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Physico-Chemical and Phytochemical Screening of Siddha Poly Herbal Formulation - *Amurthathi Chooranam*

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ABSTRACT: Urolithiasis plaques are a common urinary disease in all over the world which is defined as one or more calculi present in the urinary tract. The prevalence rate and incidence are increasing now - a - days. In Siddha medicines many formulations have been used in the treatment for Urolithiasis. *Amurthathi Chooranam* is one among the Siddha poly herbal formulation which is indicated for Urolithiasis. The aim of the study is to evaluate the physico-chemical, phyto-chemical and sophisticated analysis of Siddha poly herbal formulation "*Amurthathi Chooranam*".

***Amurthathi Chooranam* was prepared as per the Siddha literature *Anubava Vaithiya Deva Ragasiyam.* Physicochemical analysis such as water soluble extract 24.9985%, Alcohol soluble extract 5.1505%, Loss on drying 3.598%, Total ash 7.3816%, Acid insoluble Ash 3.0436%, Water soluble ash 1.2543%, phytochemical such as Alkaloids, carbohydrates, tannin, phenol, saponin, flavonoids and diterpenes are present. Other parameters like HPTLC, microbial load, specific pathogen, pesticide residues and aflatoxins were evaluated as per PLIM guidelines.**

From the outcome of results the drug *Amurthathi Chooranam* is safe, cost effective and biologically having active components which is treating for urolithiasis. This evidence based data is giving global acceptance of purity, in a prompt manner regarding *Amurthathi Chooranam*.

Keywords: *Amuthathi Chooranam*, Siddha formulation, Physicochemical, Phytochemical screening.

INTRODUCTION

Over the population globally including India, Urolithiasis has become a common disorder (Pareta *et al.,* 2011). The prevalence rate and incidence of renal stones have been immensely increasing since a decade or two (Butterweck *et al*., 2009). Multiple factors such as diet, genetic and sedentary have been suggested to be responsible for this disorder (Gindi *et al*., 2013; Heron and Yarnell 1998). Calcium oxalate and calcium phosphate are the very common components of kidney stones, while magnesium ammonium phosphate (struvite), uric acid or cystine also forms some proportion of them (Sellaturay and Fry 2008). Various kind of formulations have been employed for urolithiasis and other treatments like laproscopic removal, extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrostolithotomy and ureteroscopy etc., but not yet proved to be fruitful for proper preventive and curative of recurrence of urolithiasis (Gupta and Kanwar 2018; Arya *et al*., 2017).

Therefore, numerous alternative therapies for the treatment of kidney stones are being explored. In Siddha system *Chooranam* is one of the 32 types of internal medicines. *Amurthathi Chooranam* (AMC*)* is one among the *Siddha* polyherbal formulations which is mentioned in *Anubavavaithiya deva ragasiyam*, indicated for *Mehaneer* (Urinary disorders), *Prameham* (Urological disorders - Renal stone), *Madhumeham* (Diabetes), *Vellai* (Leucorrhoea), *Vettai* (Gonorrhoea) (Seetharam Prasath, 2014). In this study, *AMC* was filtered for standardization procedure as per PLIM guidelines. The intention of this study is to give information about the standardization of *AMC* through physico-chemical, phytochemical, HPTLC, microbial load, specific pathogens, pesticide residues and aflatoxins were evaluated.

Aim and Objective. The main aim of the study is to evaluate the physico – chemical, phyto-chemical and sophisticated analysis of Siddha poly herbal formulation "*Amurthathi Chooranam*" as per PLIM guideline.

MATERIALSANDMETHODS

Collection of the drugs:

Table 1: Ingredients of *Amurthathi Chooranam.*

Sr. No.	Tamil Name	Botanical Name	Quantity
1.	Seenthil	*Tinospora cordifolia*	35 Gram
2.	Jaathikkai	*Myristica fragrans*	5.1 Gram
3.	Jaathipathiri	*Myristica fragrans*	5.1 Gram
4.	Vaal milagu	*Piper cubeba*	5.1 Gram
5.	Elam	*Elettaria cardamomum*	5.1 Gram
6.	Kirambu	*Syzygium aromaticum*	5.1 Gram
7.	Kasakasa	*Papaver somniferum*	5.1 Gram
8.	Thalisapaththiri	*Taxus baccata*	5.1 Gram
9.	*Maasikkai*	*Quercus infectoria*	5.1 Gram
10.	*Sarkarai*	*Saccharum officinarum*	75.8 Gram

The ingredients of above mentioned formulation *Amurthathi Chooranam* in Table 1 was procured from Authenticated country drug shop, Chennai, Tamilnadu.

Identification and authentication: Identification and Authentication of all the above ingredients of *Amurthathi Chooranam* were recognized and certified by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai - 106. Specimen sample of all the above ingredients were labelled and were kept in the same as earlier mentioned for future reference.

ProcessforPurification and Preparation: All the above mentioned ingredients were purified as per Siddha classical literature and taken it into an anhydrous form pound well and grounded it in an iron mortar separately.

Purification of the *Chooranam*:

Steaming process (*Pittaviyalmurai*): This *chooranam* was purified by steam cooking with milk (*Pittaviyal murai*) as per Siddha literature. Then added equal quantity of the sugar to the above mixture and grinded well. The powder was sieved through a mesh (80-100) particle sizes and stored in a clean airtight container. It was labelled as "*Amurthathi Chooranam"(AMC)*. The prepared *Chooranam* was examined repeatedly to avoid moisture content and microbes.

Administration of the drug:

Form of the medicine: *Chooranam*

Route of Administration: Oral

Vehicle: water

Dose: 1gm, twice a day

Duration: 24 days.

Indication: *Mehaneer* (Urinary disorders), *Prameham* (Urological disorders - Renal stone), *Madhumeham* (Diabetes), *Vellai* (Leucorrhoea), *Vettai* (Gonorrhoea).

Powder microscopy: Powder microscopy was carried out and certified by Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai - 106. Sample coded as 322.02062202 for future reference. Specimen sample has been labelled and were kept in the same as mentioned earlier. Photomicrographs of diagnostic characters were taken and documented.

Physicochemical Analysis (Indian Pharmacopeia I, 2014)

The preliminary physico-chemical screening test was carried out for each extracts of *Amurthathi Chooranam* as per the standard procedures.

Allthe following studies were carried out in The TamilnaduDr.M.G.R.Medical University, Guindy, Chennai- 32 and Siddha Central Research Institute, Arumbakkam, Chennai -106.

— Total Ash
— Acid Insoluble Ash
— Water Soluble Ash
— Alcohol Soluble Extractive
— Water-Soluble Extractive
— Loss on Drying
— Reducing sugar
— Total Sugar
— pH
— Particle Size Determination (Takashi Hiroi, 2017) was done

Phyto-chemical Analysis was performed for the following tests (Protocol for Testing of Ayurvedic, Siddha and Unani medicines, 2008)

The preliminary phyto-chemical screening test was performed for each extracts of *Amurthathi Chooranam* as per the standard procedure

— Alkaloids – were performed by Mayer's Test, Dragendroff Test, Wagner Test
— Carbohydrates–Molish test, Benedict's test
— Saponin
— phenols
— Tannins
— Flavonoids – Alkaline, Lead acetate test
— Diterpenes
— Quinone
— Gum & Mucilage

TLC & HPTLC was performed (Lohar, 2008)

— Chromatogram Development was carried out
— Scanning was done

Test for Sterility was performed by pour plate method (Pour Plate Method, 2022)

— Total Bacterial Count
— Total Fungal Count

Test for Specific Pathogens mentioned in Table 2 (Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 2012).

Table 2: Details of Specific Medium and their abbreviations.

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

Test for Pesticide Residues (WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues, 2007).

— Organo Chlorine Pesticides,
— Organo Phosphorus Pesticides,
— Organo carbamates,
— Pyrethroid

Test for Aflatoxins mentioned in Table 3 were performed (Luciana de castro, 2001).

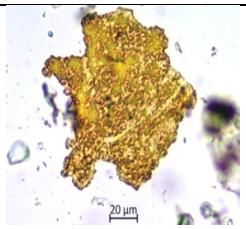
Table 3: Standards.

1	Aflatoxin B_1
2	Aflatoxin B_2
3	A flatoxin G_1
4	Aflatoxin G_2

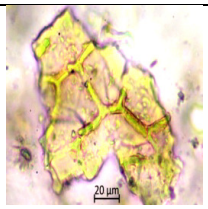
Solvent was carried out for analysing the combination of chloroform and acetonitrile.

RESULTS AND DISCUSSION

Powder microscopic studies of *Amruthathi Chooranam* as in Fig. 1 was documented as per standard procedures. Characters of *Myristica fragrans* Houtt. (seed) (Fig. 1a) and aril (Fig. 1b), *Piper cubeba* Vahl (Fig. 1c) (fruit), *Elettaria cardamomum* (Fig. 1d) (L.) Maton (seed), *Syzygium aromaticum* (Fig. 1e) (L.) Merr.&L.M.Perry, *Papaver somniferum* (Fig. 1f) L. (seed), *Taxus baccata* (Fig. 1g) L. (leaf), *Quercus infectoria* (Fig. 1h) Oliv. (gall) and *Tinospora cordifolia* (Fig. 1i) (Willd.) Miers ex Hook.f.& Thomson (extract from stem) were observed under microscope showing authenticity of the formulation.

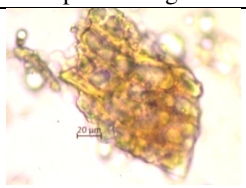


Crystalline mass of fat

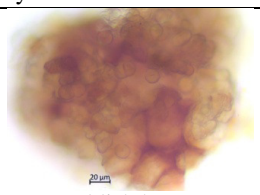


Epidermal cells of testa

Fig. 1a. Characters from *Myristica fragrans* (seed).

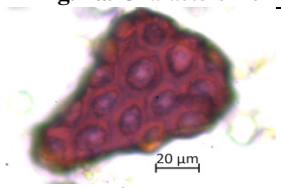


Parenchyma with oil cells

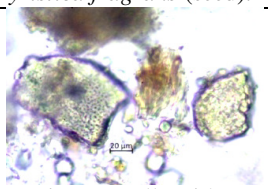


Reddish brown endosperm cells with starch and oil drops

Fig. 1b. Characters from *Myristica fragrans* (aril).

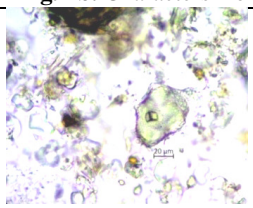


Testa fragment

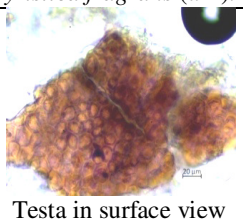


Perisperm cells with starch granules

Fig. 1c. Characters from *Piper cubeba* (fruit).

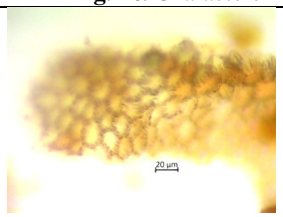


Parenchymata with crystal

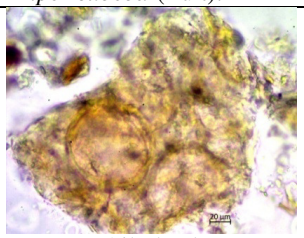


Testa in surface view

Fig. 1d. Characters from *Elettaria cardamomum* (seed).

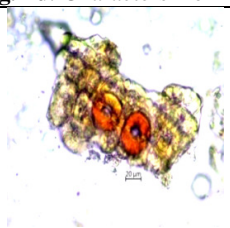


Fibrous layer in surface view

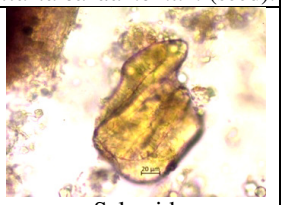


Oil gland

Fig. 1e. Characters from *Syzygium aromaticum* (flower bud).

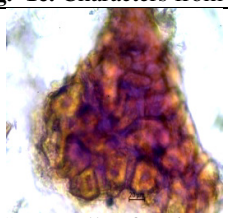


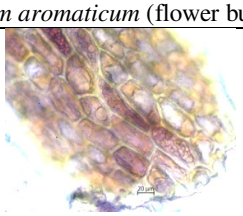
Sunken stomata



Sclereid

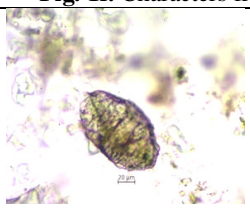
Fig. 1f. Characters from *Taxus baccata* (leaf).


Stone cells of pericarp



Cells from cotyledon

Fig. 1g. Characters from *Quercus infectoria* (gall).



Reticulate parenchyma

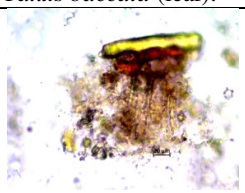
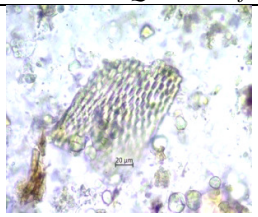
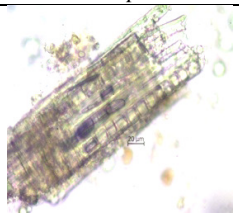

Pigmented cells from inner epidermis

Fig. 1h. Characters from *Papaver somniferum* (seed).



Vessel fragment with bordered pits



Crystal fibres

Fig. 1i. Characters from *Tinospora cordifolia* (stem).

Fig. 1. Powder microscopy of *Amurthath Chooranam.*

The *Amurthathi Chooranam* showed crystalline mass of fat, epidermal cells of testa from *Myristica fragrans* (seed), parenchyma with oil cells and endosperm cells from *Myristica fragrans* (aril), testa fragment and perisperm cells from *Piper cubeba* (fruit), parenchymatous cells of the perisperm with prisms and testa from *Elettaria cardamomum* (seed), surface view of fibrous layer and sclereidal layer from *Syzygium aromaticum* (flower bud), sunken stomata and sclereids from *Taxus baccata* (leaf), pericarp and cotyledon cells from *Quercusinfectoria* (gall), reticulate parenchyma and pigmented cells from inner epidermis from *Papaver somniferum* (seeds), bordered pitted vessels and crystal fibres from *Tinospora cordifolia* (stem) (Wallis TE, 1965; Quality Standards ICMR, 2003; Quality Standards ICMR, 2012; Quality Standards ICMR, 2010; Quality Standards ICMR, 2018).

Organoleptic Characters of *Amurthathi Chooranam*: The following organoleptic characters mentioned in Table 4 were noted in *Amurthathi Chooranam*.

Table 4: Results and Discussion of Organoleptic Characters.

Sr. No.	Parameter	Result
1.	State	Solid
2.	Nature	Fine
3.	Odour	Characteristic Agreeable
4.	Touch	Soft
5.	Flow Property	Free Flowing
6.	Appearance	Creamy in color
7.	Taste	Sweet followed by pungent

The organoleptic parameters showed that *AMC* is solid in state, fine in nature, characteristic agreeable odour, soft to touch, free flowing property, creamy in color and sweet followed by pungent in taste which stimulates the taste buds and it enhances the gastric secretions.

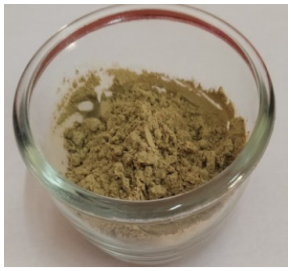


Fig. 2. *Amurthathi Choornam.*

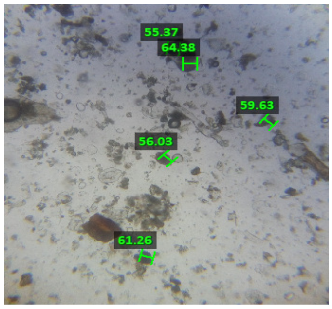


Fig. 3. Microscopic Observation of Particle Size of the *AMC*.

Microscopic Observation of Particle Size of *Amurthathi Chooranam.* Microscopic observation of the particle size indicated that *AMC*completely passed through sieve no.44 mesh, which confirms the solubility, bioavailability, processing belongings, uniformity and strength of the drug *Amurthathi Chooranam* (Takashi Hiroi, 2017). Microscopic observation of the particle size analysis exhibits that the average particle size of the *AMC* was found to be 77.71 ± 21.27 µm.

Physico-Chemical Analysis of *Amurthathi Chooranam*

Solubility. Solubility is one of the most important parameters to attain preferred concentration of the test drug in systemic circulation for desired pharmacological response. Solubility is a major task for formulation scientist. Any drug to be engaged and must be present in the form of solution at the site of absorption. Selection of the solubility improving method depends on drug property, site of the absorption and required dosage form characteristics.

Table 5: *AMC* Solubility Profile - Results and discussion.

Sr. No.	Solvent Used	Solubility/Dispensability
1.	Chloroform	Insoluble
2.	Ethanol	Soluble
3.	Water	Soluble
4.	Ethyl Acetate	Insoluble
5.	DMSO	Soluble

Solubility is the fundamental requirement for the absorption of the drug from GIT. Suitable selection of solubility enhancement method is the key to confirm the goals of a good formulation like good oral bioavailability, reduces the frequency of dosing and better patient compliances combined with a low cost production (Savjani *et al.*, 2012). The results of solubility profile and discussions of *AMC* are mentioned in Table 5.

Physico-chemical Analysis. The Physicochemical Analysis on *AMC* was performed and listed in Table 6.

Table 6: Results of Physico-chemical Analysis.

Sr. No.	Parameters	Percentage
1.	Loss on drying	3.598%
2.	Total ash value	7.3816%
3.	Acid insoluble ash	3.043%
4.	Water soluble ash	1.254%
5.	Water soluble extraction	24.998%
6.	Alcohol soluble extraction	5.15%
7.	pH value	5.90 (slightly acidic)
8.	Reducing sugar	-
9.	Total sugar	45.66%

Loss on Drying: The loss on drying value was 3.598% which indicates higher stabilityand longer shelf life of the drug *AMC*.

Total Ash: It shows the purity of the prepared drug *AMC*. The total Ash value was 7.3816%, which exhibits that *AMC* has no impurities and the drug *AMC*is safe to treat for urolithiasis.

Acid insoluble ash: The evaluation of the medication is very useful if the acid insoluble ash is low. Here, acid insoluble ash importance of *AMC* is 3.043% suggesting the less content of siliceous substances in the *AMC* (Nilakshi Pradhan *et al*., 2015; Khandelwal, 2010).

Water soluble ash: Water soluble ash of *AMC* is 1.254% which gives the crude estimate of the water soluble extractable substance present in the ash. It is more important for the biological activity.

Alcohol Soluble Extractive: Alcohol-soluble extractive value of *AMC* is 5.15% which demonstrates that the drug *AMC* has good quality and purity of the raw drug.

Water Soluble Extractive: Water-soluble extractive is a portion of the entire ash value, signifying the drug's diffusion capacity (Heamavathi *et al.,* 2022). Here, the water- soluble extractive Value of *AMC* is 24.998%, which represents easy facilitation of diffusion and osmosis mechanism.

pH: The pH of the drug is 5.9, which is slightly acidic. The acidic drug is important for bioavailability and it's effectiveness. So, the drug *AMC* will be fascinated better in the stomach (Farhud, 2015).

Total sugar. Total sugar of the drug is 45.66%

The Phyto-chemical screening of *Amurthathi Chooranam* are listed in Table 7 and their presence or absence are showed, such as the actuality of Alkaloids, Carbohydrates, Saponins, Phenols, Tannins, Flavonoids, Diterpenes, Quinones, Gum & Mucilage.

Table 7: Preliminary phytochemical screening of *Amurthathi Chooranam.*

Sr. No.	Phytochemicals	Test Name	H_2O Extract
1.	Alkaloids	Mayer's Test Dragendroff's Test Wagner's Test	-ve -ve +ve
2.	Carbohydrates	Molisch's Test Benedict Test	+ve +ve
3.	Saponin	Foam Test	+ve
4.	Phenols	Ferric Chloride Test	+ve
5.	Tannins	Gelatin Test	+ve
6.	Flavonoids	Alkaline Reagent Test Lead acetate Test	+ve +ve
7.	Diterpenes	Copper Acetate Test	+ve
8.	Quinones	Test for Quinones	-ve
9.	Gum & Mucilage	Test for Gum & Mucilage	-ve

+ve/-ve present or absent if component tested

Alkaloids: Commonly the alkaloids are bitter in taste and have distinct physiological activity.

Flavonoids. Flavonoids are largerin group of plant polyphenols with supposed advantageous effects on several common diseases. Flavonoids could effectively inhibit the formation of CaOx stones in-vitro and in-vivo, correlating with their diuretic, antioxidant, anti-inflammatory, antibacterial properties and other protective effects. Thus, flavonoid-rich plant extracts endowed with anti-urolithias is activities and probable mechanisms of actions (Zeng *et al*., 2019).

Flavonoids and saponins. These phyto-constituents offer the possibility of inhibition of calcium and oxalate deposition and crystals growth and also establish to be associated with reduced urinary creatinine level and decreasing the supersaturation of lithogenic enhancing agents.

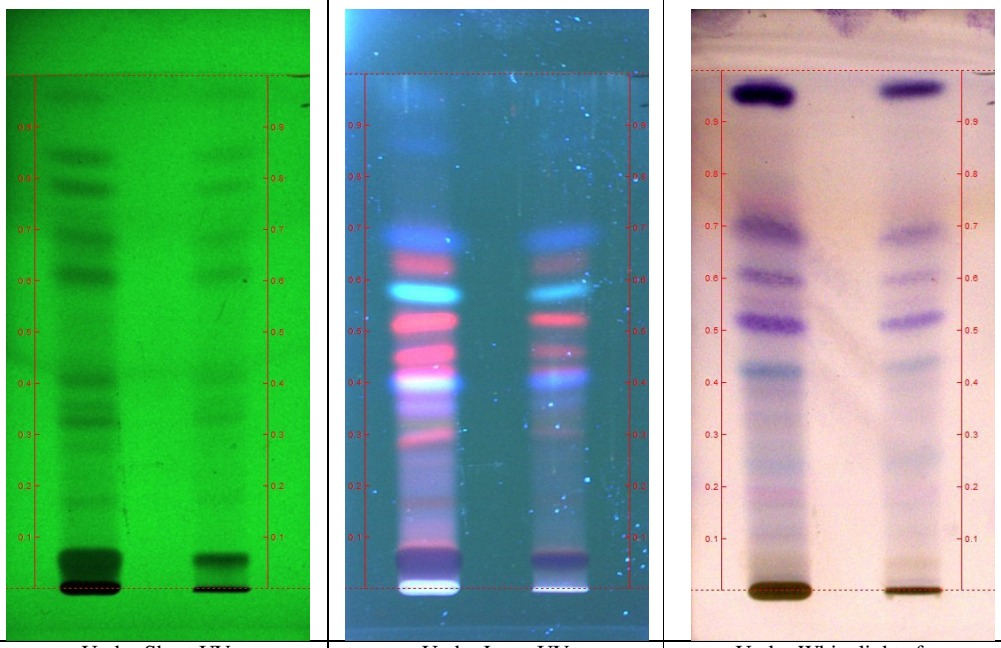
Phenol and Tannin. Polyphenolic hydrolysable tannin was demonstrated to efficiently block renal calcification (Lee *et al*., 2012).

Diterpenes. Estimation of release kinetics of CaOxinfers the effective dissolving concentration of terpenes. The test to study the dissolving ability of the kidney stone exposes the percentage of its affinity to dissolve the stones, which regulates the further techniques (Abirami *et al*., 2018).

Thin-layer Chromatography (TLC): The TLC of Ethanol extract of *AMC* (Fig. 4) showed ten spots at Rf 0.08, 0.16, 0.33, 0.37, 0.42, 0.62, 0.69, 0.80, 0.85, 0.97(all green) under UV λ254 nm; thirteen spots at Rf 0.04 (Purple), 0.09 (Fluorescent red), 0.18 (Fluorescent red), 0.30 (Fluorescent red), 0.36 (Pink), 0.39 (Fluorescent blue), 0.42 (Fluorescent red), 0.46 (Fluorescent red), 0.52 (Fluorescent red), 0.59 (Cyan blue), 0.64 (Fluorescent Red), 0.69 (Fluorescent blue), 0.90 (Fluorescent blue) under UV λ366 nm; thirteen spots at Rf 0.06 (Brown), 0.10 (Red), 0.19 (Pink), 0.24 (Sky blue), 0.33 (Red), 0.38 (Pink), 0.42 (Sky blue), 0.53 (Violet), 0.56 (Violet), 0.61 (Violet), 0.71 (Violet), 0.76 (Violet), 0.96 (Purple),under white light (after dipping in VSR).

HPTLC profile of ethanol extract under UV λ 254 nm, major peaks (Table 9) appeared at Rf 0.01 (area 3.91%), 0.03 (14.31%), 0.14 (2.16%), 0.25 (2.11%), 0.30 (9.73%), 0.38 (13.21%), 0.50 (0.74%), 0.55 (13.84%), 0.66 (6.52%), 0.70 (6.43%), 0.74 (9.69%), 0.80 (10.16%), 0.91 (5.97%), 0.98 (1.23%); under UVλ 366 nm, major peaks (Table 10) appeared at Rf 0.00 (area1.79%), 0.07 (2.84%), 0.11 (7.15%), 0.17 (5.83%), 0.24 (3.90%), 0.28 (5.33%), 0.32 (8.21%), 0.37 (17.94%), 0.44 (7.16%), 0.49 (9.46%), 0.54 (13.10%), 0.60 (3.69%), 0.64 (7.68%), 0.82 (2.51%), 0.88 (3.39%) under UV λ 520 nm, major peaks (Table 11) appeared at Rf 0.01 (area 1.23%), 0.04 (0.25%), 0.08 (1.00%), 0.14 (1.89%), 0.21 (0.64%), 0.29 (1.41%), 0.35 (9.25%), 0.46 (11.22%), 0.54 (4.30%), 0.57 (10.26%), 0.63 (30.53%), 0.86 (2.72%), 0.90 (25.30%)

Amurthathi Chooranam has been standardized as per standard testing protocol. Results of HPTLC photo documentation, Rf values, densitometric scan, fingerprint profiles are presented in respective tables, in the Table 8-11 respectively in Fig. 4-10 respectively.



Under Short UV λ=254nm	Under Long UV λ=366nm	Under White light after Derivitisation λ=520nm

Fig. 4. TLC Photo of *Amurthathi Chooranam* Extract.

Table 8: R*f* value and color of spots.

λ=254nm		λ=366nm		λ=520nm (Derivitized)	
R*f*	**Colour**	**R*f***	**Colour**	**R*f***	**Colour**
0.08	DarkGreen	0.04	Purple	0.06	Brown
0.16	Green	0.09	Fluorescent red	0.10	Red
0.33	Green	0.18	Fluorescent red	0.19	Pink
0.37	Green	0.30	Fluorescent red	0.24	Sky blue
0.42	Green	0.36	Pink	0.33	Red
0.62	Green	0.39	Fluorescent blue	0.38	Pink
0.69	Green	0.42	Fluorescent red	0.42	Sky blue
0.80	Green	0.46	Fluorescent red	0.53	Violet
0.85	Green	0.52	Fluorescent red	0.56	Violet
0.97	Green	0.59	Cyanblue	0.61	Violet
		0.64	Fluorescent Red	0.71	Violet
		0.69	Fluorescent blue	0.76	Violet
		0.90	Fluorescent blue	0.96	Purple

Table 9: Peak Table @ 254 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	106.0 AU	0.01 Rf	174.9 AU	11.32 %	0.03 Rf	3.7 AU	1735.4 AU	3.91 %
2	0.03 Rf	2.8 AU	0.07 Rf	229.4 AU	14.84 %	0.09 Rf	1.4 AU	6353.8 AU	14.31 %
3	0.14 Rf	1.7 AU	0.17 Rf	51.4 AU	3.33 %	0.20 Rf	2.2 AU	959.2 AU	2.16 %
4	0.25 Rf	0.2 AU	0.28 Rf	37.2 AU	2.40 %	0.30 Rf	19.6 AU	935.7 AU	2.11 %
5	0.30 Rf	19.7 AU	0.33 Rf	112.8 AU	7.29 %	0.37 Rf	38.6 AU	4317.8 AU	9.73 %
6	0.38 Rf	38.8 AU	0.41 Rf	140.5 AU	9.09 %	0.47 Rf	6.6 AU	5863.8 AU	13.21 %
7	0.50 Rf	4.3 AU	0.52 Rf	18.3 AU	1.19 %	0.55 Rf	1.5 AU	326.3 AU	0.74 %
8	0.55 Rf	1.6 AU	0.61 Rf	184.4 AU	11.93 %	0.63 Rf	56.8 AU	6143.4 AU	13.84 %
9	0.66 Rf	63.3 AU	0.68 Rf	95.9 AU	6.21 %	0.70 Rf	86.2 AU	2892.9 AU	6.52 %
10	0.70 Rf	86.3 AU	0.71 Rf	94.9 AU	6.14 %	0.74 Rf	51.6 AU	2854.3 AU	6.43 %
11	0.74 Rf	52.1 AU	0.77 Rf	153.1 AU	9.90 %	0.80 Rf	35.3 AU	4303.2 AU	9.69 %
12	0.80 Rf	35.5 AU	0.83 Rf	135.2 AU	8.74 %	0.89 Rf	16.7 AU	4508.6 AU	10.16 %
13	0.91 Rf	22.9 AU	0.94 Rf	61.7 AU	3.99 %	0.98 Rf	43.2 AU	2647.8 AU	5.97 %
14	0.98 Rf	43.2 AU	0.99 Rf	56.1 AU	3.63 %	0.99 Rf	22.9 AU	544.7 AU	1.23 %

Table 10: PeakTable @ 366 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	65.3 AU	0.01 Rf	198.1 AU	4.89 %	0.03 Rf	1.5 AU	2130.5 AU	1.79 %
2	0.07 Rf	1.6 AU	0.09 Rf	195.5 AU	4.83 %	0.11 Rf	81.6 AU	3384.0 AU	2.84 %
3	0.11 Rf	181.8 AU	0.12 Rf	184.3 AU	4.55 %	0.17 Rf	05.9 AU	8520.9 AU	7.15 %
4	0.17 Rf	106.4 AU	0.22 Rf	154.0 AU	3.80 %	0.23 Rf	49.7 AU	6947.8 AU	5.83 %
5	0.24 Rf	149.8 AU	0.25 Rf	158.9 AU	3.93 %	0.28 Rf	21.4 AU	4647.7 AU	3.90 %
6	0.28 Rf	121.8 AU	0.30 Rf	219.1 AU	5.41 %	0.32 Rf	44.9 AU	6348.4 AU	5.33 %
7	0.32 Rf	145.3 AU	0.35 Rf	323.3 AU	7.99 %	0.37 Rf	58.6 AU	9786.5 AU	8.21 %
8	0.37 Rf	260.1 AU	0.40 Rf	611.9 AU	15.12 %	0.44 Rf	02.0 AU	21377.5 AU	17.94 %
9	0.44 Rf	205.3 AU	0.45 Rf	326.9 AU	8.08 %	0.48 Rf	16.4 AU	8537.3 AU	7.16 %
10	0.49 Rf	116.5 AU	0.52 Rf	461.5 AU	11.40 %	0.54 Rf	20.9 AU	11277.7 AU	9.46 %
11	0.54 Rf	122.3 AU	0.57 Rf	636.8 AU	15.73 %	0.60 Rf	12.7 AU	15608.2 AU	13.10 %
12	0.60 Rf	113.5 AU	0.61 Rf	155.2 AU	3.83 %	0.64 Rf	15.6 AU	4401.3 AU	3.69 %
13	0.64 Rf	115.9 AU	0.67 Rf	258.0 AU	6.37 %	0.73 Rf	42.6 AU	9155.7 AU	7.68 %
14	0.82 Rf	35.6 AU	0.87 Rf	81.2 AU	2.01 %	0.87 Rf	76.9 AU	2992.6 AU	2.51 %
15	0.88 Rf	77.3 AU	0.89 Rf	82.8 AU	2.05 %	0.95 Rf	41.2 AU	4041.8 AU	3.39 %

Table 11: PeakTable@ 520 nm.

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	105.8 AU	0.01 Rf	144.6 AU	6.74 %	0.03 Rf	1.8 AU	1055.4 AU	1.23 %
2	0.04 Rf	0.0 AU	0.06 Rf	12.0 AU	0.56 %	0.08 Rf	0.2 AU	213.1 AU	0.25 %
3	0.08 Rf	0.3 AU	0.10 Rf	50.3 AU	2.34 %	0.13 Rf	2.0 AU	858.7 AU	1.00 %
4	0.14 Rf	0.3 AU	0.19 Rf	63.1 AU	2.94 %	0.21 Rf	3.9 AU	1620.2 AU	1.89 %
5	0.21 Rf	4.3 AU	0.24 Rf	19.8 AU	0.92 %	0.26 Rf	9.5 AU	546.7 AU	0.64 %
6	0.29 Rf	4.4 AU	0.33 Rf	40.2 AU	1.88 %	0.34 Rf	31.7 AU	1210.6 AU	1.41 %
7	0.35 Rf	31.7 AU	0.42 Rf	186.8 AU	8.71 %	0.46 Rf	27.3 AU	7937.2 AU	9.25 %
8	0.46 Rf	27.4 AU	0.51 Rf	291.1 AU	13.57 %	0.53 Rf	41.8 AU	9620.0 AU	11.22 %
9	0.54 Rf	142.0 AU	0.55 Rf	151.8 AU	7.08 %	0.57 Rf	12.1 AU	3689.3 AU	4.30 %
10	0.57 Rf	112.8 AU	0.60 Rf	226.9 AU	10.58 %	0.63 Rf	26.7 AU	8795.3 AU	10.26 %
11	0.63 Rf	126.6 AU	0.69 Rf	288.1 AU	13.43 %	0.81 Rf	88.7 AU	26180.6 AU	30.53 %
12	0.86 Rf	81.7 AU	0.87 Rf	83.4 AU	3.89 %	0.90 Rf	62.1 AU	2335.1 AU	2.72 %
13	0.90 Rf	62.4 AU	0.94 Rf	587.0 AU	27.36 %	0.99 Rf	13.4 AU	21702.3 AU	25.30 %

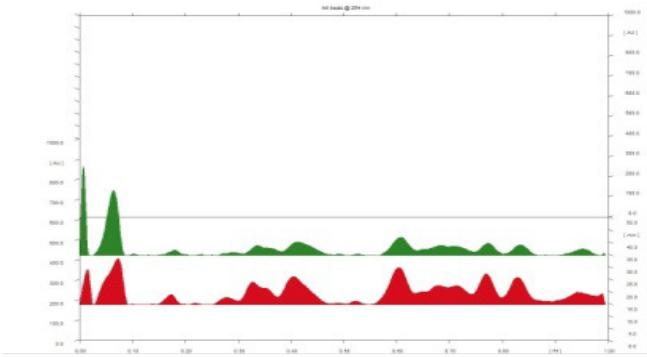


Fig. 5. 3D Chromatogram of 254 nm.

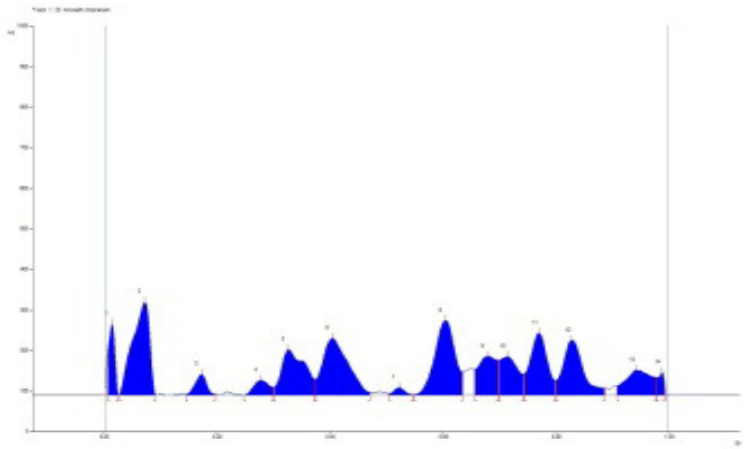


Fig. 6. HPTLC finger print profile of Ethanol extracts canning 254 nm.

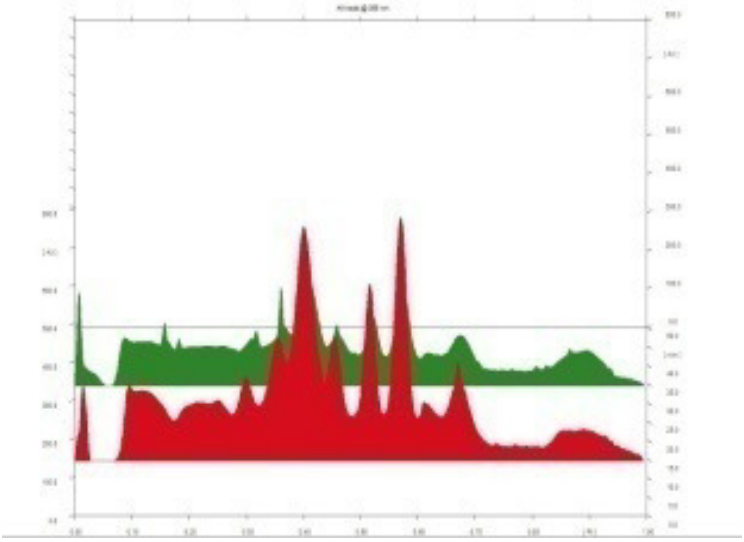


Fig. 7. 3D Chromatogram of 366 nm.

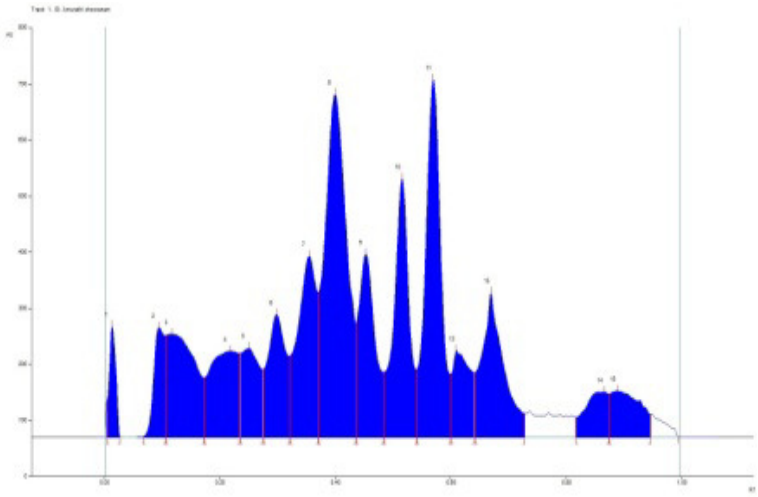


Fig. 8. HPTLC finger print profile of Ethanol extract scanning 366 nm.

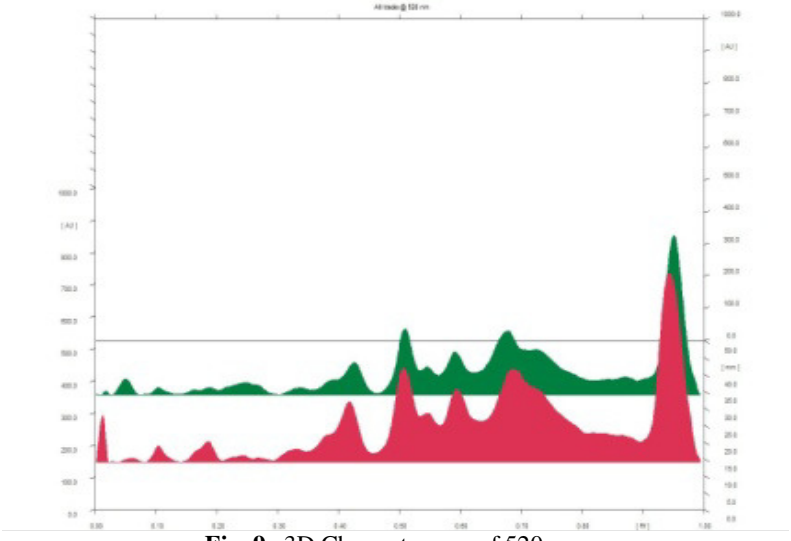


Fig. 9. 3D Chromatogram of 520nm.

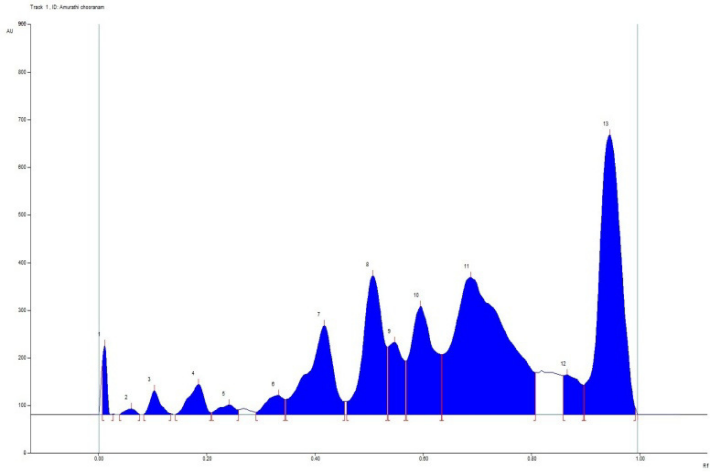


Fig. 10. HPTLC finger print profile of Ethanol extract scanning 520 nm.

— HPTLC finger printing analysis of the specimen establishes the existence of fourteen notable peaks at 254 λ nm, fifteen peaks at 366 λ nm, thirteen notable peaks at 520 λ nm indicates the presence of versatile phyto components.

— Rf value of the peaks differs from 0.01 to 0.98 at 254 λ nm, 0.00 to 0.88 at 366 λ nm and 0.01 to 0.90 at 520 λ nm

— The approach of fingerprint analysis through HPTLC has become the effective technique for quality control of herbal drugs due to its simplicity, flexibility and reliability. It play vital role for identification, authentication and quality control of herbal drugs. The development of chromatographic finger prints acts as a remarkable role in the quality control of complex herbal medicines (Attimarad *et al.,* 2011; Lalhriatpuii, 2020).

— This approach was employed successfully to show a chemical fingerprint for authentication and reliable verification of the existence of bioactive compounds in the trial drug *AMC*. So, important phytochemicals were presented in the drug *AMC* was strengthened by TLC and comparing the Rf of corresponding spot with that of standards.

— The drug *AMC* possesses anti-urolithiatic property. Hence, the result supports the drug *AMC* is very useful in the treatment of urolithiasis.

STERILITY TEST BY POUR PLATE METHOD

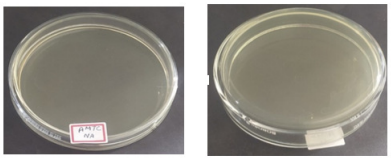


Fig. 11. Nogrowth/colonies were observed in any of the plates inoculates with the test sample *AMC*.

Observation. No growth was observed after incubation period. It reveals the absence of specific pathogens.

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

The results of Table 12, showed that the drug *AMC* is free from the microorganisms and the absence of total bacterial and fungal count which may shows that the drug *AMC* has good quality and safer drug to treat Urolithiasis (Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard 2012)

Table 12: Test for microorganisms.

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	

Specific Pathogen

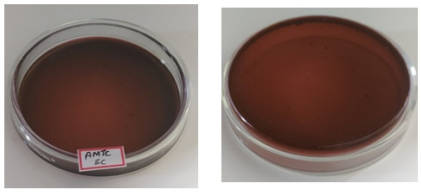


Fig. 12. Culture plate with *E-coli (EC)* specific medium.

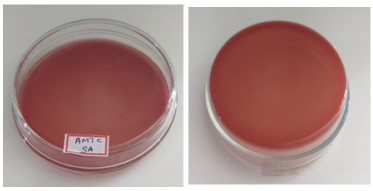


Fig. 13. Culture plate with *Salmonella (SA)* specific medium.

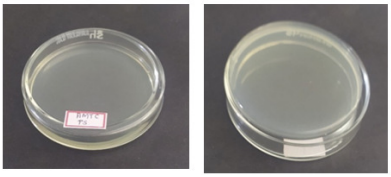


Fig. 14. Culture plate with *Pseudomonas Aeruginosa (PS)* specific medium.

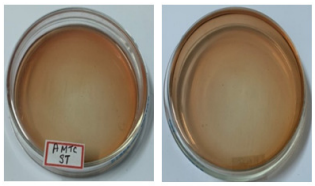


Fig. 15. Culture plate with *Staphylococcus Aureus (ST)* specific medium.

Observation. No growth was observed after incubation period. It reveals the absence of specific pathogens

Result. No growth/colonies were observed in any of the plates inoculated with the sample *AMC* as shown in Fig. 11.

The specific pathogen test results mentioned in Table 13 revealed that the drug *AMC* were able to prevent the growth of the microorganisms such as *E. coli, Salmonella species, Staphylococcus aureus, Pseudomonas aeruginosa* as shown in Fig. 12-15 respectively and it shows that the drug *AMC* used to reduce the morbidity and mortality from chronic diseases (WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues, 2007).

Table 13: Test for pathogens.

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

Pesticide Residue: The results as mentioned in Table 14 exposed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample *AMC*. So, *AMC* has no toxicity and bioaccumulation. Hence, the trial drug *AMC* is a safer drug for human health to treat urolithiasis (Protocol for Testing of Ayurvedic, Siddha and Unani medicines, 2008).

Table 14: Test Result Analysis of the Sample *AMC*.

Pesticide Residue I. Organo Chlorine Pesticides	Sample *AMC*	AYUSH Limit (mg/kg)
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	0.2mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
IV. Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Aflatoxins: The results shown in Table 15 indicates that there were no spots being identified in the test sample loaded on TLC plates when compared to the standard which indicates that the test sample *AMC* was free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2. So, the drug *AMC* is non-toxic, there is no contamination and it does not act as a carcinogen. This trial drug *AMC* is safe to treating urolithiasis (Protocol for Testing of Ayurvedic, Siddha and Unani medicines, 2008).

Table 15: Aflatoxins Report of the Sample *AMC*.

Aflatoxin	Sample *AMC*	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)

CONCLUSIONS

Over a number of decades, urolithiasis has become the most important threats in human health globally, because of the lack of effective treatments, greater recurrence rates and it's multiple etiology factors. Standardization is the most powerful but we are least using tools. Documentation of the above results and discussions confirms that the *Amurthathi Chooranam* has potent drug indicated for urolithiasis. Physicochemical analysis such as water soluble extract 24.9985%, Alcohol soluble extract 5.1505%, Loss on drying 3.598%, Total ash 7.3816%, Acid insoluble Ash 3.0436%, Water soluble ash 1.2543%, which reveals that the drug has good quality and purity. It specifies no impurities in the raw drug *A MC*. The stability and shelf life of the drug *AMC* is good so very safe to treat urolithiasis. Powder microscopic analysis strengthens the drug has no adulterations and there are no impurities. Phytochemical such as Alkaloids, carbohydrates, tannin, phenol, saponin, flavonoids and diterpenes are having reduction of super saturation of lithogenic enhancing properties.

Other parameters like HPTLC, demonstrates a chemical fingerprint for authentication and reliable verification of the existence of bioactive compounds in *AMC*. So, the presence of important phytochemicals in *AMC* was strengthened by TLC and comparing the Rf of corresponding spot with that of standards. The drug *AMC* possesses anti-urolithiatic property. Sterility test reveals, *AMC* is free from microorganisms. So the drug has good quality and safety. A specific pathogen test showed that the drug *AMC* prevents the growth of

microorganisms which shows that the drug *AMC* can be used to decrease the morbidity and mortality from chronic diseases. The aflatoxin assay results revealed that the drug *AMC* is non-toxic, no contamination and it does not acts as a carcinogen. The pesticide precipitate test demonstrated that the drug *AMC* has no toxicity and bioaccumulation. Hence, the drug *AMC* is safe to treat urolithiasis. From the outcome of results the drug *Amurthathi Chooranam* is safe and cost effective and biologically having active components which is treating for urolithiasis.

FUTURE SCOPE

The study is also explaining the toxicity profile of the test drug as recommended by the concerned authorities and international guidelines. The successful completion of the toxicity profile and Anti urolithiatic, diuretic and nephro-protective potency of the study drug *Amurthathi Chooranam* by preclinical testing may further lead to global accreditation of this Siddha Drug after suitable clinical studies.

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Conflict of Interest. None.

REFERENCES

Abirami, R., Parimala, B., Swathipriya, R and Sethuram, M. (2018). In vitro inhibition of urolithiasis using terpene isolated from scopariadulcis. *International Journal of Pure and Applied Mathematics, 119*(15), 551-563.

Arya, P., Pandey, S. and Verma, V. (2017). Kidney stone formation and use of medicinal plants as antiurolithiatic agents. *UJPR, 2*, 42-48.

Attimarad, M., Mueen Ahmed K. K., Aldhubaib B. E and Harsha, S. (2011). High-performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery. *Pharma Methods, 2*(2), 71 – 74.

Butterweck, V. and Khan S. R. (2009). Herbal Medicines in the Management of Urolithiasis: Alternative or Complementary. *Planta Med, 75*, 1095-1103.

Farhud, D. D. (2015). Impact of lifestyle on health. *Iranian Journal of Public health*, *Nov, 44*(11*),* 1442.

Gindi, S., Methra, T., Chandu, B. R., Boyina, R. and Dasari, V. (2013). Antiurolithiatic and invitro anti-oxidant activity of leaves of Ageratum conyzoidesinrat. *World J Pharm Pharm Sci, 2*, 636-649.

Gupta, S. and Kanwar, S. S. (2018). Phyto-molecules for Kidney Stones Treatment and Management. *Biochem Anal Biochem, 7*, 362.

Heamavathi, S., Kumar, J., Shankar, S., Saravanadevi, M. D. and Velpandian, V. (2022). Evaluation of phytochemical, physicochemical and biochemical analysis of saaranai ver chooranam (the root of Trianthemadecandralinn,) -a Siddha herbal medicine. *Int J. Pharm.Drug, Anal, 10*(2), 27-37.

Heron, S. and Yarnell, E. (1998). Recurrent kidney stones: A naturopathic approach. *Altern Complement Ther*, *4,* 60-67.

Indian Pharmacopeia I, Volume I, Government of India, Ministry of Health and Family welfare (2014). *Indian Pharmacopoeia commission.*

Khandelwal, K. R. (2010). *Practical Pharmacognosy Techniques and Experiments.20, Pune, India. Nirali Prakashan. 23*(1,17), *25*(1,9).

Lalhriatpuii, T. (2020). HPTLC Fingerprint in Herbal Drug Formulations. In: Sen S., Chakraborty R. (eds) *Herbal Medicine in India. Springer, Singapore,* 337 - 362.

Lee, H. J., Jeong, S. J., Park, M. N., Linnes, M., Han, H. J, Kim, J. H, Lieske, J. C. and Kim, S. H. (2012). Gallotannin suppresses calcium oxalate crystal binding and oxalate-induced oxidative stress in renal epithelial cells. *Biol Pharm Bull, 35*(4), 539-544.

Lohar, D. R. (2008). Protocol for testing of Ayurvedic, Siddha and Unani Medicines. Ghaziabad. Government of India, Department of AYUSH, Ministry of Health & Family Welfare. *Pharmacopoeial Laboratory for Indian Medicines*, *21*, 40-47.

Luciana de castro (2001). 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, 21,* 1.

Nilakshi Pradhan., Jyoti Gavali and Nitin Waghmare (2015). WHO (World Health Organization) Guidelines for Standardization of Herbal Drugs. *International Ayurvedic Medical Journal, 3*(8), 2239.

Pareta, S. K., Patra, K.C. and Harwansh, R. (2011). In-vitro calcium oxalate crystallization inhibition by Achyranthesindicalinn. Hydroalcoholic extract: An approach to antilithiasis. *Int J Pharm Bio Sci*, *2*, 432-437.

Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. (2012). Wayne, PA, Clinical and Laboratory Standards Institute. *11th ed.CLSI document M02-A11.*

Pour Plate Method (2022). Procedure, Uses, (Dis) Advantages. *Microbe Online. Available from: https://microbeonline.com*

Protocol for Testing of Ayurvedic Siddha and Unani Medicines (2008). Ghaziabad. Department of AYUSH, *Pharmacopoeial Laboratory for Indian Medicines*. 49-50

Quality Standards. ICMR. (2003). 1, 97,153-5.

Quality Standards. ICMR. (2010). 8, 314-24.

Quality Standards. ICMR. (2012). 10, 286.

Quality Standards. ICMR. (2018). 16, 205-13.

Savjani, K. T., Gajjar, A. K. and Savjani, J. K. (2012). Drug solubility: importance and enhancement techniques. *ISRN Pharm. 2012*, 195727.

Seetharam Prasath, J. (2014). *Anubava Vaithiya Deva Ragasiyam.* Rathna Nayakkar and Sons, Chennai, Thirumagal Vilasa Press, 684.

Sellaturay, S. and Fry, C. (2008). The metabolic basis for urolithiasis. *Surgery*, *26,* 136-139.

Takashi Hiroi. (2017). Measurement of Particle Size Distribution in Turbid Solutions by Dynamic Light Scattering Microscopy. *J Vis Exp, 119*, 548-585.

Wallis, T. E. (1965). Analytical Microscopy - Its aims and methods in relation to foods, water, spices and drugs. *Third Edition. Boston. Little, Brown and Company.*

WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues.*WHO Guidelines* 2007*, WHO Geneva.*

Zeng, X., Xi, Y. and Jiang W. (2019). Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: A review. *Crit Rev Food SciNutr, 59*(13), 2125-2135.

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