Research Article

Int J. Pharm. Drug. Anal, Vol: 10, Issue: 2, 2022; 38-45

International Journal of Pharmaceutics and Drug Analysis

Available at www.ijpda.com ISSN: 2348:8948

Pharmaceutical Standardization of Siddha Herbo-Mineral formulation of Kodi Pavala Chunnam

Govindaraj B [*1], Vajahath Ali A[2], Saravana Devi M.D[3], Velpandian V [4]

[*1] PG Scholar, Post Graduate Department of Gunapadam (Pharmacology), Govt. Siddha Medical College, Chennai, Tamilnadu, India

[2] PG Scholar, Post Graduate Department of Gunapadam (Pharmacology), Govt. Siddha Medical College, Chennai, Tamilnadu, India

[3]Professor, Head of the department, Post Graduate Department of Gunapadam (Pharmacology), Govt.Siddha Medical College, Chennai, TN, India

[4]Professor, PG, Gunapadam Department (Pharmacology), Govt.Siddha Medical College, Chennai, TN, India

Received: 23 March 2022 Revised: 05 April 2022 Accepted: 20 May 2022

Abstract

Background: The present study has anticipated the perspective of physicochemical characteristics of the traditional Siddha medicine Kodi pavala chunnam. It is a herbo mineral formulation that treats Cholelithiasis & its related symptoms. Materials and Methods: Kodi Pavala Chunnam was prepared as per the formulae in the Siddha literature, Noigaluku Siddha parigaaram part 2. The prepared drug underwent physicochemical, biochemical, organoleptic, and phytochemical analysis and HPTLC. Results: Loss on drying at 105°C was 0.8667 ± 0.4041, Total ash (%) was 55.67 ± 9.603, Acid insoluble ash (%) 0.64 ± 0.02, Water soluble extractive was 3.1 ± 1.044, Alcohol soluble extractive (%) was 0.2533 ± 0.0611, the PH was eight which indicates alkaline nature of the drug. From the results, the drug had no microbial contamination. Heavy metals and Pesticide residues were detected in below the quantification limit. **Conclusion:** The outcome indicates that the medication is of expected standard and can be utilized as a standardization parameter in laying pharmacopeia standards. The results obtained in the above investigations concluded that the drug is effective biologically active components that may help treat Cholelithiasis and its related ailments.

Keywords: *Cholelithiasis, Kodi pavala chunnam, herbo mineral, Siddha medicine, alkaline.*

***Corresponding Author**
Govindaraj B

DOI: https://doi.org/10.47957/ijpda.v10i2.503

Produced and Published by
South Asian Academic Publications

Introduction

The traditional Siddha system of medicine possesses tremendous administration in treating various diseases **[1].** A herbo mineral formulation named *Kodi pavala chunnam* helps treat Cholelithiasis and related ailments such as cramping pain, indigestion, nausea, and vomiting. Chunnam is one of the Thirty-two forms of enteral medicines are mentioned in Siddha literature, manafactured by applying heat and calcination following the procedure mentioned in the formulations. Medicinal products such as herbs or minerals, either individually or combined, are ground in calves by adding juices, then dried and kept in moose (Crucible), which is kept in the furnace and made into fine powder. It becomes red by mixing with water and turmeric as it has lime (Calcium). The life period of chunnam is 500 years **[2].** It possesses alkaline properties likely limestone (Chunnam). However, the scientific evidence of *Kodi pavala chunnam* had not yet been reported. Hence the current study was done to identify the

physicochemical, biochemical, and phytochemical analysis of Siddha formulation *Kodi pavala chunnam* according to PLIM guidelines.

Materials and Method

The test drug *Kodi pavala chunnam* is a poly-herbal formulation mentioned in the *Siddha* literature "*Noikalukku Siddha Parigaram*-part -2" **[3]** indicated as Gall bladder stone and other hepatic lesions. Ingredients of *Kodipavala chunnam* were tabulated in Table-1.

Table-1: Ingredients of Kodi *pavala chunnam*

S. No	Name of Drugs	Botanical Name	Part Used	Quantity
1.	Coral	Corallium rubrum	Whole part	35grams
2.	Thaivelai	Cleome gynandra	Leaves	Q.S
3.	Lemon	Citrus limon	Fruit	Q.S

The Drug Materials Are Collected

The raw drug *coral* was obtained from the Ramasamy Chettiyar Store, Parry's Corner, Chennai, Tamilnadu. Thaivelai (Cleome gynandra) and Elumitchai (Citrus limon) were collected in and around Dharmapuri district, Tamilnadu.

Drugs' Identification and Authentication

Botanists and experts from the Govt Siddha Medical College's PG Gunapadam (pharmacology) Department in Arumbakkam, Chennai, identified and validated all of the substances. Corallium rubrum, Cleome gynandra, and Citrus limon specimens were tagged 1011/PGG/321912103/GSMC-CH/2019-2022 to 1013, respectively. For future reference, the labelled specimens are stored in the PG Gunapadam laboratory.

Purification of Medications

The purification process [4] was done as per Indian material medica. The coral was taken into the mud pot, and lime juice was added to the mud pot until the coral got immersed. Then the mouth of the mud pot was closed with the other proper mud plate. This was kept alone for 24 hours; then, coral was taken from the mud pot, washed with hot water, and allowed to dry well—the dried coral was stored in an airtight container.

Preparation of Kodi Pavala Chunnam

35gm of purified coral was taken and ground with lemon juice for one day, made into small pellets, and dried. Enveloped by a paste of *Cleome gynandra* leaves and allowed to dry, subjected to incineration with 300 cow dung cakes in a pit. After being cooled, the product obtained was taken into a dish, sprinkled some boiled cow's milk over it, and kept aside. The following day morning end product Chunnam obtained was grounded and taken, which was kept in an airtight container.

Indication

As per literature, evidence will be given 2 to 4 grains (130mg-260mg) twice a day with cow's butter or honey for gall bladder stones and other hepatic lesions**.**

Qualitative Analysis Investigation

The analysis of the trial drug KPC was evaluated as per the PLIM (Pharmacopoeial Laboratory for Indian Medicines) guidelines. Physico-chemical, Phyto-chemical analysis, Biochemical analysis, Heavy metal analysis, TLC&HPTLC analysis and Sterility method, specific pathogen test, Pesticide residue analysis, and Aflatoxins assay were done at Noble Research Institute, Perambur, Chennai.

Properties for Organoleptic

The test medication's organoleptic properties were evaluated. The colour, aroma, taste, texture, particle size, and other properties of 1 gm of the test medication were evaluated in daylight with the naked eye. The result was noted.

Physico-Chemical Evaluation [5], [6]

- Physicochemical Analysis was done
- Finding total ash was done
- Determination of acid-insoluble was done
- Acid soluble extractive was done
- Water-soluble determination extractive was done
- pH determination also done
- solubility test was done
- Particle size determination was done

Phytochemical Analysis was done for the following tests [7]

- Alkaloids analysis – was performed with Mayer's Test
- Coumarins
- Saponin--Foam's Test

- Tannins-Fecl3 Test
- Glycosides - Borntrager's Test
- Flavonoids- Ammonia Test
- phenols - Lead acetate test
- steroids
- Triterpenoids -Burchard test
- Cyanins
  A. Anthocyanin
  B. Betacyanin
- Carbohydrates - Benedict's test
- Proteins -Biuret Test
- TLC [14]
- HPTLC was done [8]
- Chromatogram Development was done
- Scanning was done
- Biochemical analysis was done**[15]**
- Heavy Metal Analysis By AAS Standard was done [9]
  Standard preparation
- As & Hg- 100 ppm sample in 1mol/L HCl
- Cd & Pb- 100 ppm sample in 1mol/L HNO3
- Test for Sterility was done by Pour Plate Method [10]
- Specific Pathogen [11]

Methodology for Specific Pathogen

Table 2: A description of a particular media and its acronym

Organism	Abbreviation	Medium
E-coli	EC	EMB Agar
Salmonella	SA	Deoxycholate agar
Staphylococcus Aureus	ST	Mannitol salt agar
Pseudomonas Aeruginosa	PS	Cetrimide Agar

- Pesticide Residue was done[12], [13]
- Test for aflatoxins was done [14]

Results and Discussion

Results of Organoleptic Characters

Organoleptic characters showed that KPC is a fine powder, whitish colour, odourless, and bitter taste. Test drug KPC is shown in fig.-1. The results are tabulated in table-3.

Fig-1. Kodi Pavala Chunnam (KPC)

Table 3: Organoleptic Characters of Kodi *Pavala Chunnam*

S.No	Parameter	Result
1	State	Solid
2	Nature	Very fine
3	Odor	None
4	Touch	Soft
5	Flow property	Free-flowing
6	Appearance	Whitish
7	Taste	Bitter

Solubility Profile

Table 4: Solubility Profile of *k Kodipavala chunnam*

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

Results of Physicochemical Parameters

Table 5: Results of physicochemical analysis of *KPC*

S.No	Parameter	Mean (n=3) SD
1.	Loss On Drying At 105 °C (%)	0.8667 ± 0.4041
2.	Total Ash (%)	55.67 ± 9.603
3.	Acid Insoluble Ash (%)	0.64 ± 0.02
4.	Water Soluble Extractive (%)	3.1 ± 1.044
5.	Alcohol Soluble Extractive (%)	0.2533 ± 0.0611

Qualitative Phytochemical Screening of *KPC*

The Qualitative Chemical and Phytochemical Analysis results indicate that the medication KPC shows the presence of Alkaloids, Phenol, Tannin, Protein, and Saponins. The results were tabulated in the table,

Table 6: Result of qualitative Phytochemical Screening

S.No	Test	Observation
1	ALKALOIDS	+
2	PHENOL	+
3	TANIN	+
4	PROTEIN	+
5	SAPONINS	+

+ Indicates Positive

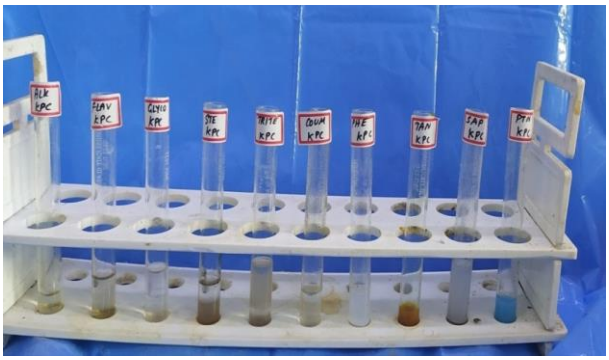


Fig 2. Qualitative Chemical and Phytochemical Analysis of KPC

HPTLC Analysis of KPC

The sample's HPTLC fingerprinting analysis reported the existence of two significant peaks, indicating the available of two different Phyto components. The peaks' Rf values range from 0.02 to 0.77.

Fig 3: TLC Chromatogram of KPC

TLC Visualization of KPC at 366 nm

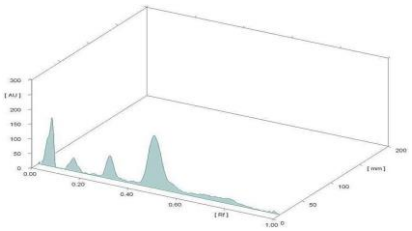


Fig 4. HPTLC Chromatogram

3D-Chromatogram

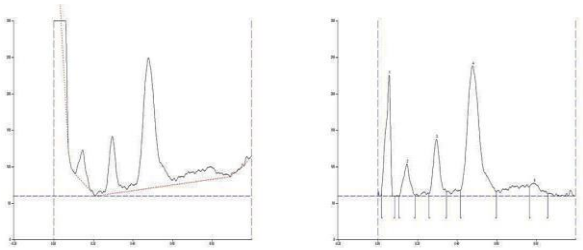


Fig 5. HPTLC fingerprinting

Table 7: HPTLC Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	3.6	0.06	166.0	34.24	0.08	0.0	1885.2	19.58
2	0.11	0.2	0.15	44.4	9.16	0.19	1.0	677.5	7.04
3	0.26	3.6	0.30	78.0	16.09	0.35	5.8	1267.0	13.16
4	0.42	8.1	0.48	178.7	36.86	0.60	6.5	5339.5	55.45
5	0.77	13.4	0.79	17.8	3.66	0.86	3.3	460.3	4.78

AAS's Heavy Metal Analysis

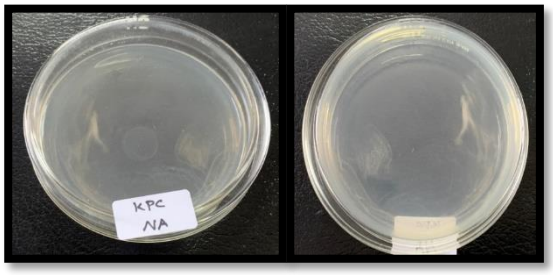
The present investigation revealed that the KPC included no traces of heavy metals such as arsenic, mercury, lead, or cadmium. The observed results of heavy metals analysis were tabulated in Table-8.

Table 8: Results of Heavy Metal Analysis of *KPC*

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	PDF	Ten ppm
Arsenic	193.7 nm	PDF	Three ppm
Cadmium	228.8 nm	PDF	0.3 ppm
Mercury	253.7 nm	PDF	One ppm

PDF- Below Detection Limit

Test for Sterility Using the Pour Plate Method



Observation

Following the incubation time, no growth was seen. Shows that a certain pathogen is not present.

Result

In any plate inoculated with the KPC, there was no growth/colonies.

Table 9: Result of sterility test of KPC

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	

Results for Specific Pathogen

The test results of specific pathogen screening for *E.coli, Salmonella, Staphylococcus Aureus* and *Pseudomonas Aeruginosa* in test drug KPC reveal no traces of the four mentioned specific pathogens. The results were tabulated in Table-10—culture plates in Fig 6, Fig 7, Fig 8, and Fig 9.

Table 10: Result of Specific Pathogen of KPC

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

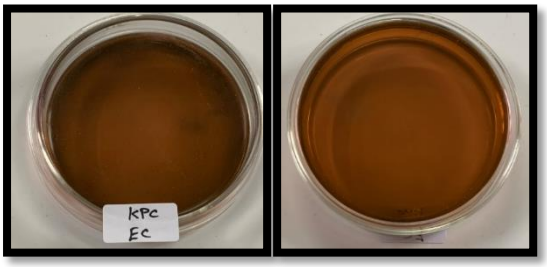


Fig 6. E. coli (EC) specific media in a culture plate

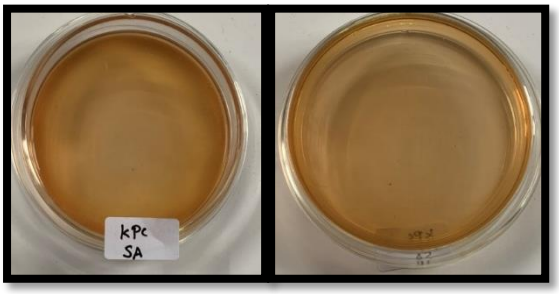


Fig 7. Salmonella-specific media on a culture plate

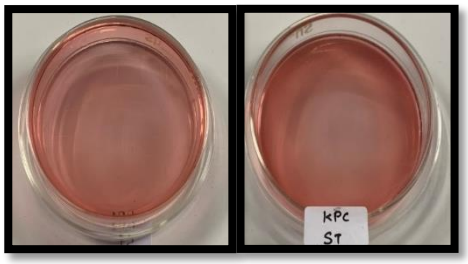


Fig 8. Staphylococcus Aureus (ST) specific media on a culture plate

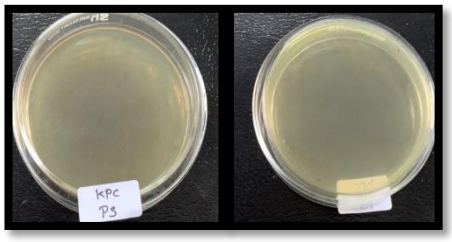


Fig 9. Pseudomonas Aeruginosa (PS) specific media on a culture plate

Results for Pesticide Residue

Analysis of pesticide residues reveals that KPC contains no traces of residues of organochlorine, organophosphorus, and pyrethroids. The observed results of pesticide analysis were tabulated in table-11.

Table 11: Result of Pesticide Residue of *KPC*

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

BQL- Below Quantification Limit

Result for Aflatoxin by TLC (B1, B2, G1, G2)

The results showed that no spots were identified when KPC loaded on TLC plates compared to the standards,

indicating that the KPC was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2.

Table 12: Aflatoxin Study of KPC

Aflatoxin	Sample KPC	AYUSH Specification Limit
B1	Not Detected	0.5 ppm
B2	Not Detected	0.1 ppm
G1	Not Detected	0.5 ppm
G2	Not Detected	0.1 ppm

Results for Acid and Basic Radicals

Results for Acid Radicals

The biochemical analysis reveals the presence of carbonates and phosphates in KPC. These were tabulated in table-13.

Table 13: Results of Acid Radicals of KPC

Specific Radical	Test Report
Test for carbonates	Positive
Test for phosphates	Positive

Results for Basic Radicals

The biochemical analysis of the KPC reveals the absence of essential radicals.

Discussion

The drug KPC was an excellent powder whitish in color with odorless. Organoleptic properties, color, texture, taste, and delicate powder nature indicate the ingenuity, purity, and quality of the test drug *Kodi Pavala Chunnam.* The loss on drying value denotes the drug's moisture content, which was evaluated as 0.8667 ± 0.4041%. The moisture content of the herbal medicine should be minimized as it encourages the growth of living organisms, fungi, or insects and causes deterioration following hydrolysis [16]. For herbal drugs, the moisture content must be less than 14% [17]. The total ash value of the test drug KPC was 55.67 ± 9.603%. The value of insoluble acid ash was 0.64 ± 0.02%, indicating that the drug contains siliceous matter in not significant amount. Ash value is one of the most significant measurable factors for the standardization of the herbal drug. A high ash value represents the presence of more inorganic residues such as phosphates, carbonates, and silicates [18]. The water and alcohol soluble extractive values were determined as 3.1 ± 1.044% and 0.2533 ± 0.0611%, respectively. Extractive values are helpful in the determination of the amount of the phytoconstituents exist in the herbal drug and helpful in evaluating the percentage of chemical soluble in a particular solvent [19]. The pH value is eight, which indicates that the drug is weekly alkaline. Solubility property impacts the bioavailability and pharmacological efficacy of a drug. KPC is highly soluble in water, soluble in ethanol, dimethyl sulfoxide, and insoluble in chloroform and ethyl acetate [20]. Qualitative Preliminary Phytochemicals test result reveals the presence of alkaloids, phenol, tannin, saponin, and protein in the test drug KPC. Phytochemicals are chemical compounds present in plants and act as pharmacologically active substances helpful in treating human diseases as medicinal ingredients and nutrients. These phytochemicals are responsible for the potential therapeutic properties of KPC [21]. Alkaloids are nitrogen-containing bases used as anti-cancerous, sedatives, anti-microbial, and insecticidal. Phenols possess anti-bacterial and anti-fungal activity [22]. Tannins are polyphenolic compounds that regulate glucose levels in the blood by stimulating receptor cells to bind carbohydrates [23]. HPTLC fingerprinting analysis of the sample KPC reveals the existence of different phyto components. Test for heavy metals report that the test drug KPC has lead, arsenic, cadmium, and mercury below the detection limit. Results of the present investigation have clearly shown that the sample KPC has no traces of heavy metal lead. Heavy metal represents a metallic chemical element with a relatively high density and is poisonous even at minute concentrations. Some metals like mercury, lead, arsenic, and cadmium cause poisonous and carcinogenic effects when administrated even at low concentrations [24]. No growth/colonies were observed in any plates injected with the test sample KPC indicates that the test drug is sterile and free from bacterial and fungal contamination. KPC has pesticides below the quantified limit which means there is no pesticide contamination. Pesticides are chemical compounds used to control or eradicate pests. According to their chemical structure, they are classified into Organo Chlorine, Organo Phosphate, and Pyrethroids. When these pesticides are used on herbal plants during agricultural practices will remain in plants that become a significant source of contamination of herbal medicines. As pesticides cause several health issues, it is necessary to determine the number of pesticides in herbal medicines [25]. When comparing the test sample packed on TLC plates to the standard, no sites were discovered, indicating that the sample was not contaminated with Aflatoxin B1, Aflatoxin B2,

Aflatoxin G1, or Aflatoxin G2. Aspergillus flavus, Aspergillus parasiticus, and other Aspergillus species create aflatoxins, which are harmful fungal secondary metabolites. The aflatoxins in herbal remedies must be determined since they are very poisonous, carcinogenic, mutagenic, teratogenic, and hepatotoxic [26]. The presence of carbonates and phosphates is revealed by an acid radical test. Carbonate maintains pH and acid balance in numerous bodily regions as bicarbonate ions.

Conclusion

Although there are numerous medicines mentioned in the Siddha literature, there is no specific potent medicine mentioned to treat cholelithiasis. At the same time, many medicines mentioned in Siddha literature proved helpful in treating cholelithiasis but lacked scientific validation and standardization. But standardizing the treasurable medicines present in the Siddha literature, we can treat the cholelithiasis effectively. From the results obtained in the above investigations, it was concluded that the Siddha herbo mineral formulation *KPC* having biologically effective active components, may help in treating cholelithiasis and its related ailments. Investigation of those specifications utilising cutting-edge analytical techniques helps in the standardization of *KPC*. Hence, this present investigation has generated evidence-based data regarding purity, standard, physicochemical, phytochemical, and biochemical nature of the formulation *KPC*.

Acknowledgements

I am so grateful to Tamil Nadu Dr. MGR Medical University, Chennai, Principal and faculties of PG Gunapadam Department of, Govt. Siddha Medical College, Chennai.

Conflict of Interest

No conflict of interest.

Funding

No Funding

Author Contribution

All authors contributed equally.

References

1. Introduction and history of Siddha [Internet]. National Health Portal. 2015. Available from: https://www.nhp.gov.in
2. R.Thiagarajan B.I.M.Gunapadam Thathu Jeeva Vaguppu 4th Edition 2004; Published By Indian Medicine and Homeopathy Dept.Chennai 106;p.
3. M.Shanmugavel H.B.I.M.Noigalukku Siddha Parigaram Part-2.1993; Department of Indian medicine and Homeopathy, Chennai-106;p.73-74.
4. Aanaivaari Anadhan. Sarakku Suththi Muraigal. Department of Indian medicine and Homeopathy, Chennai-106; 2008.p. 6-13.
5. .India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopoeia commission, 2014.
6. Protocol for Testing of Ayurvedic Siddha and Unani medicines [Internet]. Ghaziabad: Department of AYUSH, Pharmacopoeial Laboratory for Indian Medicines; 2008. P. 49-50. Available from: https://www.researchgate.net
7. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975.p. 36-45
8. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas.2nd ed. Heidelberg: Springer-Verlag Belgium; 2002. p. 305, 227.
9. Protocol for Testing of Ayurvedic Siddha and Unani medicines [Internet]. Ghaziabad: Department of AYUSH, Pharmacopoeial Laboratory for Indian Medicines; 2008. p. 69-73.Available from: https://www.researchgate.net
10. Pour Plate Method: Procedure, Uses, (Dis) Advantages • Microbe Online [Internet]. Microbe Online. 2022 [cited 13 April 2022]. Available from: https://microbeonline.com
11. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-11th ed. CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
12. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues [Internet]. 2007. Available from: https://www.who.int
13. Protocol for Testing of Ayurvedic Siddha and Unani medicines [Internet]. Ghaziabad: Department of AYUSH, Pharmacopoeial Laboratory for Indian Medicines; 2008. p. 94-97.Available from:

https://www.researchgate.net

14. Castro L de, Vargas EA. Determining aflatoxins B1, B2, G1 and G2 in maize using florisil clean up with thin layer chromatography and visual and densitometric quantification. Food Sci Technol [Internet]. 2001;21(1):116. Available from: http://dx.doi.org/10.1590/s0101-20612001000100024
15. Khandelwal K. Practical Pharmacognosy. Maharashtra: Niral Prakashan; 2008.
16. Nilakshi Pradhan, Jyoti Gavali,Nitin Waghmare. WHO (World Health Organization) Guidelines for Standardization of Herbal Drugs. International Ayurvedic Medical Journal. 2015;3(8):2239.
17. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. 20th ed. Pune, India: Nirali Prakashan; 2010. p. 23.1-23.17, 25.1-25.9
18. Aziz N, Wal P, Wal A, et.al. Evaluation of a Poly-herbal Powder for Treatment of Diabetes Mellitus. Indian Journal of Pharmaceutical Sciences. 2019;81(6).1072
19. Kaneria M, Chanda S. Phytochemical and Pharmacognostic Evaluation of Leaves of Psidium guajava L. (Myrtaceae). Pharmacog J. 2011;3(23):41–45
20. Savjani KT, Gajjar AK, Savjani JK. Drug Solubility: Importance and Enhancement Techniques. ISRN Pharmaceutics. 2012;2012:195727:2
21. L Patwekar, S., Arvind B, S. and Manoj, G., et.al 2016. Standardization of herbal drugs: An overview. The Pharma Innovation Journal, 4, p.100.
22. Babu, Ashok, Mahalakshmi, Vasudevan. Medicinal uses of plant secondary metabolites: A short review. International Journal of Zoological Investigations [Internet]. 2021;7(2):855–8.
23. Medicinally Important Phytochemicals: An Untapped Research Avenue. Research & Reviews: Journal of Pharmacognosy and Phytochemistry. 2016;4(1).40
24. S.Shaban n. Beni-Suef University Journal of Basic and Applied Sciences. 2016;5:103-104.
25. Siddique N, Mujeeb M, Ahmad S, Panda B, Makhmoor M. Determination of Aflatoxins in Medicinal Plants by High-Performance Liquid Chromatography–Tandem Mass Spectrometry. J Pharm Pharm Sci.2013;16(2):321
26. Seethalakshmi B, Kavitha K. Qualitative Analysis of Inorganic Acid, Basic Radicals, and Estimation of Biomolecules in the Flower Extract of *Butea Superba Roxb*. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(10):161.