al 2019).preliminary screening extract of PDK is similar to above except cardiac glycosides and terpenoids. In the present study specific pathogenic bacteria are absent in PDK formulation. It is as per WHO norms. So it proves PDK is free from microbial contamination. The finding of this study also highlighted the safety of PDK. The information obtained from preliminary phytochemical screening will be useful in finding out the reality of the drugs.

References

1. India Pharmacopeia I Volume I, Govt. of India, Ministry of Health & Welfare, Indian Pharmacopeia Commission, 2014.
2. Pharmacopoeial Laboratory for Indian Medicine (PLIM), Guideline for standardization and evaluation of Indian Medicine include drug of Ayurveda, Unani & Siddha systems, Dept. AYUSH Ministry of Health & Family welfare, Govt. of India.
3. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975:36-45
4. Lukasz Komsta, Monika Waksmundzka-Hajnos, Joseph Sherma. Thin Layer Chromatography in Drug Analysis . CRC Press, Taylor and Francis.
5. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305, 227.
6. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues, WHO Geneva. 2007.
7. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines. Ministry of AYUSH. 2007.
8. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up with Thin Layer Chromatography and Visual and Densitometric Quantification. Ciênc. Tecnol. Aliment. vol. 21 no.1 Campinas. 2001.
9. Sowmiyamaity, Arunsharma, Evaluation of Antioxidant and characteristic of phenolic constituent of *Phyllanthus amarus*, Journal of Agricultural and Food Chemistry 61(4), Jun 2013.
10. Priya.K, Preethy John, Phytochemical analysis of *Eclipta prostrata*, International Journal of Current Microbiology and Applied Sciences, Nov 2018.

© 2022, IJCRCPS. All Rights Reserved

11. Patilkalpana, Wadekarraju, Morpho-anatomy, physiochemical and phytochemical standardization with HPTLC fingerprinting of *Trichosanthus lobate*, Journal of Natural Remedies, Feb 2017.
12. Gayathri N, Shanmugapriya C, Evaluation of siddha herbo formulation (valikana kudineer) for its physio and phytochemical analysis, World journal of pharmaceutical and Medical Research, Oct 2018.
13. Gayathri V, Victoria S, Formulation and standardization of Kana Mantha Mathirai, International Journal of Current Research in Chemistry and Pharmaceutical Science, 2021.
14. Meenakshisundaram M, Logamanian M, Physiochemical standardization of siddha syrup preparation Madhulaimanapagu, OSR Journal of Dental and Medical Science, May 2018.

Access this Article in Online	
	Website: www.ijcrcps.com
Quick Response Code	Subject: Siddha Medicine
DOI: 10.22192/ijcrcps.2022.09.03.001	

How to cite this article:
Vijayalakshmi V, Victoria S, Shanmugapriya C, Manju Hemamalini N. (2022). Formulation and Standardization of Siddha drug - Paandu Kudineer (PDK). Int. J. Curr. Res. Chem. Pharm. Sci. 9(3): 1-11.
DOI: http://dx.doi.org/10.22192/ijcrcps.2022.09.03.001

© 2022, IJCRCPS. All Rights Reserved