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Original Article

Quality Standards of *Athiyadhi Kashayam* - A Polyherbal Classical Siddha Formulation with Potent Anti-Diabetic Ingredients

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ABSTRACT

Background: Diabetes mellitus is considered a major disease because it is a silent killer associated with many co-morbidities. The recent days' changes in habits, lifestyle, food habits, stress, etc., have affected good health of people and hence they are seeking traditional medicines like Siddha which are time tested. *Athiyadhi Kashayam* (AK) is an important Siddha polyherbal formulation containing 5 barks *Atthi* (*Ficus racemosa*), *Aavarai* (*Senna auriculata*), *Sarakkondrai* (*Cassia fistula*), *Naaval* (*Syzygium cumini*) and *Kadal azhinjil* (*Salacia oblonga*). The compound formulation AK is a combination of antidiabetic ingredients due to which it is a good medicine for diabetes mellitus and co-morbidities. The current study is the first attempt to standardize the formulation as per pharmacopeial standards and to justify the formulation as a potent antidiabetic herbal drug. **Materials and Methods:** Pharmacognostic, physiochemical and HPTLC studies were carried out as per standard procedures mentioned in Pharmacopoeias. Literature on phytochemistry and pharmacology of each ingredient were compiled to justify the antidiabetic potential of the medicine. **Results:** Macroscopy, microscopy, powder microscopy, physiochemical analysis and HPTLC was matched with the characters of these ingredients as reported in pharmacopoeias. The earlier reports have revealed the potential phytochemicals in the ingredients revealing its possible benefits for diabetes and related comorbidities. **Conclusion:** Botanical and chemical standards for quality control of AK have been obtained following standard testing protocol which will give monographic data on identity and quality parameters of this unexplored Siddha medicine. The formula composition from the 5 ingredients with potential antidiabetic properties and managing other comorbidities makes this formula a potent medicine for antidiabetic applications.

Keywords: *Madhu megam*, Monograph, Non-communicable diseases, Quality of life, Standardization.

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INTRODUCTION

Diabetes mellitus is considered a major disease because it is a silent killer associated with many co-morbidities. Worldwide, in 2021, 537 million people suffered from diabetes and is expected to be 643 million in 2030, in 2040 the number will increase to 783 million.[1]

Siddha system medicine has a long tradition and uniqueness; it is integrated into the daily life of the people. In recent days' changes in habits, lifestyle, food, increased stress, etc. have affected the quality of life, therefore, nowadays people have been seeking traditional medicines to lead a healthy life. The Siddha system has many drugs for co-morbidity, for example, during the dengue fever and COVID-19 pandemic *Nilavembu kudineer* and *Kabasura kudineer* played major roles in boosting immunity and recovery from acute fever, body pain, mucus, sneezing and cough. Likewise, there are different medicines in Siddha mentioned for treating non-communicable diseases.

Siddhars (ancient Tamil Siddha practitioners) recommended many formulations for curing non-communicable diseases based on their experience. Siddhar Therayar, one of the pioneers of the Siddha medicine, described *Madhumegam* as *Neerinai perukkal noi* (clinical conditions associated with increased urinary output) and *Nerinai arukkal noi* (clinical conditions associated with low urinary output). *Madhumegam* is classified under *Neerinai*

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perrukkal noi because of the etymology that Mellitus means sweetness which is equivalent to *Madhu*. Diabetes means passing like a fountain connecting to urinary diseases that manifest as excessive urination or decreased urination.[2]

Athiyadhi Kashayam (AK) is one of the most useful Siddha polyherbal formulations mentioned for treating diabetes mellitus. AK is mentioned in *Mega Nivarana Bothini Ennum Neerizhivu Maruthuvam*, for treating *Madhumegam*.[3] AK contain 5 plant barks such as *Atthi* (*Ficus racemosa*), *Aavarai* (*Senna auriculata*), *Sarakkondrai* (*Cassia fistula*), *Naaval* (*Syzygium cumini*) and *Kadal Azhinjil* (*Salacia oblonga*). The *F. racemosa* stem bark is traditionally used for piles, bleeding, diabetes, amebic dysentery, dysentery and leucorrhoea. *C. auriculata* stem bark is used in penile irritation, urine infection, spermatorrhea, body heat, diabetes and menstrual disorders. *C. fistula* stem bark used as laxative, for menstrual disorders, diabetes, digestive disorder, stomach ulcer, intestine ulcer, imbalance of the three humors and anorexia. *S. cumini* bark is used as stomachic, diuretic, tonic, for menstrual disorders, cough, mouth sore, asthma. *S. oblonga* root bark is used for fever, diabetes, dysentery, Ivy disease and dehydration.[4] The compound formulation AK may significantly reduce the diabetes mellitus and co-morbidities, hence this study is carried out to derive quality standards for the first time for *Athiyadhi Kashayam* as per pharmacopeias.

MATERIALS AND METHODS

Collection and Authentication

Authentic raw drugs were collected from Chennai herbal drug market, Tamil Nadu, India. The plant samples were authenticated at Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai-600106. The voucher specimen 1, *Atthi* (*Ficus racemosa* L.) (EST40); 2, *Aavarai* (*Cassia auriculata* L.) (EST61); 3, *Sarakkondrai* (*Cassia fistula* L.) (EST24); 4, *Naaval* (*Syzygium cumini* (L.) Skeels) (EST65); 5, *Kadal azhinjil* (*Salacia oblonga* Wall) (DRT57) was deposited in the department museum of SCRI (CCRS), Chennai, India for future reference.

Macro-microscopic Authentication

The organoleptic character like colour, odour, taste, texture, fracture and surface characters were performed as per standard procedures.[5] For authentication of the dried bark ingredients, materials were soaked in water for 24 h and free hand sections were taken to record the microscopic features. The section was stained with 4% of saffranin and 0.4% astra blue, mounting medium used was 80% glycerin. The anatomy sections were photographed under different magnifications with the help of Zeiss Axiolab 5 trinocular microscope fitted with Axiocam 208 color camera.[6] All the barks were washed, air dried, powdered and passed through sieve #80. A pinch of AK powder was mounted on a glass slide with a free drop of glycerol after clearing with saturated solution of chloral hydrate solution. The diagnostic microscopic characters were observed and documented using Nikon Eclipse trinocular microscope fitted with Axiocam ERC5s camera.[7]

Preparation of formulation (AK)

All the Ingredients are washed out in running tap water and shade dried; equal quantity of the raw ingredients (Table 1) weighed and ground to coarse powder; the powders were passed through sieve #40 to mix all ingredients well.[8]

Physicochemical Analysis

All the chemicals and solvents used were AR grade (Merck). Preliminary physicochemical analysis like total ash, acid in soluble ash, alcohol soluble extractive, water-soluble extractive, loss on drying and pH were carried out for AK as per the standard procedures.[5]

Phytochemical Analysis

The preliminary phytochemical screening for phenol, tannin, flavonoids, triterpenoid, proteins, glycosides, reducing sugar, anthraquinones, quinones, alkaloids, saponins, cardiac glycoside, steroids and coumarins was done for methanol extracts of AK following standard procedures.[5]

HPTLC

CAMAG, Switzerland make ATS4 was used for methanol extracts application on TLC plate; development was performed on twin trough chamber (10 × 10 cm); for obtaining densitograms Scanner 4 with Vision CATS software was used; visualizer was used for photo documentation under UV-visible conditions; TLC plate heater was used for colour development and derivatising agent Vanillin (1 g) Sulphuric Acid (5%) in ethanol (VSA) used for visualization. The mobile phase used was mixture toluene: ethyl acetate: methanol: formic acid (7.5:2.5:0.5:0.5 *v*/*v*/*v*/*v*); and stationary phase was aluminium plate (Merck) pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness.[9]

RESULTS

Macroscopy identity

F. racemosa

Cut pieces of bark varying in size from 1 to 2.5 cm thick, curved and channeled, exterior surface silver brown to dirty white, patches of exfoliating bark filling surface is yellowish brown in color, inner surface slightly reddish brown; external surface rough, inner surface longitudinal fibrous wrinkle; fracture heard and fibrous; odor nil, taste astringent (Figure 1B).

S. auriculata

Cut pieces of bark varying in size, up to 10 cm long and 1 to 1.5 mm thick, shows mostly double quill and few are curved, outer surface reddish brown with light dirty brown 1mm width lenticels; a few barks having exfoliating dirty brown papery scales patches of

rhytidome on outer surface; inner surface slightly reddish brown with longitudinal striation; external surface rough, fracture short and splintery; odor nil, taste astringent (Figure 1D).

C. fistula

Cut pieces of bark flat and slightly curved, up to 9 cm long, 5 cm wide, 0.4 to 0.8 cm thick, exterior dirty white with lenticels and patches of exfoliating blackish brown rhytidome; inner surface pale reddish brown, cut surface reddish brown; externally rough with warty patches, internally parallel striations of fibrous wrinkle; fracture hard and fibrous; odor nil, taste astringent (Figure 1E).

S. cumini

Cut pieces of bark flat and slightly curved, varying in length, breadth up to 8 cm, 0.5 to 2.5 cm thick; exterior pale silver brown to dirty white, longitudinal fissure and wrinkles with lenticels and patches of exfoliating bark; inner surface slightly reddish brown; external surface rough, inner surface longitudinal fibrous; fracture heard and fibrous; odor nil, taste astringent (Figure 1A).

S. oblonga

Cut pieces of bark varying in size up to 4 cm long, 2 cm in wide, 1 to 1.5 mm in thickness; mostly flat and slightly curved and slightly twisted; external surface rough, fracture short and splintery with pale white fibrous; outer exfoliating bright yellow papery scales patches of rhytidome, surface is yellowish brown; inner surface pale reddish brown with longitudinal striations; odor not characteristic, taste astringent (Figure 1C).

AK course powder is dull reddish brown in color with yellow-colored papery fragments; touch rough, odour nil, taste strong astringent and bitter (Figure 1E).

Anatomy

F. racemosa

TS of bark shows 1 to 4 band of periderm, outer periderm consists up to 30 cells high outer exfoliating phellem embedded with brownish content followed by phellogen and phlloderm which are formed of collapsed cells embedded with stone cells; the cells contain brownish content and cluster crystals of calcium oxalate; inner periderm consists 25 to 35 cells high phellem and non-storied phellogen followed by 6 to 12 layered narrow secondary cortex and a very wide zone of secondary phloem composed of usual elements, phloem rays are mostly uni to tri-seriate few are multi-seriate, thin-wall phloem parenchyma cells are oval to circular in shape, phloem fibres lignified, thick-wall with very narrow lumen: laticifers with small granular masses found in phloem; thin walled parenchyma cells embedded with orange or reddish brown content, round to oval simple and compound starch grains, cubical and rhomboidal calcium oxalate crystals; some lignified cortical cells shows pits and found in large groups and often scattered singly and in small groups (Figure 2E).

S. auriculata

TS of bark shows outer narrow 8 to 15 rows of tangentially running rectangular exfoliating cork cells embedded with reddish brown content followed by distinguished single layered cork cambium

Table 1: Physicochemical parameters of *Athiyadhi Kashayam*

Name of the Experiment	Mean value
Loss on drying at 105°C	10.37%
Total ash	5.20%
Acid insoluble ash	0.88%
Water soluble extractive	13.43%
Alcohol soluble extractive	14.97%
pH value (10% solution)	5.42

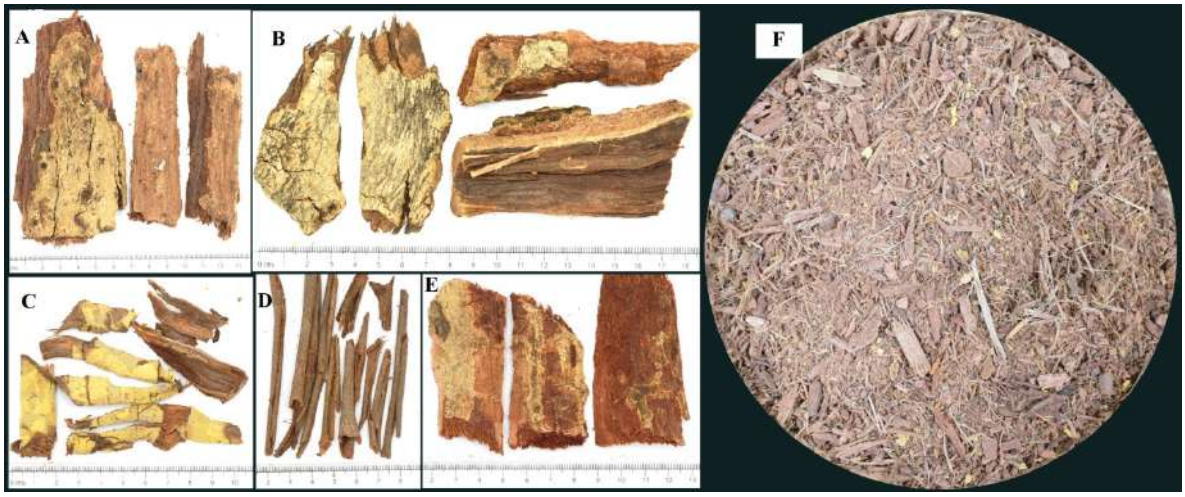


Figure 1: Macroscopy of *Athiyadhi Kashayam* ingredients. A, *Syzygium cumini*; B, *Ficus racemosa*; C, *Salacia oblonga*; D, *Senna auriculata*; E, *Cassia fistula*; F, the formulation (AK).

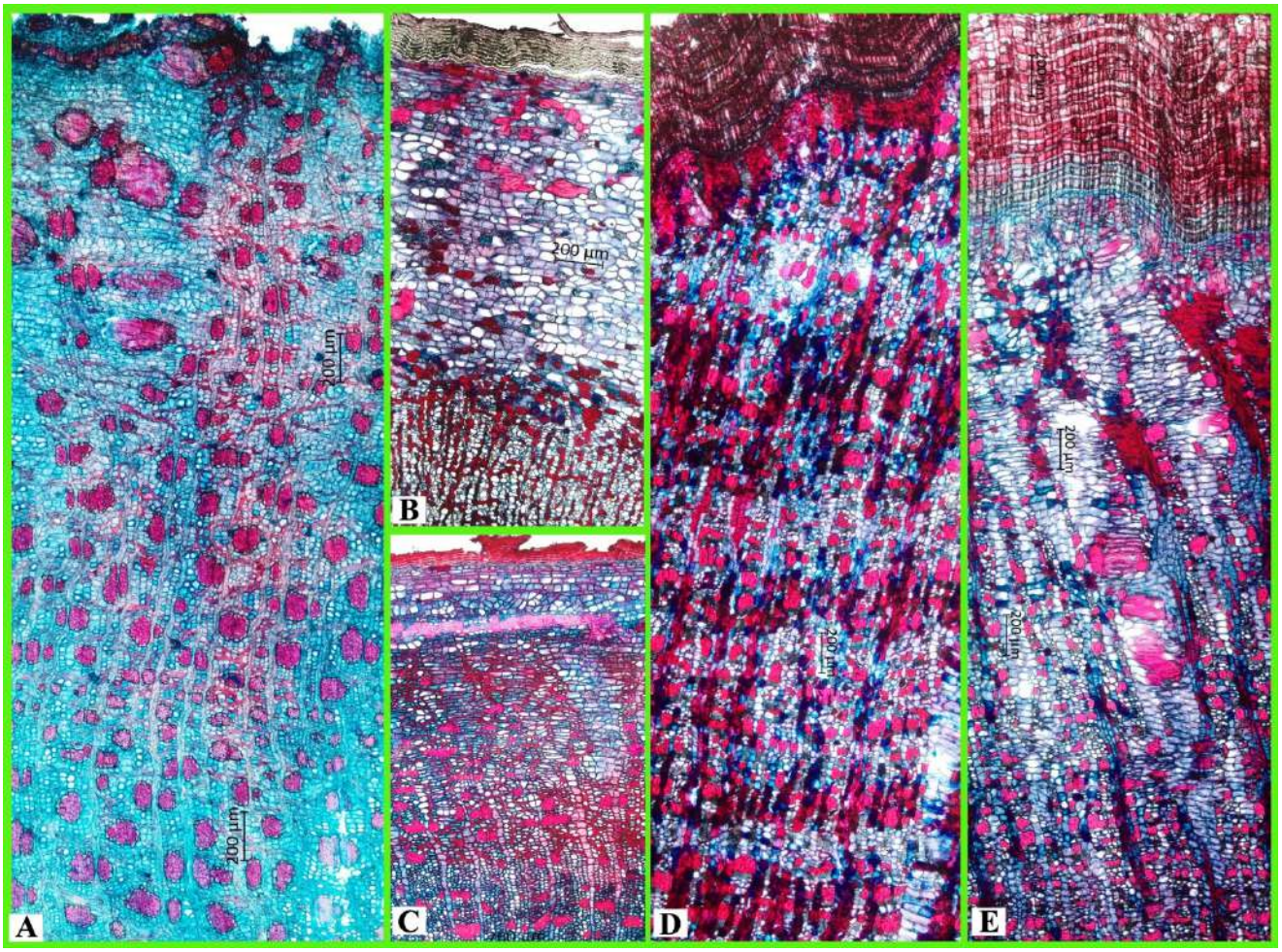


Figure 2: Detailed TS of of *Athiyadhi Kashayam* ingredients. A, *Cassia fistula*; B, *Salacia oblonga*; C, *Senna auriculata*; D, *Syzygium cumini*; E, *Ficus racemosa*.

and narrow band of rectangular cells of phelloderm. Cortex consists polygonal shaped parenchyma cells embedded with discontinuous band of thick walled, pitted stone cells; tangentially running dilated parenchyma tissue is seen intervened by conical stand of phloem tissue towards the periphery. Phloem consists group of fibres, parenchyma cells and other usual elements embedded with starch grains and thin band of ceratenchyma (Figure 2C).

C. fistula

Detailed TS of the bark shows very narrow band of suberized exfoliating cork cells embedded with brownish content, followed by narrow secondary cortex embedded with a band of sclerenchymatous stone cells and large group of sclerieds, underneath this lie wide zone of phloem region embedded with group of phloem fibres intertwined irregular line of uni to bi-seriate, rarely tri-seriate phloem rays; throughout the outer phloem tissue irregularly running band of ceretenchyma is embedded; simple to few 2 to 3 component starch grains, prismatic and cluster crystals of calcium oxalate, gum like content are observed; inner phloem consists less number of phloem fibre bundle, ceratenchyma and other ergastic substances (Figure 2A).

S. cumini

Detailed TS of the bark shows narrow zone of suberized rhytidome which is differentiated into exfoliating upper cork and the middle area consists of compressed parenchyma cells embedded with reddish-brown content and group of lignified cells; inner cork consists tangentially running wavy band of rectangular cell having distinct phellogen, narrow compressed parenchymatous secondary cortex; wide zone of secondary phloem consists of sieve cells, ray cells, polyhedral thin-walled parenchyma, phloem fibres, diffused isolated and group of stone cells and sclereids; phloem rays running in wavy line, mostly uni to bi-seriate few are tri and tetra seriate; some places of phloem region having spindle shaped dilated rays consists tangentially running rectangular non-storing parenchymatous cells; throughout the section present few reddish-brown content, rosette crystals of calcium oxalate and simple, round to oval starch grains (Figure 2D).

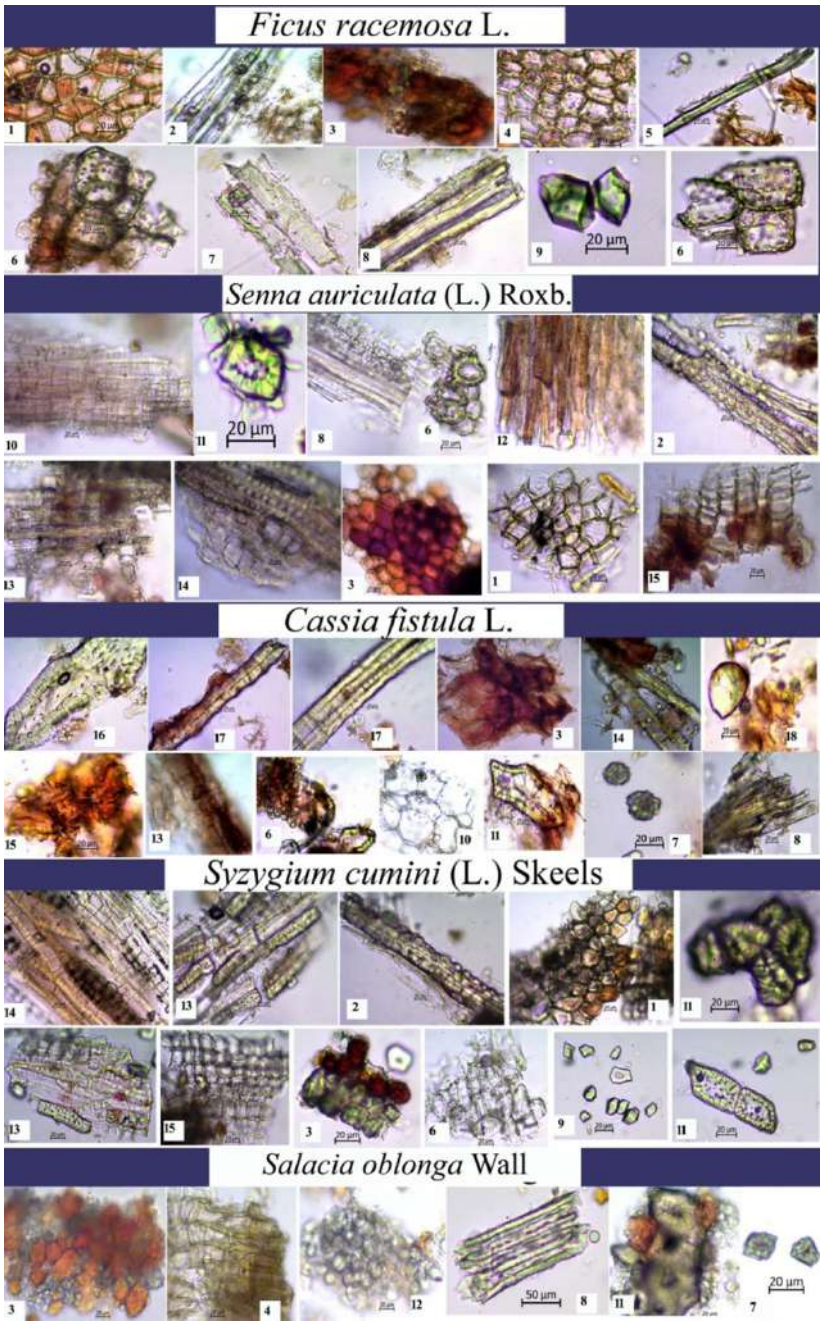


Figure 3: Powder microscopy of *Athiyadhi kashayam*. 1, cork cells in surface view; 2, crystal fibre; 3, parenchyma cells with brownish contents; 4, thick walled parenchyma; 5, cortical fibre; 6, pitted parenchyma; 7, cluster and rosette crystals of calcium oxalate; 8, phloem fibre; 9, prismatic crystals of calcium oxalate; 10, thin walled parenchyma; 11, stone cells; 12, cortical parenchyma; 13, tangential longitudinally cut phloem tissue; 14, radial longitudinally cut phloem tissue; 15, cork cells in surface view; 16, sclereids; 17, fibre sclereids; 18, oil globule.

S. oblonga

TS of bark shows outer narrow 8 to 15 rows of tangentially running rectangular exfoliating, thick-walled suberized cork cells embedded with reddish brown content; distinct single layered cork cambium is seen followed by wide zone of cortex consisting dilated, polygonal, thin-walled parenchymatous cells embedded with isolated and group of stone cells and sclereids; rosette and cluster crystals of calcium oxalate, round to oval simple starch grains few are having up to six component are seen in the parenchyma cells; phloem consists diffused, thin-walled bast fibres, uni and bi-seriate phloem rays; sieve elements and parenchyma cells with brownish content (Figure 2B).

Powder microscopy

AK shows cork cells, parenchyma cells with brownish content, parenchymatous cells, pitted parenchyma, fibres, prismatic and cluster crystals from *Ficus racemosa*; cork cells in sectional and surface view, cortical cells, cells with brownish content, parenchymatous cells, pitted parenchyma cells, crystal fibres

Table 2: Phytochemical parameters of *Athiyadhi Kashayam*.

Phytochemical parameters	Result
Phenol	+
Tannin	+
Flavonoids	+
Triterpenoid	-
Proteins	-
Glycosides	-
Reducing Sugar	+
Anthraquinones	-
Quinones	+
Alkaloids	+
Saponins	+
Cardiac glycoside	+
Steroids	-
Coumarins	-
Acids	+

and stone cells from *Senna auriculata*; cork cells, parenchyma with crystals, stone cells, sclereids, fibre bundle, oil drops and cluster crystals from *Cassia fistula*; cork cells, parenchyma, cells with reddish brown content, stone cells, sclereids, crystal fibres and prismatic crystals from *Syzygium cumini*; thick walled parenchymatous cells, cells with starch grains, fibre bundle, stone cells, brownish content, cluster crystals from *Salacia oblonga* (Figure 3).

Physicochemical and Phytochemical studies

Physico-chemical standards of AK are summarized in Table 1. The preliminary phytochemical screening shown presence of class of secondary metabolites as summarized in Table 2.

HPTLC of Methanol Extract

The TLC of methanol extract of AK (Figure 4) showed 7 spots under UV λ 254 nm; 15 spots under UV λ 366 nm; 15 and 9 spots appeared under UV λ 366 nm after dipping in VSR and under white light (λ 540 nm) respectively (Table 3).

The HPTLC densitogram showed 7 peaks at UV 254 nm, 4 being major at R_f 0.013 (51.65%), 0.19 (15.20%), 0.37 (12.30), 0.51 (11.67%) (Figure 5). Densitogram at 366 nm showed 5 peaks 4 being major with R_f values at 0.012 (47.22%), 0.099 (19.64%), 0.37 (11.99%), 0.51 (12.59%) (Figure 6). After derivatization, under UV λ 540 nm visible spectrum, showed 9 peaks 5 being major at R_f 0.09 (15.17%), 0.16 (18.02%), 0.20 (20.47), 0.28 (13.31%), 0.34 (17.01) (Figure 7). HPTLC fingerprinting investigation of the formulation demonstrated a chemical fingerprint for authentication and reliable verification of the existence of bioactive compounds in the test drug.

DISCUSSION

Authentication is one of the most important tools per proceeding pre-clinical and clinical evaluation.[10] The botanical and analytical parameters documentation will be helpful for standardization of formulation as per Pharmacopoeia norms.[11] Before any drug is explored for pre-clinical and clinical safety and efficacy studies, deriving its quality standards is a must.[12,13] The standardization research on this Siddha medicine has given set of quality standards which is must before the drug is added to Pharmacopoeia. The standards derived in this study will be a base for future standardization studies on this polyherbal formulation.[14]

Note on antidiabetic potential of ingredients

AK is an herbal formulation containing 5 different plant barks used in diabetes. Previous studies on *C. fistula* stem barks have shown a higher antioxidant potential;[15] and due to presence of *β*-sitosterol, it reduced total cholesterol, triglycerides and LDL-cholesterol level and increase HDL cholesterol content; it is also antihyperlipidemic, antilipidemic; hexane extracts possess strong antidiabetic activity as it has shown to significantly decrease blood glucose levels.[16] Aqueous extracts of stem bark administered to rats with streptozotocin-induced diabetes, it reduced the serum blood glucose concentrations, induced favorable changes in body weight, improved transaminase activity, achieved a better lipid profile.[17] The methanolic extracts also showed improvement in parameters like glycosylated hemoglobin and insulin profile as well as regeneration of beta cells of pancreas.[18]

Clinical trials on *F. racemosa* bark extract showed the blood glucose level reduction and the drug demonstrated good antidiabetic, hypolipidemic, antioxidant properties in streptozotocin-induced diabetic rats.[19,20] Isolated *β*-sitosterol,[21] lupeol,[22] *β*-sitosterol have shown potential anti-diabetic activities.[23] Lupeol showed elevated serum insulin level and concomitant reduction of serum glucose,[24] ethanolic extract significantly reduced the blood glucose level and restored the status of lipids and lipoproteins to near the normal range.[25] Aqueous extract of bark given orally two times for 15 days, in combination with oral hypoglycemic drug, decreased the level of blood glucose in human.[26]

Root bark extract *S. oblonga* was shown to exhibit hypoglycemic, antioxidant, antimicrobial, cytoprotective, antidiabetic and immunomodulatory activities.[27-31] *S. oblonga* bind to the intestinal enzymes alpha-glucosidases and turn carbohydrate into glucose, also variety of pharmacological activities antidiabetic and anti-oxidative activities are reported.[32] The aqueous extract decreased plasma glucose level when administered to the Obese Zucker Rat (OZR)[33] and decreased glycemia.[34] A study on 66 diabetic patients study revealed significant reduction in the postprandial insulin response.[35] In an anti-glycemic activity clinical trial involving 43 healthy human subjects, 1000 mg of aqueous extract decreased plasma glucose by 27% and serum

Table 3: R_f values of TLC fingerprint profiling methanol extract of *Athiyadhi Kashayam* formulation

254 nm		254 nm		254 nm (Derivatized)		254 nm (Derivatized)	
Color	R_f value (s)	Color	R_f value (s)	Color	R_f value (s)	Color	R_f value (s)
-	-	-	-	Brown	0.02	-	-
-	-	-	-	Brown	0.05	-	-
-	-	Blue	0.06	-	-	Red pink	0.06
Green	0.075	-	-	-	-	-	-
-	-	-	-	-	-	Red pink	0.08
-	-	yellow	0.09	Blue	0.09	-	-
Green	0.10	-	-	-	-	-	-
-	-	-	-	-	-	Red pink	0.11
-	-	-	-	Brown	0.12	-	-
Green	0.14	-	-	-	-	-	-
-	-	-	-	-	-	Red pink	0.15
-	-	-	-	Brown	0.16	-	-
Green	0.18	Blue	0.18	-	-	-	-
-	-	-	-	Brown	0.20	Red pink	0.20
-	-	Blue	0.22	-	-	-	-
-	-	-	-	Brown	0.23	-	-
-	-	-	-	-	-	Red pink	0.24
-	-	-	-	Brown	0.27	-	-
-	-	-	-	-	-	Red pink	0.28
-	-	-	-	Blue	0.30	-	-
-	-	Blue	0.31	-	-	-	-
-	-	-	-	Brown	0.33	-	-
-	-	-	-	-	-	Red pink	0.34
Green	0.37	-	-	-	-	-	-
-	-	Blue	0.40	-	-	-	-
-	-	-	-	-	-	Red pink	0.43
-	-	-	-	Gray	0.44	-	-
-	-	Blue	0.45	-	-	-	-
-	-	Blue	0.48	-	-	-	-
-	-	-	-	Blue	0.50	-	-
Green	0.51	-	-	-	-	-	-
-	-	Green blue	0.52	-	-	-	-
Green	0.55	Red	0.55	-	-	-	-
-	-	-	-	Gray	0.56	-	-
-	-	Red	0.57	-	-	-	-
-	-	Blue	0.60	-	-	-	-
-	-	Blue	0.68	Gray	0.68	-	-
-	-	Green	0.72	-	-	-	-
-	-	-	-	Gray	0.74	-	-
-	-	Red	0.78	-	-	-	-

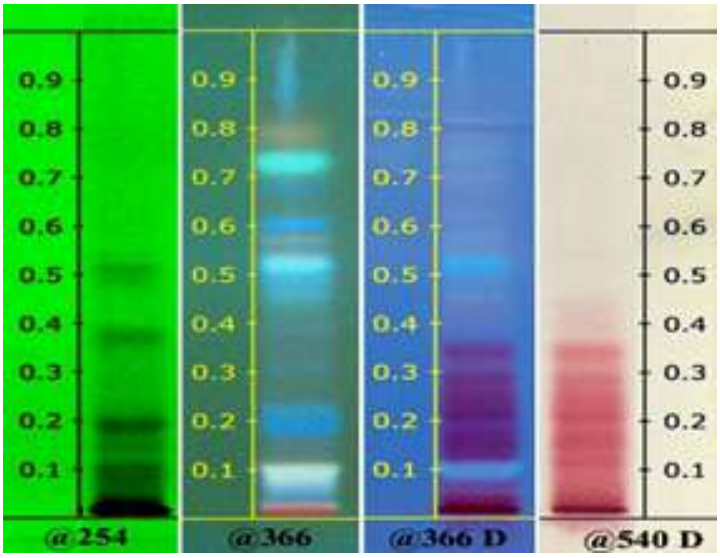
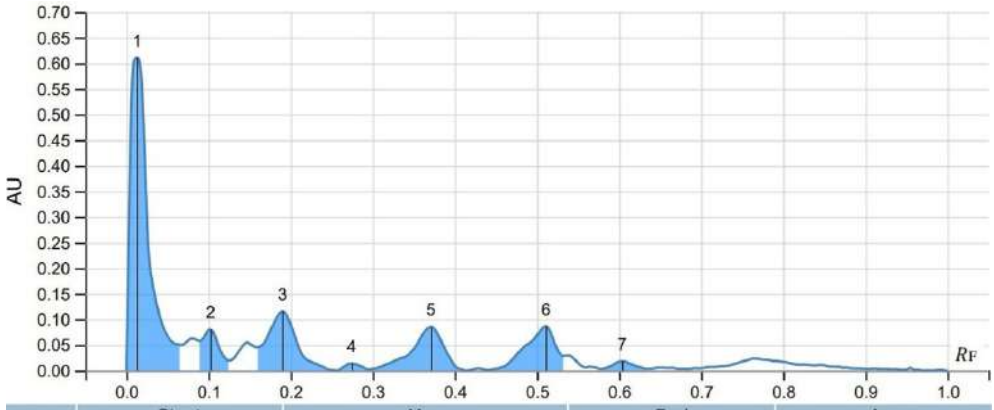


Figure 4: TLC plate of methanol extract of *Athiyadhi Kashayam* formulation.



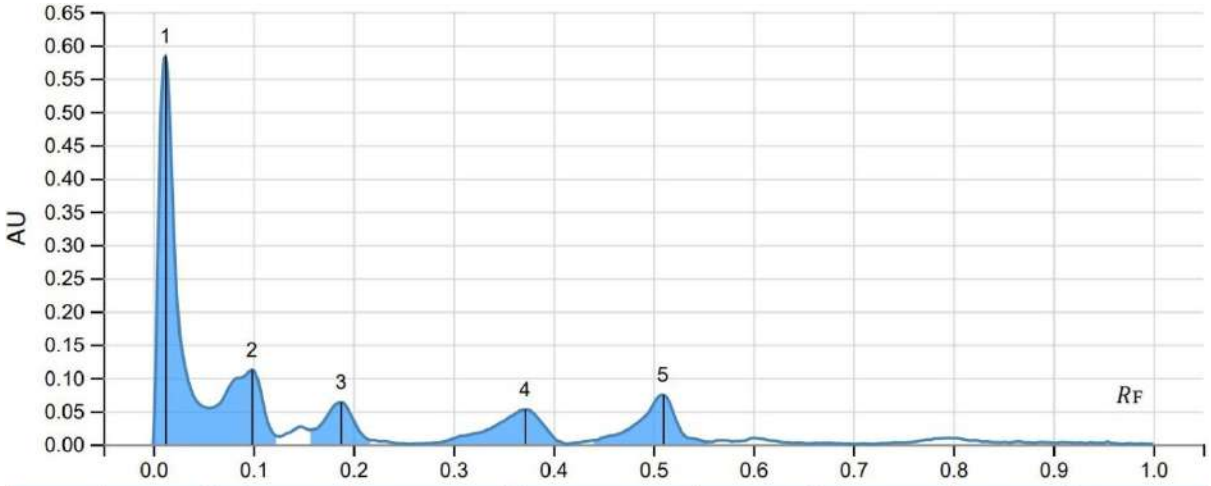
Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.013	0.6106	60.48	0.066	0.0500	0.01616	51.65
2	0.089	0.0578	0.102	0.0804	7.96	0.126	0.0195	0.00196	6.27
3	0.159	0.0457	0.190	0.1154	11.43	0.251	0.0006	0.00476	15.20
4	0.256	0.0000	0.274	0.0137	1.36	0.295	0.0021	0.00032	1.01
5	0.295	0.0021	0.371	0.0854	8.46	0.413	0.0000	0.00385	12.30
6	0.440	0.0008	0.511	0.0863	8.55	0.533	0.0283	0.00365	11.67
7	0.578	0.0030	0.604	0.0178	1.77	0.638	0.0036	0.00060	1.90

Figure 5: HPTLC densitogram of methanol extract of *Athiyadhi Kashayam* formulation at 254 nm.

insulin by 35% over a 2 hr time span, while increasing exhaled hydrogen by 60% and also no adverse effects were reported in this studies.[36]

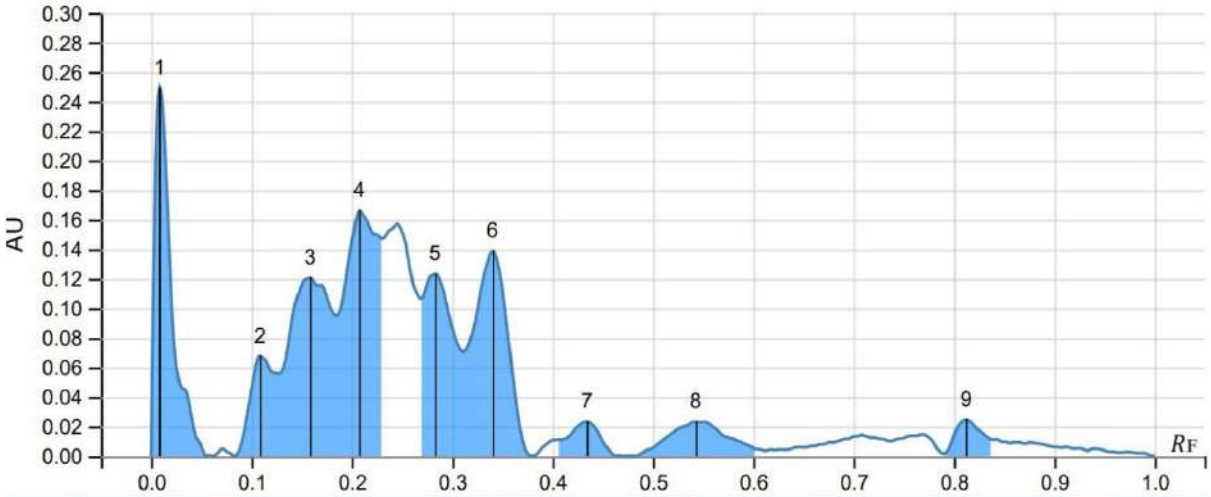
The *S. cumini* stem bark contain betulinic acid, *β*-sitosterol, quercetin, kaempferol, ellagic acid, gallic acid, myrecetin etc.,[37] Aqueous extract of stem bark showed significant decrease in serum glucose and cholesterol levels and increase in total red blood cells[38] and T lymphocytes levels in alloxan induced diabetic rats.[39] Ethyl acetate and water fractions showed excellent antioxidant, free radical scavenging activities,[40] and anti-diabetic activities.[41] It also exhibited their potency against H5N1 virus.[42] Methanolic and aqueous extracts were found to be diuretic in Wistar albino rats at the dose of 500 mg/kg body weight.[43]

The methanolic extract of *C. auriculata* stem bark showed a significant reduction in blood glucose levels in diabetic rats with a deviation of more than 80% when compared that on first day and the drug also showed antifertility activity.[44,45] Oral administration of extracts decreased the serum cholesterol, triglycerides, LDL and VLDL and increased the HDL cholesterol;[46] decreased the urea, uric acid and creatinine levels.[47] Excellent recovery of renal function and regenerative capability of the renal tubules was reported.[48,49] Stem bark has been reported to contain rutin, quercetin, kaempferol[50,51] which exhibit antihyperglycaemic and antioxidant activity in STZ-induced diabetic rats.[52,53] Besides, quercetin has also been reported to possess anti-hyperglycemic potential due to its pleiotropic mechanisms.[54]



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.012	0.5828	65.98	0.056	0.0539	0.01211	47.22
2	0.056	0.0539	0.099	0.1115	12.62	0.126	0.0115	0.00504	19.64
3	0.157	0.0214	0.188	0.0628	7.11	0.218	0.0060	0.00220	8.56
4	0.271	0.0009	0.372	0.0519	5.88	0.415	0.0000	0.00308	11.99
5	0.438	0.0052	0.510	0.0743	8.41	0.557	0.0032	0.00323	12.59

Figure 6: HPTLC densitogram of methanol extract of *Athiyadhi Kashayam* formulation at 366 nm.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.009	0.2491	26.59	0.055	0.0002	0.00486	15.17
2	0.083	0.0000	0.109	0.0676	7.22	0.127	0.0557	0.00189	5.91
3	0.127	0.0557	0.159	0.1207	12.89	0.184	0.0949	0.00577	18.02
4	0.184	0.0949	0.207	0.1661	17.73	0.230	0.1471	0.00656	20.47
5	0.270	0.1065	0.283	0.1232	13.15	0.311	0.0705	0.00427	13.31
6	0.311	0.0705	0.340	0.1388	14.82	0.379	0.0000	0.00545	17.01
7	0.402	0.0108	0.434	0.0234	2.50	0.463	0.0002	0.00085	2.66
8	0.482	0.0000	0.543	0.0232	2.48	0.602	0.0047	0.00159	4.97
9	0.789	0.0016	0.812	0.0247	2.63	0.839	0.0110	0.00079	2.46

Figure 7: HPTLC densitogram of methanol extract of *Athiyadhi Kashayam* formulation at 540 nm (Derivatized with VSR).

So, the current research confirms that the *Athiyadhi Kashayam* has potential medicinal properties to be indicated for diabetes.

CONCLUSION

Quality analysis of *Athiyadhi Kashayam* has been taken up to build standards for assessing its grade and righteousness. The analytical parameters, TLC image documentation and HPTLC finger printing profile are essential in improving Pharmacopoeial norms for AK. This study will contribute to the quality standards and antidiabetic potential exploration of this unexplored Siddha poly-herbal formulation. Evaluating its safety and effectiveness with future studies will give further proof on use of this Siddha medicine indicated in classical Siddha literature.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AK: *Athiyadhi Kashayam*; **D:** Derivatized; **HPTLC:** High-performance thin layer chromatography; **TLC:** Thin layer chromatography; **VSR:** Vanillin Sulphuric Acid.

SUMMARY

Diabetes mellitus is considered a major disease because it is a silent killer associated with many co-morbidities. Siddha system medicine has a long tradition and uniqueness as it is integrated into the daily life of the people. The Siddha system has many co-morbidity drugs just like *Athiyadhi Kashayam* (AK) which has potent medicinal properties to be indicated for diabetes. AK is an herbal formulation of five different plant barks used for diabetes patients. The current research confirms the macroscopic; microscopic structures of the five ingredients; powder microscopic features to differentiate cellular identity; analytical parameters, TLC image documentation and HPTLC finger printing profile which are essential in improving Pharmacopoeial norms for AK. The botanical and analytical parameters documentation will be helpful for standardization of this formulation as per Pharmacopoeia norms.

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