QUALITATIVE SCREENING OF TRADITIONAL POLY HERBAL FORMULATION *ELATHY CHOORANAM*

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***Abstract*- The present study highlight the qualitative parameters of the traditional poly herbal formulation *Elathy chooranam* indicated for jaundice. *Elathy chooranam* was made as per the Siddha literatrure *Anubava vaithiya theva ragasiyam* (part-3) . *Elettaria cardamomum, Cuminum cyminum , Phyllanthus niruri and Saccharum officinarum* were the ingredients of the preparation. The prepared drug underwent organoleptic evaluation, physiochemical, HPTLC, heavy metal analysis, microbial load, specific pathogen, pesticide residue, aflotoxins, phytochemical, biochemical analysis. The results acquired in the above standardization parameters that explained in this article.**

***Keywords*: *Elathy chooranam*, Poly herbal formulation, Qualitative parameters**

INTRODUCTION

The major sign of liver disease is Jaundice (Hyperbilirubinemia) which is known as yellowish discolouration of skin and mucous membrane[1]. Whatever the system of medicine we are taking first of all its toxic substances affect the liver. So, we must protect the Liver by giving Hepatoprotective drugs.According to WHO, Liver diseases are the 10 th most common cause of mortality in India. Common liver diseases prevalence in Tamil nadu are alcoholic liver disease, chronic viral hepatitis due to Hepatitis B and C virus and fatty liver[2]. Much more drugs are available to treat the Jaundice and other hepatic diseases like *Eclipta prostrata, Tinospora cordifolia, Acacia sinuate, Prenma tomentosa etc*..[3] Chooranam is named for a powder made by a single or a blend of herbal drugs. Based on above facts, one of the Polyherbal preparation *"ELATHY CHOORANAM"* mentioned in Siddha literature "*Anubava vaithiya theva ragasiyam(Part-3).Elakkai*(*Elettaria cardamomum), Seeragam(Cuminum cyminum), Keezha*nelli (*Phyllanthus niruri) and Sarkkarai (Saccharum officinarum)* were the ingredients of the preparation. This formulation is used for *(Jaundice-Kaamaalai)* is yet remained unexplored for its exact qualitative parameters in terms of scientific research according to PLIM guidelines. So, I interested to standardization the "*ELATHY CHOORANAM"* a unique herbal preparation for its hepato-protective activity.

MATERIALS AND METHODS

Selection of the drug

Elathy chooranam was taken as a sample medicine for its activity against Jaundice as mentioned in *Anubava vaithiya theva ragasiyam(Part-3)* (Pg .no: 388)

Ingredients of *Elathy chooranam* [4]

Table no: 1 Ingredients of ELC

S. no	Common name	Botanical name	Quantity
1	*Elakkai*	*Elettaria cardamomum*	One part (100g)
2	*Seeragam*	*Cuminum cyminum*	One part (100g)
3	*Keezhanelli*	*Phyllanthus niruri*	One part (100g)
4	*Sarkkarai*	*Saccharum officinarum*	One part (100g)

Drug collection

Phyllanthus niruri collected from Vilupuram district. *Elettaria cardamomum, Cuminum cyminum, Saccharum officinarum* were bought from raw drug store at Parry's corner, Chennai

Identification and attestation of drugs

Drugs were identified and attested by the Botanist and Gunapadam(Pharmacology) experts. Each sample has been labeled as 1096-1099/PGG/320220100506/GSMC-CH/2020-2023 The identified product samples were maintained in the PG Gunapadam laboratory for future references.

Method of Drug Purification

Purification method was carried out according to the literature *Sarakkugalin suthi seimuraigal* [5]. *Elettaria cardamomum* was roasted in the pan and their outer layer was removed. *Cuminum cyminum* was cleaned without any impurities and roasted in the pan. *Phyllanthus niruri* was well cleaned without any impurities. *Saccharum officinarum* was powdered and sieved with cloth.

Method of Drug Preparation

All the drugs were taken as per the quantity and pounded in iron motor . The fine powder filtered through mesh size 92 .The chooranam was purified by Pittavial process.Then dried and stored in an air tight glass container for further evaluation.

Dosage: 1-2 g (Once a day- Morning)

Adjuvant: Cow's milk

Indication : Kaamalai (Jaundice)

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 ELC 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 [6-8]

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 [9]

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[10,11]

Bio chemical Assessment of Basic and Acidic Radicals [12]

For spotting of carbonate, sulfate and phosphate

Heavy Metal Analysis through Atomic Absorption Spectroscopy (AAS) [13]

Lead, Arsenic, Cadmium and Mercury were tested

Microbial load (Sterility Test) [14]

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

Test for Specific pathogen [15]

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[16,17] and Aflotoxin Assay [18] **were evaluated**

RESULTS

Results of Organoleptic Properties

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

Figure 1.ELC drug (end product)

Table no.2 Organoleptic properties

S. No	Specifications	Properties
1	Nature	Fine
2	State	Solid
3	Taste	Bitter
4	Odour	Pleasant
5	Flow	Non- free flowing
6	Colour	Greenish brown
7	Texture	Soft

Results of Physicochemical Assessment

Loss on drying was evaluated as 7.149%. This denoted the moisture content. Total ash value was determined to be 10.11%. It indicate the presence of inorganic matter of the preparation. Water soluble and alcohol soluble extractive values were noted to be 30.006% and 17.73% respectively. The acid insoluble ash was 4.37% . P^H value determined as 6.5(acidic nature) .
The results are mentioned in Table no: 3

Table no: 3 Results of Physico chemical Assessment of ELC

S. no	Tests	Obtained values
1	Loss on drying at 105ºc	7.149%
2	Ash- total	10.11%
3	Acid insoluble ash	4.37%
4	Water soluble extractive	30.006%
5	Alcohol soluble extractive	17.73%
6	P^H	6.5
7	Particle size	22.71± 6.13µm

Determination of Particle size
Average particle size was noted to be 22.71± 6.13µm. Size range between 13µm to 31µm.

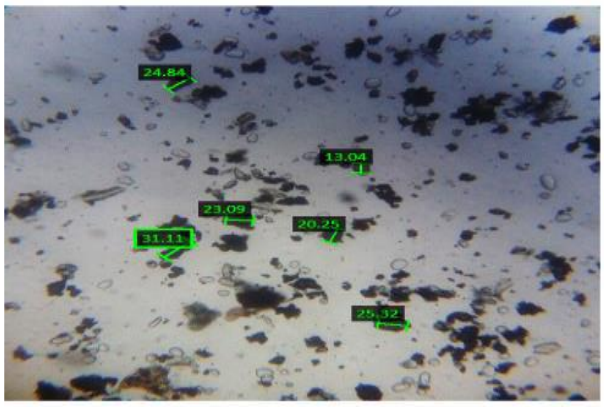


Figure no:2 Microscopic observation of Particle size for ELC

Results of Solubility Assessment of ELC
Results mentioned in table no:4

Table no: 4

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

Phyto chemical assessment of ELC
Presence of biologically significant phytochemicals evaluated through qualitative phytochemical analysis of ELC. Results are mentioned in table no : 5

Table no: 5. Results of Phyto chemical assessment

S. no	Analysis	Test name	Inference
1	Alkaloids	Wagner's Test	Positive
2	Carbohydrates	Molich's test Benedict Test	Positive Positive
3	Saponin	Foam Test	Positive
4	Phenols	Ferric chloride Test	Positive

5	Tannins	Gelatin Test	Positive
6	Flavanoids	Lead acetate	Positive
7	Diterpenes	Copper Acetate test	Positive
8	Quinones	Test for Quinones	Positive
9	Gum & mucilage	Test for Gum & mucilage	Positive

Figure no :3 Results of phytochemical analysis of ELC

HPTLC

Reveals eight peaks correlating with eight variable phyto components present. Rf value of the peaks ranges from 0.02 to 0.91 in 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 ELC at 366nm

Figure: 4 TLC Chromatogram of ELC

3D- Chromatogram

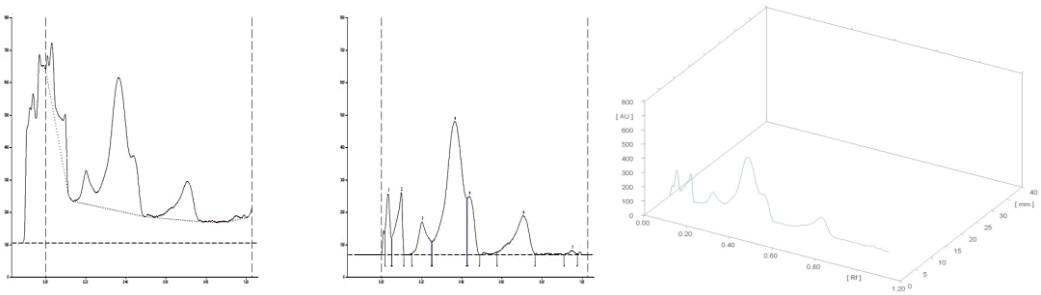


Figure no .4 .HPTLC finger printing of Sample ELC

Table no :6 Peak table

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.02	66.6	0.03	190.6	15.72	0.05	51.7	2066.9	5.84
2	0.05	53.4	0.10	193.5	15.96	0.11	0.4	3195.5	9.03
3	0.15	3.9	0.20	102.6	8.46	0.25	41.3	2591.0	7.32
4	0.25	41.7	0.37	411.5	33.95	0.43	174.4	18947.8	53.54
5	0.43	174.9	0.44	180.4	14.88	0.49	0.1	3286.2	9.29
6	0.58	8.7	0.71	120.6	9.95	0.77	2.4	5075.9	14.34
7	0.91	0.0	0.95	13.2	1.09	0.98	3.5	224.4	0.63

Biochemical analysis

Carbonate, sulfate and phosphate has been present in the results of bio chemical analysis. Results are tabulated in table no.7

Test for Acid radicals

Table no :7 Results for acid radicals

S. NO	PARAMETER	OBSERVATION	RESULT
1	Test for carbonate	Presence of brisk effervescence	Positive
2	Test for sulfate	Presence e of white precipitate	Positive
3	Test for phosphate	Presence of yellow precipitate	Positive

Heavy Metal Analysis by Atomic Absorption Spectroscopy (AAS)

Heavy metal traces of Lead, Arsenic, Cadmium and Mercury were nil in the sample

Table no: 8 Results of Heavy Metal Analysis of ELC

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	BDL	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL- Below detection limit

Microbial load (Sterility test)

No growth or colonies were noticed on pour plate. Results were tabulated in table no.9

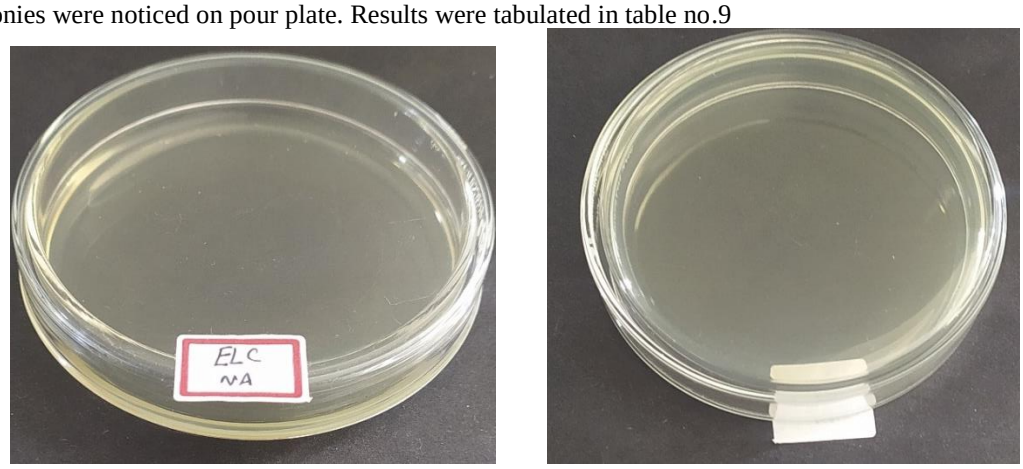


Figure no :5.Pour plate Method for Microbial method

Table no: 9 Results of Microbial load by pour plating method

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	

Test for Specific Pathogen

Result

Any growth and colonies did not reveal in the inoculated plates. Results were tabulated in table no 10 & figures no : 6

Table. No: 10 Result For Specific Pathogen of ELC

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

Figure no :6.Culture plate with E-coli (EC) specific medium

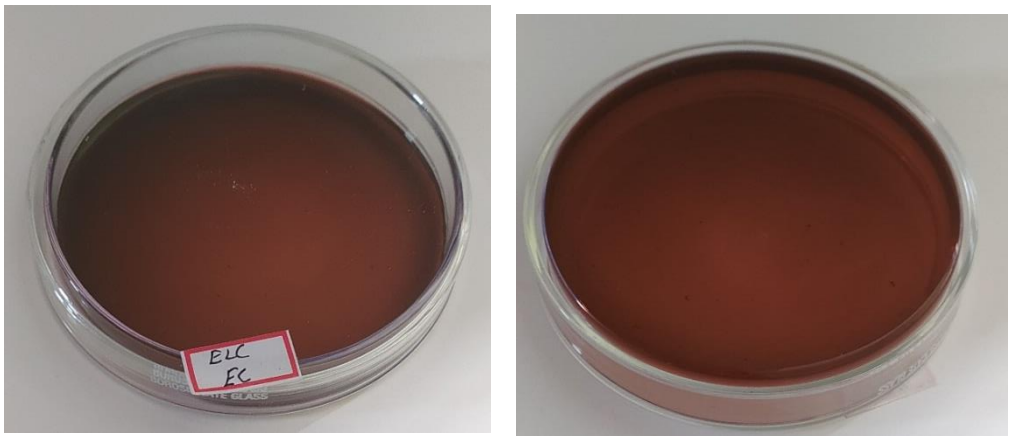
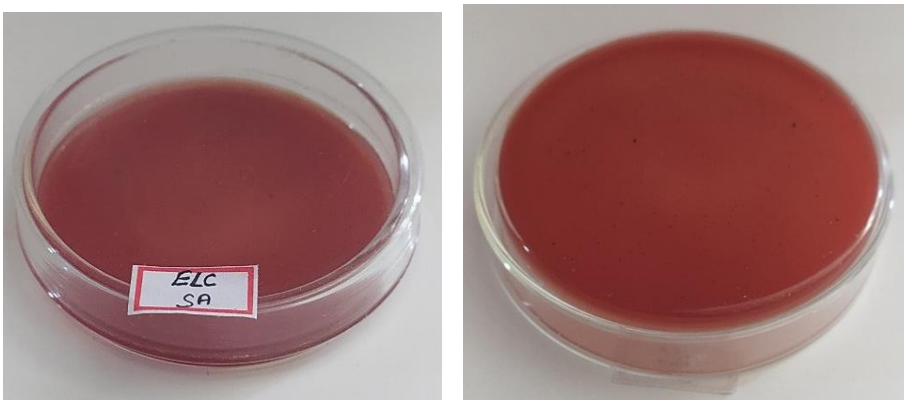


Figure no :7 Culture plate with Salmonella (SA) specific medium



Figur no :8 Culture plate with Staphylococcus Aureus (ST) specific medium

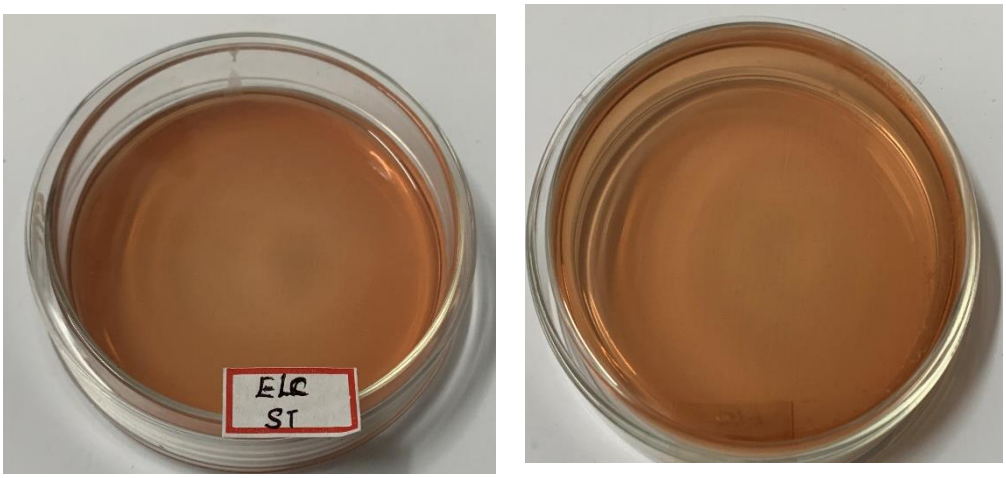
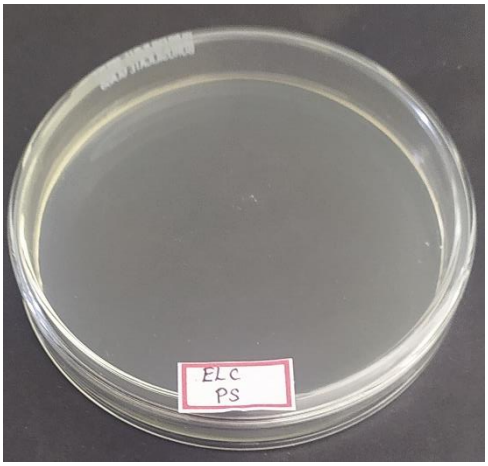
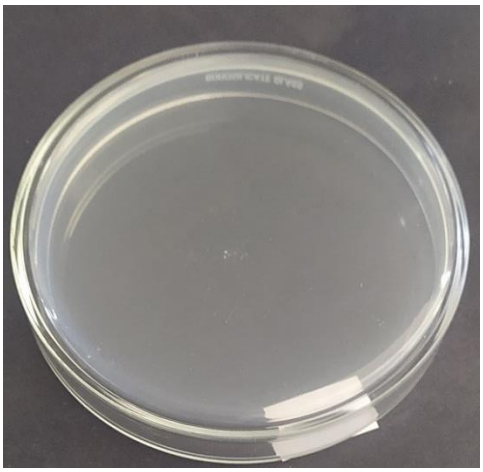


Figure no :9Culture plate with Pseudomonas Aeruginosa (PS) specific medium





Pesticide Residue Analysis

Organochlorine, Organophosphorus, Organocarbamates and Pyrethroids were below the quantification limit. Results are tabulated in table no:11

Table no: 11 Results of pesticide residue of ELC

Pesticide Residue	Sample ELC	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

Aflotoxin assay

The results revealed no spots identified in the test sample loaded on TLC plates when compared to the standard which denotes that the sample ELC were free from Aflotoxin B1, AflotoxinB2, AflotoxinG1, AflotoxinG2. Results are tabulate in table no:12

Table no:12 Results of Aflotoxins

Aflotoxin	Sample of EC	AYUSH specification unit
B1	Not detected	0.5ppm(0.5mg/kg)
B2	Not detected	0.1ppm(0.1mg/kg)
G1	Not detected	0.5ppm(0.5mg/kg)
G2	Not detected	0.1ppm(0.1mg/kg)

Disscussion

Elathy Chooranam is Solid in nature, Greenish Brown in Colour, Bitter in taste, Fine powder in texture, Pleasant odour, non Free Flowing and soft on touch. So the drug is good in nature. Hence, the drug *ELC* is safe to consume. Loss on drying was indirectly indicate the moisture content and the value was 7.149% denoting a stability and good shelf life. The total ash value was 10.11% , it indicates the purity of *ELC* that test explains the presence of minerals. The acid insoluble ash value was indicate the presence of siliceous matter which is 4.37% of this drug suggesting the good quality. Diffusion capacity indicates the Water soluble extractive which was to be 30.006%. Alcohol soluble extractive value was 17.73%. It expressed that the drug has purity and good quality. The P^H value of the drug was 6.5.Indicates the good oral bioavailability.

Solubility is a big challenge for pharmaceutical scientist. Any drug to be absorbed must be present in he form of solution at the absorption site [19]

The plants and fibres have natural bioactive compound of phytochemicals acts against conditions like Liver disease. Alkaloids, Carbohydrates, Saponins, Phenol, Tannin, Flavanoid, Diterpens, Quinones, Gum and mucilage will increase the therapeutic efficacy

of the drug. Hepatoprotective effects of natural compounds have been frequently attributed to their anti oxidant properties and the ability to mobilize endogenous antioxidant defense system[20]
Liver protective plants contain a variety of chemical compounds such as phenols, lignans, essential oils, triterpenes, glycosides, alkaloids, carotenoids, flavonoids and xanthines[20a]
There were seven prominant peaks in the HPTLC fingerprint in the chloroform extract corresponds to the presence of seven versatile phytocomponents present with in it. Rf value of the peaks ranges from 0.02 to 0.91 in 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. The acid radical test indicates the presence of Carbonate, Sulfate and phosphate. Calcium carbonate increase the calcium level in the body. Calcium is a versatile secondary messenger that regulates multiple hepatic functions, including lipid and carbohydrate metabolism , as well as bile secretion and choleresis[21]. Presence of sulfate is essential for liver protection by reducing increased serum enzymes of Liver. Dietry Calcium and Phosphate precipitate in the small intestine to form insoluble amorphous Calcium phosphate (ACP). The ability of ACP to bind and inactive luminal bile acids might have an effect on cholesterol metabolism. Results of the present investigation have clearly shows that the sample has no traces of heavy metals such as Mercury , Cadmium, Lead and Arsenic .The absence of heavy metal ensures the quality and safety of the test drug. No bacterial and fungal growth were observed in any of the plates inoculation with the test sample ***ELC***. This revealed that the drug is free from the viable microorganisms and the absence of total bacterial and fungal count which may indicates that the drug ***ELC*** has good quality and safer drug to treat Jaundice. The sample drug was free from specific pathogens like *Escheria coli*, *Pseudomonas aeruginosa*, *Salmonella*, *Staphylococcus aureus* were within the limit. The Organochloride pesticides, organophosphorus pesticides and organocarbamates and pyrethroid were also present within Below the limit of quantification expressing the collection of wild plants as per good collection practices. Since aflotoxin B1, B2, G1 and G2 value reveals less than 0.5ppm, 0.1ppm, 0.5ppm and 0.1 ppm respectively were found to be below the limit of the drug would be administered internally.

Conclusion

Those acquired results of physicochemical, phytochemical, biochemical assessment, microbial load, test for specific pathogen, pesticide residue and aflotoxin will be a helpful tool for standardization and quality control analysis of the traditional polyherbal formulation *Elathy chooranam*. The detect p^H value was 6.5 denoting the weekly acidic nature of the drug. The phytochemical assessment determine the increased polar secondary metabolites such as alkaloids, glycosides, flavonoids, phenol, saponin, diterpinoids, triterpinoids, tannins.This discovers that the above trial drug Elathy chooranam was safe to take as a internal medicine. Hereafter, pharmacological studies accomplish the medicinal value of the sample drug.

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Conflicts of interest

No conflicts of interest

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