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EVALUATION OF PHYTOCHEMICAL, PHYSICOCHEMICAL AND BIOCHEMICAL ANALYSIS OF *SIDDHA* POLYHERBAL FORMULATION *THATHU BHUSTIKU CHOORANAM*

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ABSTRACT

Thathu bhustiku Chooranam is a Siddha polyherbal formulation indicated for treating male infertility. Chooranam is named for a powder made by a single or a blend of herbal parts like leaf, stem, root etc. Male infertility is increasing nowadays. Life style changes, food habits, disturbed sleep pattern, and stress all constitute male infertility. Systemic diseases like long standing hypertension and diabetes have important role in the development of male infertility. Treatment for male infertility is still unsatisfactory and AYUSH systems of Medicine advise change in the life style along with rejuvenating herbals Thathu bhustiku Chooranam is a poly herbal drug with rejuvenating potency and indicated for Thathu nattam (male inferlity). The present study aims to endow up-to-date standardized data of Thathu bhustiku chooranam (TBC) indicated for Aan maladu in Anuboga vaidhiya birahmma ragasiyam. The study show the distinct observations such as physicochemical, phytochemical, biochemical, analysis of heavy metal, microbial load, specific pathogen, pesticide residue, aflatoxin parameters are evaluated as per PLIM guidelines. The obtained results are within normal limits in this study, which is briefly described in this paper.

KEYWORDS: Thathu bhustiku chooranam, aan maladu, male infertility, physicochemical standardization, Phytochemical evaluation.

1. INTRODUCTION

Siddha medicine is a comprehensive system of medicine, described by Siddhars. Siddha Medicine emphasizes the theory of Interdependent phenomenon of the Universe and the Human Body and applies this law of nature in diagnosis and treatment of disease. "The Body is in the Cosmos, The Cosmos is in the body, whatever is present in the Cosmos, Is present in the Human body".[1]

The concept of Disease, according to the Siddha system, is explained through the Theory of Three Humours (Thiri dosha Theory). Siddha system of medicine classifies three humours namely Vali (Vatham), Azhal (Pitham), Aiyam (Kapham) and it is called this as Uyir Thathu. which are vital forces for life. Derangements in these humours causes diseases in the gross body. During every moment of life of human being, the three humours are in action through the 5 elements of nature. The three humours must have a harmonious relationship to keep the body and mind in healthy state. When they are disturbed, they bring about disease's peculiar to their influence.[2]

Apart from this it also acts as a Prophylaxis to prevent diseases. ―An Ounce of Prevention is Worth a pound of cure". There are so many ways such as Kayakalpam, Yoga, Pranayama to prevent diseases by maintaining theses humours in their optimal range. Pathyam (diet) plays a unique role in siddha medicine to prevent further complications of the existed diseases.

A remarkable increase in the usage of medicinal plant products in the form of plant extracts and their active components etc. have been observed in the past decade, among the world population as a primary health care aid. Yugi muni one of the great saint of siddha medicine classified the diseases into 4448.[3]

AAN MALADU is one among them and it is a broad term which indicates the ailments which causes to degrade the normal seminal findings. According to Yugi muni, the character of semen in aanmaladu exhibit the characteristics such as absence of sweetness, buoyancy on water and less viscus.[4]

Sukkilam is one among the seven udalthathukkal ie, constituents of gross body and it is affected in Aanmaladu. It can be termed as male Infertility in modern science. Most cases of

male infertility are due to decrease in sperm count or low sperm motility. Male Infertility is the inability of a male who is sexually active, but unable to make conception of non-contraception female to achieve pregnancy within one year of marriage. In former days infertility was considered only accompanied with women. But nowadays it is also accompanied by men. Male Infertility is blamed in 50 % of cases where couples could not conceive naturally.

As per WHO guidelines a report with count less than 15 million / ml is oligozoospermia. The most common problems a man faces are low sperm count, morphology abnormalities and motility of sperm.[5] Herbal preparations are useful in decreasing the abnormalities in semen. The study drug Thathu bhustiku chooranam which contains 3 active herbals for rejuvenation and for male infertility is taken for qualitative analysis.

The study show the distinct observations such as physicochemical, phytochemical, biochemical, analysis of heavy metal, microbial load, specific pathogen, pesticide residue, aflatoxin parameters are evaluated as per PLIM guidelines. The obtained results are within normal limits in this study, which is briefly described in this paper.

2. MATERIALS AND METHODS

Selection of the drug

In this research work, the "THATHU BHUSTIKU CHOORANAM", a poly herbal formulation, has been selected to evaluate Dhadhu Balaveenam (Oligospermia), mentioned In "Anuboga vaidhiya birahmma ragasiyam, Pg no; 77.

Ingredients of the Thathu bhustiku chooranam.[6]

Table no 1: Ingredients of the Thathu bhustiku chooranam (TBC).

S.No	NAME OF THE DRUG	SCIENTIFIC NAME	QUANTITY
1.	*Neermulli virai*	*Hygrophilla auriculata*	*1 Palam (*35 gm*)*
2.	*Salaam pisin*	*Polygonatum verticillatum*	*1 Palam (*35 gm*)*
3.	*Karuvappatai*	*Cinnamomum verum*	*1 Palam (*35 gm*)*

Source of Collection

The drug was purchased from authorized country Raw Drug Store Ramasamy chetty shop, Parrys corner Chennai.

Identification and Authentication of the drug

The collected raw materials and plants were identified and authenticated by Botanist and faculties of Gunapadam department, Government Siddha Medical College Chennai, Tamilnadu. Purification of the drug.

Purification process were done as per classical Siddha literature Sarakkugalin suthi seimuraigal.[7]

Preparation of Thathu bhustiku Chooranam

The above given ingredients were taken in an equal quantity, then pounded into fine powder. The obtained powder was then stored in clean air-tight container and named as Thathu bhustiku chooranam.

Storage of the drug

The prepared test drug was stored in a clean, dried, air tight container. The contents were explore frequently to avoid moisture and microbes.

ADMINISTRATION OF DRUG

Form of the medicine : Chooranam
Route of Administration : Enteral route
Dose : 1/2 Palam (17.5 gram)
Adjuvant : Goat's milk
Indication : Thaathu nattam

Fig no. 1 Ingredients of Thathu bhustiku chooranam.

Fig no : 1 (a) Neermulli virai-*Hygrophila auriculata.*

Fig 1: (b) karuvapattai- cinnamomum verum.

Fig 1: (c) Salampisin- Polygonatum verticillatum.

Organoleptic Properties

The state, nature, odour, feel and other macroscopic features were pointed from the preparation.

Below analysis were done in Noble Research solution, Perambur, Chennai. Analysis of TLC was performed through PLIM guidelines. Analysis of Physico chemical, Phytochemical, Bio chemical, Heavy metals, Sterility test, High performance Thin Layer Chromatography, Specific pathogen, Pesticide residue analysis, Aflotoxin assay were done.

Physico chemical Assessment[8-10]

Establish the percentage of loss on drying, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive. PH was find out. The particle size was discovered through microscopic method.

Phyto chemical Assessment[11]

Tests were helps to establish alkaloids, saponins, tannins, glycosides, flavonoids, phenols, steroids, triterpenoids and carbohydrates. Chromatographic assessment helps to evaluation of botanical materials and quality control analysis.[12,13]

Bio chemical Assessment of Basic and Acidic Radicals[14]

For spotting of carbonate, sulfate and phosphate.

Heavy Metal Analysis through Atomic Absorption Spectroscopy (AAS)[15]

Lead, Arsenic, Cadmium and Mercury were tested.

Microbial load (Sterility Test)[16]

For identification of organism, the pour plate method was implemented. Then counted the CFU accordingly.

Test for Specific pathogen[17]

Cetrimide agar, EMB agar, Mannitol salt agar, Deoxylate agar and was a specific medium used for identification of specific pathogen like Pseudomonas Aeruginosa, E.coli, Staphylococcus aureus, Salmonella respectively.

Pesticide Residue Assessment[18,19] **and Aflotoxin Assay**[20] **were evaluated.**

3. RESULTS

Result of Organoleptic Character

Finely powdered Thathu bhustiku chooranam was greenish brown in colour with pleasant odour and non free flowing nature. Results mentioned in Table no:2.

Fig no. 2: TBC Prepared drug.

Table 2: Organoleptic Characters of TBC.

S.NO	DESCRIPTION	RESULTS
1	State	Solid (Fine powder)
2	Nature	Fine powder
3	Odour	Strong Aromatic
4	Touch	Soft

5	Flow Property	Non free flowing
6	Appearance	Pale Brownish
7	Taste	Bitter

RESULTS FOR PHYSICOCHEMICAL

The physicochemical parameters of TBC were determined and the results given in Table:3.

Table 3: Results of Physicochemical Analysis of *TBC.*

S.NO	PARAMETERS	PERCENTAGE
1	Loss on drying	7.7 ± 1.127
2	Total ash value	4.733 ± 0.70
3	Acid insoluble ash	0.032 ± 0.01
4	Water soluble ash	15.2 ± 0.2
5	Alcohol soluble extraction	7.83 ± 0.87
6	pH	7.4

Determination of Particle size

Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be 79.27 ± 18.66µm.

Results of Solubility Assessment of TBC

Results mentioned in table no:4.

Table 4: Solubility Profile of *TBC.*

S.NO	SOLVENT USED	SOLUBILITY/ DISPERSIBILITY
1	Chloroform	In soluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	In soluble
5	DMSO	Soluble

Results for Analysis of phytochemical

The phytochemical parameters of TBC were determined and the results are tabulated in Table: 5.

Table 5: Result of Qualitative Phytochemical Screening.

S.NO	TEST	OBSERVATION
1.	Alkaloids	+
2.	Coumarins	+
3.	Glycosides	+
4.	SteroidS	+
5.	Tannin	+
6.	Saponin	+

7.	Phenols	+
8.	Flavonoids	+
9.	Triterpenes	+

+ Indicates positive

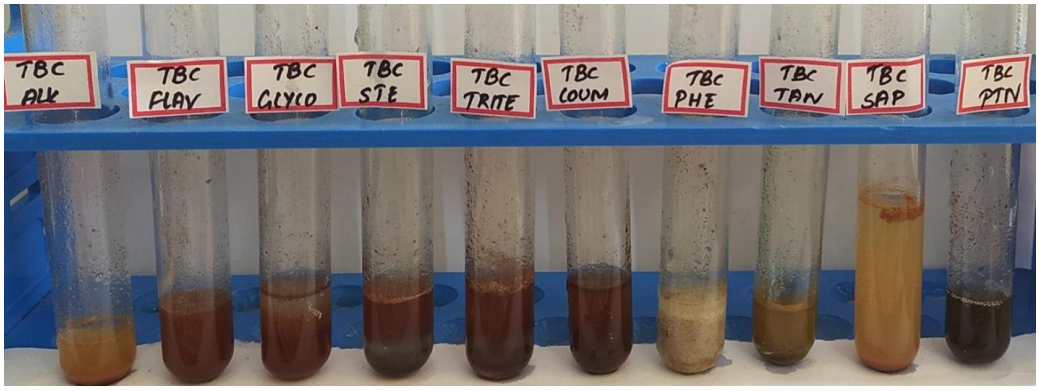


Figure no : 3 Qualitative Phytochemical Investigation.

HPTLC

Reveals eight peaks correlating with eight variable phyto components present. Rf value of the peaks ranges from 0.03 to 0.81in which highest concentration of the phytoconstituents was found to be 33.95% and 15.96% with its corresponding Rf value were found to be 0.252 and 0.05 respectively.

TLC Visualization of TBC at 366 nm

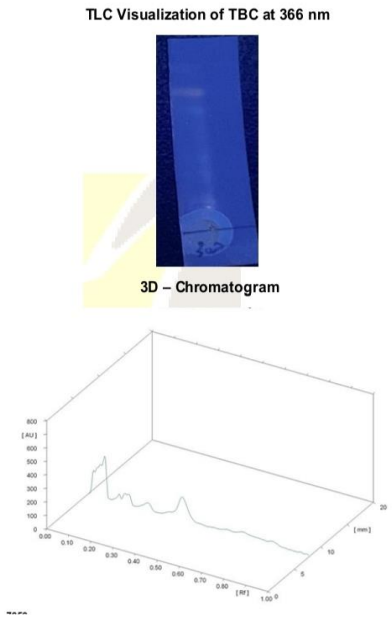
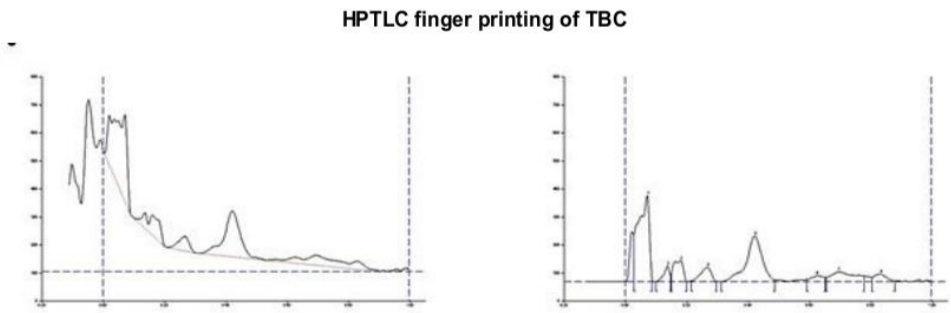


Fig no. 4: TLC Chromatogram of TBC and 3D chromatogram.



Peak Table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	167.9	0.07	308.1	41.59	0.09	6.0	5171.5	37.38
2	0.10	0.1	0.14	56.1	7.57	0.15	25.0	476.9	3.45
3	0.15	30.2	0.18	75.1	10.14	0.20	1.0	1126.0	8.14
4	0.22	3.6	0.27	52.2	7.04	0.30	0.7	854.4	6.18
5	0.31	0.2	0.42	163.8	22.11	0.49	6.3	3955.8	28.60
6	0.59	7.6	0.63	21.7	2.93	0.65	16.0	411.5	2.97
7	0.65	16.2	0.69	35.7	4.82	0.78	20.5	1309.7	9.47
8	0.81	16.1	0.83	28.2	3.80	0.88	4.0	527.7	3.81

Fig no. 5,6 HPTLC finger printing of sample TBC and 6 peak table.

REPORT

HPTLC finger printing analysis of the sample reveals the presence of eight prominent peaks corresponds to the presence of three versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.03 to 0.81.

Results for Biochemical Analysis Test for Acid Radicals Specific Radical

Test report

Test for carbonates Positive – indicates the presence.

Test for sulfates Positive – indicates the presence.

Results for Heavy Metal Analysis by Atomic Absorption Spectroscopy (AAS)

Heavy metal analysis of ASWC was determined and results are given in Table: 7

Table 7: Results of Heavy Metal Analysis of *TBC.*

Name of the Heavy Metal	Absorption Max A max	Result Analysis	Maximum Limit
Lead	217.0 nm.	0.811 PPM	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Arsenic	253.7 nm	0.167 PPM	1 ppm

Results of the current study include show that the model has no hints of heavy metals such as Lead, Arsenic, Mercury, and Cadmium.

BDL – Below Detection Limit

Microbial load (Sterility test)

No growth or colonies were noticed on pour plate. Results were tabulated in table no.8.

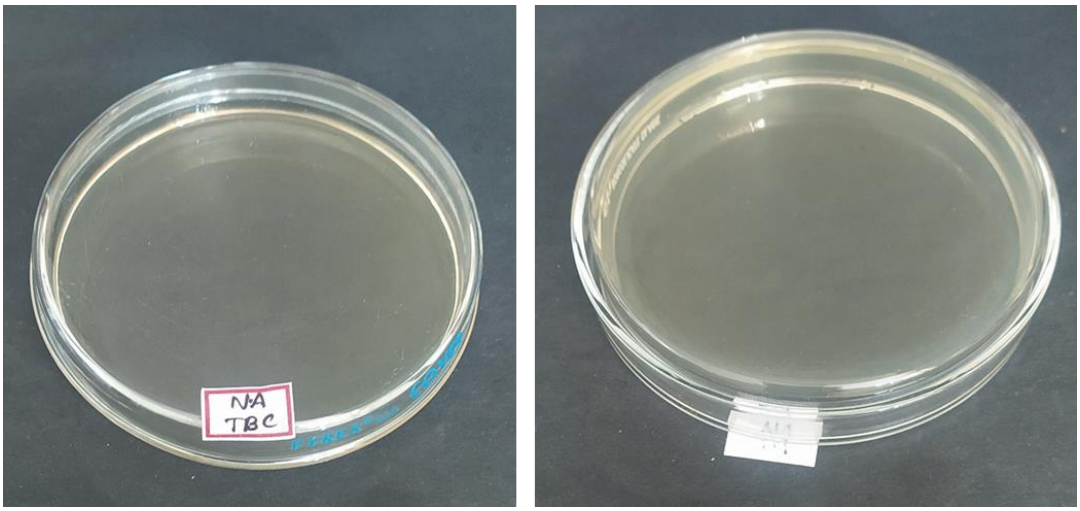


Figure no. 6: Pour plate Method for Microbial method.

Table 8: Result of Sterility test of *TBC.*

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

Results for Specific Pathogen

Table 9: Result of specific pathogen of *TBC.*

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

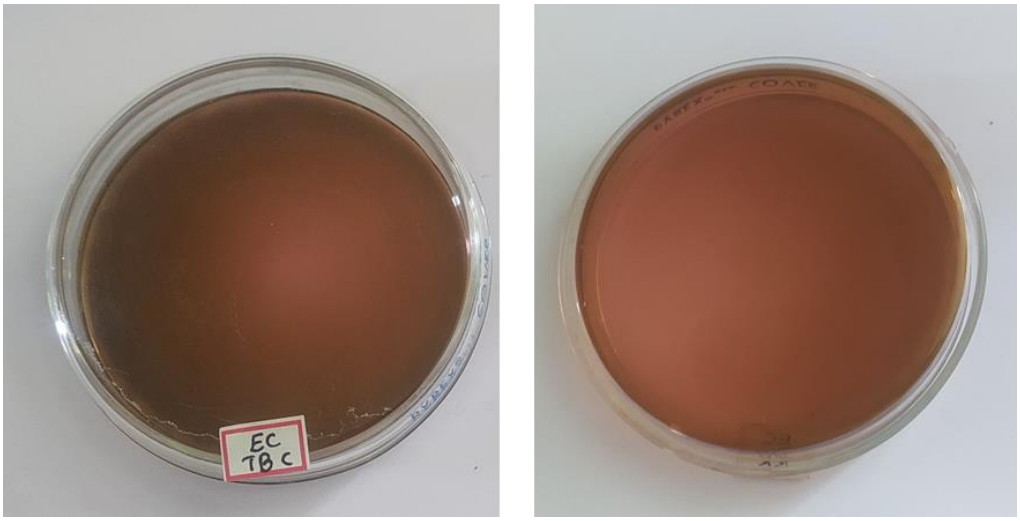


Fig no. 7: Culture plate with E-coli (EC) specific medium.

Fig no. 8: Culture plate with Salmonella (SA) specific medium.

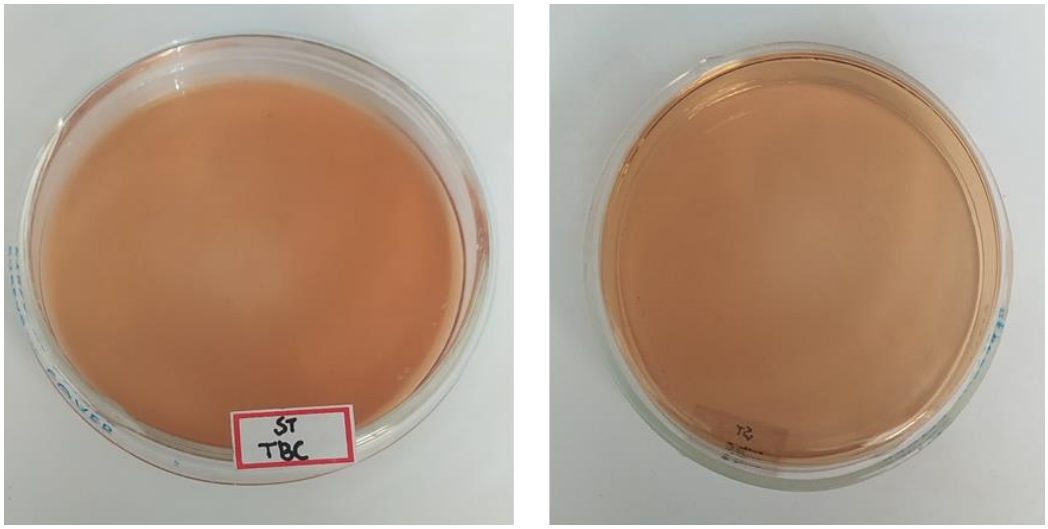


Fig no. 9: Culture plate with Staphylococcus Aureus (ST) specific medium.

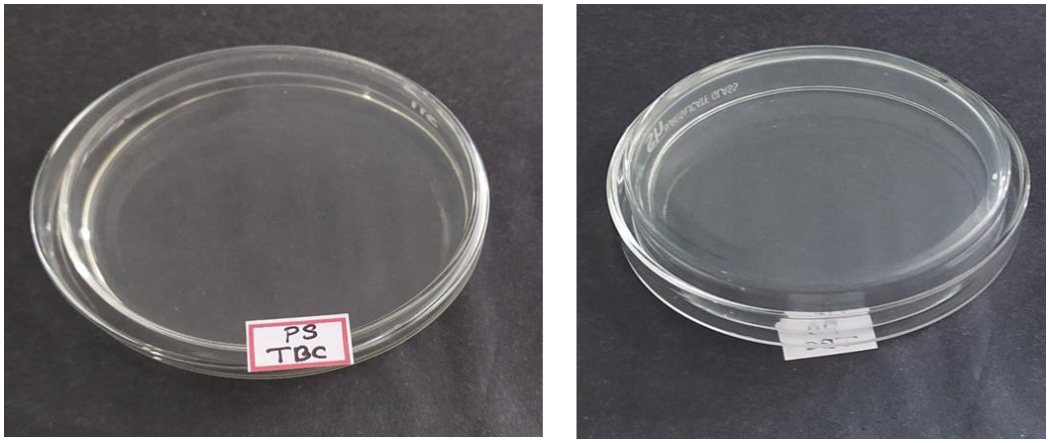


Fig no. 10: Culture plate with Pseudomonas Aeruginosa (PS) specific medium.

Results for Pesticides residues

The results demonstrated no traces of pesticide residues such as Organochlorine, Organophosphorus, Organo carbamates, and pyrethroids in the sample supplied for analysis. The outcomes are tabulated in Table: 10.

Table no 10: Result of Pesticide Residue of *TBC*.

Pesticide Residue	Sample TBC	AYUSH Limit (mg/kg)
1.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
11.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2mg/kg
Dichlorovos	BQL	1mg/kg
3. Organo carbamates		
Carbofuran	BQL	.1mg/kg
4.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 *TBC* loaded on TLC plates compared to the standards, indicating that the *TBC* was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2.

Table 11: Aflatoxin Assay for *SOVC.*

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

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.

DISCUSSION

Standardization of herbal formulations is essential to assess the drug's grade, effectiveness, and potency. The standardization of Thathu bhustikun chooranam was acquired via numerous methods by dissecting the organoleptic characters, physicochemical qualities, and elements current in the drug. The organoleptic parameters like State, Nature, Odor, Touch, Flow property, and appearance show that it is solid, soft to touch, pale brownish with a characteristic odor. This drug TBC is excellent and safe to consume. The results received from the physicochemical study of Thathu bhustikun chooranam (TBC) apparently show that soluble in primary solvents proves the efficacy of solubility in the stomach indirectly and improves the bioavailability. The pH of the drug is 7.4, which is basic. The base drug is essential for bioavailability and its effectiveness. So, the drug TBC will be absorbed better in the stomach.[20] The loss on drying value was 5.9% indicates the stability and shelf life of the drug TBC are good. The total Ash value was 9.5%, which indicates that the drug TBC has no impurities. It is safe to treat male infertility; the acid insoluble ash value of TBC was 0.004% which was less than 1% suggesting the less content of siliceous matter in the Chooranam. The watersoluble extractive is 10.73%, conveying easy facilitation of diffusion and osmosis mechanism, and the alcohol- soluble extractive is 6.933%, which reveals that the drug has good quality and purity. It indicates no impurity in the raw drug TBC.

The result of the phytochemical analysis indicates the presence of Alkaloids, Steroids, Triterpenoids, Phenol, Tannin, Saponins, Sugar. Alkaloids have potent spermatogenic, aphrodisiac, and antioxidant effects.[21] The presence of alkaloids in TBC confirms the male infertility potency of the drug. The drug contains phenols, it confirms the male infertility potency of the drug The drug contains steroids, that can regulates the hypothalamic-pituitary-

gonadal axis in turn regulate the testostoreone production and spermatogenesis.[21] Presence of Tannins ensures that antioxidant property of drug.

HPTLC fingerprinting study of the example demonstrates four major peaks compared to four versatile Phyto-components present within it. Rf significance of the peaks varies from -0.03to 0.81 So, the presence of medicinally important phytochemicals in the sample drug TBC was strengthened by TLC and comparing the Rf of the corresponding spot with that of standards. Biochemical analysis shows the presence of carbonates and sulfate that plays an immense role in biosysnthesis and detoxification via sulfation of many endogenous and exogenous compounds. Heavy metal analysis results include establishing that the sample TBC contains no traces of heavy metals like Lead, Arsenic, Mercury, and Cadmium. These outcomes indicate that the trial medication is positively secure as it has heavy metals below detection limitations. This reveals the drug is safe to consume. The sterility test outcome shows no evolution/colonies in any plates inoculated with the trial sample TBC. This demonstrated that the medication TBC is free from microorganisms and the absence of total bacterial and fungal count, which indicates that the drug TBC is of good quality and safety. A specific pathogen test showed that the drug TBC prevented the growth of microorganisms such as E.coli, Salmonella species, Staphylococcus aureus, and Pseudomonas aeruginosa, indicating that the drug TBC can be used to reduce the morbidity and mortality from chronic diseases. The aflatoxin assay outcomes revealed no spots seen in the test sample TBC crowded TLC plates compared to the standard, which indicates that the sample TBC was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, and Aflatoxin G2. So, this drug TBC is non-toxic, there is no contamination, and it does not act as a carcinogen. The pesticide precipitate outcomes demonstrated that the drug TBC has no hints of pesticide remains, such as Organochlorine, Organophosphorus, and Pyrethroids. So, this drug TBC has no toxicity and bioaccumulation. Hence, the drug TBC is a safer drug for human health in treating thrombosis.

5. CONCLUSION

It can be concluded that the analysis of Thathu bhustikun chooranam has been taken out to propose benchmarks for assessing its grade and righteousness. The analytical parameters, TLC image documentation, and HPTLC fingerprinting profile choice be essential in improving its Pharmacopoeial norms. As a result, Thathu bhustikun chooranam, a Siddha poly-herbal formulation, is subjected to many studies to validate its effectiveness and safety

via a defined standardization process. It is advised to bring the formulation to the subsequent investigation status via pharmacological studies and clinical trials.

Conflict of Interest

The authors declared No conflict of interest.

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Author Contribution

All authors contributed equally.

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