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Pharmacognostical characterization of *Aavarai kudineer* - A poly herbal preparation

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Abstract

Calibration of drugs and medical preparations are essential parameter in scientific scenario and it helps to improve quality and efficacy of drugs. *Aavarai Kudineer* is a poly herbal Siddha drug and it has been highly prescribing for the condition of *Madhumegam* (Diabetes mellitus). There are number of formulations of *Aavarai Kudineer* documented in literature, whereas scientific studies has not been reported. The current study was design to analyze *Aavarai Kudineer* scientifically for develop a standardization to the formula. Precursor mixture (coarse powder) of *Aavarai Kudineer* and aqueous extract of *Aavarai Kudineer* were tested. Pharmacognostical analysis including preliminary phytochemical study was done. The findings of the study could be useful in diagnostic keys for identification and preparation of aqueous extract of *Aavarai Kudineer*.

Keywords: *Aavarai Kudineer*, hypoglycemic drug, pharmacognostical study, standardization

Introduction

The traditional systems of medicine have received significant popularity in all over the globe as the drugs have curative property, less toxic and minimal side effects [1]. Siddha system of medicine is one of the ancient traditional systems of medicine and it is believed that the Sages laid the foundation for this system. It has great potential of medicinal resource repository goes back to B.C 10 000–B.C 4000 [2]. Ancient Siddha text books documented 4448 variety of diseases included *Madhumegam* [3].

Madhumegam or *Neerilivu* can be compared with diabetes mellitus [4]. It is one of the common metabolic disorder and raised as a global problem as the morbidity of diabetes mellitus increasing day by day [5]. The World Health Organization estimates, there will be 300 million diabetic patients in worldwide by the year of 2025⁶. A number of hypoglycemic herbal drugs and herbal formulations are available in the traditional medicinal practice and they have been widely prescribe by the physicians [5].

Aavarai Kudineer (AK) is one of such poly herbal formulation described in Siddha Materia Medica. The ancient Siddha medical literature the *Theraiyar kudineer* written by the ancient saints describes the constituents and indications of AK. AK is a common drug for diabetes mellitus in the Siddha system of medicine and have been prescribing by the traditional medical institutions. It is comprising of seven medicinal plants namely; leaves of *Senna auriculata* (L.) Roxb. (*Avirai*) and *Cassia fistula* L. (*Kondrai*), Seeds of *Syzygium cumini* (L.) Skeels. (*Naval*), wood of *Salacia reticulate* Wight. (*Kadalazhinjil*), root of *Saussurea lappa* Clarke. (*Koshtam*), rhizome of *Cyperus rotundus* L. (*Koraikizhangu*) and bark of *Terminalia arjuna* (Roxb.) Wight & Arn. (*Marutham*) [7].

Previous studies have shown the multi-dimensional therapeutic properties including hypoglycemic activity of the constitutional plants of AK [5, 6]. A previous *in-vivo* study confirms the anti-hyperglycemic potential of AK [8].

Differences was observed in the constituent plants and parts used to prepare the AK in literature [4-6, 9]. A number of constituent plants of AK is replaced by another plant species; i.e., *Salacia prinoides* and *Costus spiciosus* were used instead of *S. reticulata* and *S. lappa* respectively [4, 5, 9]. In addition discrepancies were noted with the parts used to prepare AK; i.e., whole plant of *S. auriculata* was used instead of leaves of *S. auriculata* [4], flowers and bark of *C. fistula* used for leaves of *C. fistula* [4, 6, 9] and rhizome of *C. spiciosus* used for instead of *S. lappa* [6].

Physical, chemical and biological analysis are necessary to identify the drug and study its pharmacological activities. In addition it is used to detect adulteration^[10]. Standardization, formulation and quality control measures of a medicine is essential parameters for maximizing pharmacological properties. Little published information on the extent of its scientific studies and it is essential need to standardize the AK for effective and efficient usage, in current scenario. Pharmacognostical characters is useful to standardize a drug. The current study was design to evaluate the pharmacognostical characterization of AK to develop scientific evidence.

Materials and methods

Plant materials

Constituent plants (dried parts) of *Avarai kudineer* as *Senna auriculata* (L.) Roxb. (Fabaceae), *Cassia fistula* L. (Fabaceae), *Cyperus rotundus* L. (Cyperaceae), *Saussurea lappa* Clarke. (*koshtam*) (Asteraceae), *Terminalia arjuna* (Roxb.) Wight & Arn. (Combretaceae), *Salacia reticulata* Wight. (Clestraceae) and dried seeds of *Syzminum cumini* (L.) Skeels. (Myrtaceae) were obtained from Siddha Medical shop in Nargarkovil, Tamil Nadu, India.

Preparation of precursor mixture of AK and aqueous extract (*Kudineer*)

Preparation of precursor mixture of AK

Dried leaves of *S. auriculata* and *C. fistula*, dried seeds of *S. cumini*, wood of *S. oblongata*, rhizome of *C. rotundus*, root of *S. lappa* and bark of *T. arjuna* were washed with distilled water and dried at room temperature in aerated condition. Above described dried constituents parts of AK were crushed individually to make small particles. The small particles were weighted separately (each 5g) using the electronic balance (SHIMADZU 0.0001-250 g) and mixed together.

Preparation of aqueous extract of AK

An aqueous extract of AK was prepared by boiling the precursor mixture of AK (35 g) with 1300 mL of distilled water to 1/8 of the initial volume, meeting the conditions of *Kudineer* preparation. Prepared aqueous extract of AK was stored in air tight, clean and labeled bottle at room temperature and subjected to the studies.

Morphological and microscopic characters

Medicinal plant materials were identified macroscopically and touch based on colour, surface characters, texture and special appearance of cut surface. Microscopic characters were tested using Optical microscope. Transverse sections of all plant parts were made using scalpels. The sections were mounted with glycerine in a slide (carefully avoided entrapment of air bubbles when mounting). Saffranin was added as a staining agent to identify the different tissues¹⁰ and the special characters (cells) were identified and recorded.

Calculation of Ash value

Empty weight of the crucible was measured using the electronic balance (Shimadzu 0.0000 - 250 g). The precursor mixture of AK (2g) was measured with crucible (A g). The crucible was placed into the furnace (Biolinkz) and burnt 5 hours at 450 °C. The following day the crucible was taken from the furnace and kept in the desiccator for 30 minutes. Finally crucible was removed from the desiccator and the weight of crucible measured (B g) and recorded. Ash value was calculated using the recorded weights, where A - crucible

with precursor mixture of AK, B - crucible weight after burnt, ash value = $(A-B) / 2 \text{ g} \times 100$ ^[11].

Calculation of Acid insoluble ash

Empty weight of the crucible was measured using the electronic balance (SHIMADZU; 0.0000– 250g) (A g). Above prepared ash (0.2 g) was mixed with of 25 mL of 1 N HCl in a beaker and transferred into a conical flask. The conical flask was heated for 5 minutes at 40 °C using mantle (Equitron). The solution was filtered using Whatman filter paper (40 size). The filter paper with the residuals (insoluble in acid) was folded and kept into the crucible. Then the crucible was kept in the furnace (Biolinkz) for 5 hours at 650 °C. The following day the crucible was removed from the furnace and weighted (D g). The weight was recorded and the acid insoluble ash value calculated using the recorded weights, where A - crucible weight, D - crucible weight after burnt, 2 g of precursor mixture of AK used to calculate the ash value and acid insoluble ash = $(D - A) / 2 \text{ g} \times 100$ ^[11].

Calculation of Loss on drying at 110 °C

Empty weight of the beaker was measured using the electronic balance (A g). The precursor mixture of AK (2 g) was measured with empty beaker (B g). The beaker was placed into the hot air oven, and burnt 5 hours at 110 °C. Finally the beaker was kept in the desiccator for 30 minutes and the weight measured (C g). Loss on drying at 110 °C was calculated using the recorded weights, where A - weight of the empty beaker, B - weight of the empty beaker with precursor mixture of AK and C - beaker's weight after burnt and loss on drying at 110 °C = $(B - A) / (B - C) \times 100$ ^[12].

Total solids of aqueous extract of *Avarai kudineer*

Aqueous extract of AK (2 mL) was subjected to the Refractometer (Atago) and the reading recorded.

Calculation of pH of aqueous extract of AK

Initially microprocessor pH meter (Hanna) was run with the buffer solutions of pH 7 and pH 4 respectively. Then the meter was run with the aqueous extract of AK and reading recorded.

Calculation of extractive values

Calculation of water soluble extractive

The precursor mixture of AK (5 g) was placed into an iodine flask and 100 mL of distilled water added into the flask. The iodine flask was kept in the shaker (Rivotex) for 7 hours. 10 mL of above mixture were filtered using Whatman filter paper (125 mm \varnothing) and transfer into the weighted (A g) beaker. The beaker was kept in hot air oven for 1 hour at 110 °C and allowed to cool at the room temperature. Finally the beaker was weighted (B g) and recorded. Water soluble extract was calculated using the recorded weights, where A - weight of the empty beaker, B - beaker weight after kept in hot air oven and water soluble extractive = $(B - A) / 5 \times 100 / 10 \times 100$ ^[13].

Calculation of alcohol soluble extractive

The precursor mixture of AK (2.5 g) was placed into an iodine flask and 50 mL of ethanol (99.9%) added into the flask. The iodine flask was placed in shaker (Rivotex) for 7 hours and the mixture filtered in to a beaker using Whatman filter paper (125 mm \varnothing). A conical flask was measured (A g) and 10 mL of above filtrate placed into the flask. The flask was kept in hot air oven for 1 hour at 110 °C and allowed to cool at room temperature. Then the flask was weighted (B g)

and recorded. Acid soluble extractive was calculated as $(B - A) / 2.5 \times 50 / 10 \times 100$

Identification of Heavy metals of precursor mixture of AK and aqueous extract of AK

The precursor mixture of AK (0.5 g) was measured into a 250 mL beaker. 25 mL of dil. HCl and 25 mL dil. HNO₃ were added into the beaker individually and kept in the water bath (105-110 °C) until the volume reduced to 25 mL and then allowed to cool in room temperature (30 minutes). The solution was filtered in to a graduated stranded cylinder and makeup in to 100 mL by using distilled water and shaken well. Then 2 mL of above solution was taken into a graduated standard cylinder and makeup in to 50 mL by using distilled water. Then the solution was subjected to Atomic absorption spectrometer (AA240) and the readings recorded.

Preliminary phytochemical analysis

Alkaloids: The aqueous extract of AK (2 mL) was taken into the test tube and Hager's reagent added (Hager's test) [14]. Colour changes were observed. The interaction was defined as presence of alkaloids if the colour changed to yellow [14].

Flavonoids: The aqueous extract of AK (2 mL) was added into a test tube. 10% of sodium hydroxide and dil. HCl were added individually (alkaline reagent test). Colour changes were observed. The interaction was defined as presence of flavonoids if the intense yellow colour turns to colourless when dil. HCl added.

Sterols: Residue of aqueous extract of AK (2 mg) was taken into a test tube. 2 mL of chloroform and 2 mL of Conc. H₂SO₄ were added from the side of the test tube individually. The test tube was shaken for 2-3 minutes (Salkowski test) [15]. Colour changes were observed. The interaction was defined as presence of sterols if red colour appears at the lower layer [15].

Saponins: Residue of aqueous extract of AK (2 mg) was taken into a test tube and shaken vigorously with 20 mg of sodium bicarbonate and 20 mL of water (foam test) [16]. Changes were observed. The interaction was defined as presence of saponins if honeycomb like froth appears [16].

Tannin: Aqueous extract of AK (5 mL) was taken into a test tube and added 1 ml of 10% solution of potassium dichromate (potassium dichromate test) [16]. Colour changes were observed. The interaction was defined as presence of tannin if appear dark brown colour [16].

Protein: Aqueous extract of AK (1 mL) was taken into a test tube and 1 mL of conc. HNO₃ added. The test tube was heated: at 30-40 °C for 2-3 minutes and allow to cool at room temperature. Then 1 mL cons. NaOH was added. (xanthoproteic test). Colour changes were observed. The interaction was defined as presence of protein if yellow colour appeared and disappeared when added the NaOH.

Sugar: Aqueous extract of AK (2 drops) was taken into a test tube. 0.5 mL of dil. HCl and 0.5 mL of distilled water were added into the test tube individually and kept in water bath

(105 -110 °C) for 5 minutes. Allow to cool at room temperature for 20 – 30 minutes. NaOH, 0.5 mL of copper sulphate pentahydrate and 0.5 g sodium pellets were added individually (Fehling's test). Colour changes were observed. The interaction was defined as absence of sugar if the colour not change [17].

Thin layer chromatography analysis

Precursor mixture of AK (15 g) was placed into an iodine flask. 50 mL of methanol (CH₃OH) were added into the flask, then mixed well and heated using a water bath (90-95 °C) for 15 minutes. The extract was filtered using Whatman filter paper (125 mm \varnothing). The extract spotted on pre-coated aluminum foil plate (10X20 cm)-stationary phase, using micropipette and marked as origin line with led pencil. The plates were placed vertically on the mobile phase tank with its lower edge immersed in selected mobile phase. Toluene: ethyl acetate (9:1) and toluene: ethyl acetate: formic acid (9:1:1) were used as mobile phase. The bands were observed using a viewing cabinet (CAMAG) with UV lamp (CAMAG – equipped to emit light under 254 and 366 nm wavelengths). The retention factor (R_f) value were calculated for every spots as the distance moved by the compound divided by the distance moved by the solvent front [5].

Antimicrobial study

Pour plate method was used to test the antimicrobial study. 10g of agar (*Escherichia coli* – Eosin Methylene Blue agar, *Salmonella* spp. – brilliant green agar, *Staphylococcus aureus* –mannitol salt agar, *Pseudomonas aeruginosa* – cetrimide agar base) was suspended in a 90 mL of sterile saline water blank to make a microbial suspension. Serial dilution was made by using aqueous extract of AK. Finally 1mL of prepared dilutions were added with 15–20 mL molten (45 °C) sterile agar individually and allowed to cool at room temperature. The plates were incubated, in an inverted position at incubator at 37 °C for 24 hours. The test was performed in triplicate to ensure reproducibility of results. The number of colonies appearing on dilution plates were counted, averaged and multiplied by the dilution factor to find the number of colony of the sample. Calculate the number of organism per gram or milliliter of the samples by applying the following formula, Number of colony / gram or milliliter = Number (average of 3 replicates) of colonies X Dilution factor weight of sample.

Results

The results of the pharmacognostical analysis as macroscopic, microscopic, physiochemical, screening for heavy metals, preliminary phytochemical and microbial screening of AK described below.

Macroscopic and microscopic characters

That the precursor mixture of AK power is a brown colour dried powder contains dried pieces of leaves, bark, rhizome and wood and aqueous extract of AK is dark brown liquid. Special cells/tissues, relative positions of the cell, shape and size determined microscopically.

The Table 1, showed the macroscopic and microscopic appearance of the part used of constituent plants of AK.

Table 1: Macroscopic and microscopic appearance of the parts used of constituent plants of AK

Plants	Part used	Macroscopic appearance	Microscopic appearance
<i>S. auriculata</i>	Leaves	Brownish green leaves, oval-oblong, 2–4 cm	Simple trichome 200–350 μm length. Straight wall showed prismatic crystal
<i>C. fistula</i>	Leaves	Brownish green leaves, ovate or ovate-oblong, 10–15 cm	Trichome bent at the terminal end in upper epidermal and warty with bulbous base in lower epidermal
<i>C. rotundus</i>	Rhizome	Dark reddish/blackish brown tuber, bulb like structure, roots present	Dark brown, starch grains
<i>S. lappa</i>	Root	Dark brown, stout, slender	Irregular yellowish-orange resin masses and oil globules
<i>T. arjuna</i>	Bark	Outer surface pale greenish yellow; inner surface pinkish	Rosette and cluster crystals
<i>S. reticulata</i>	Wood	Golden yellowish bark, inner surface reddish reticular formation	Simple pitted and xylum vessels. Prismatic crystals (X shape)
<i>S. cumini</i>	Seeds	Yellowish brown, ovoid-oblong, smaller in size	Oval to rod and triangle shaped starch cells. Different shape of stone cells

Physico- chemical evaluation of AK

Total ash and acid insoluble ash of AK calculated and the Table 2 indicated the ash values. Loss on drying at 110 °C, total solid and pH of aqueous extract of AK were 6.62%, 4% and 4.93 respectively. The extractive value of AK in water and alcohol listed in Table 3.

Table 2: Ash value of Avarai Kudineer

Parameter	Precursor mixture of AK
Total ash	10.21%
Acid insoluble ash	1.24%

Table 3: Extractive value of Avarai Kudineer

Solvents	Precursor mixture of AK
Water	19.13%
Alcohol	13.76%

Table 4 indicated the availability of heavy metals as mercury, arsenic and lead in precursor mixture and aqueous extract of AK.

Table 4: Test for heavy metals in Avarai Kudineer.

Heavy metals	Precursor mixture of AK(ppm)	Aqueous extract of AK(ppm)
Mercury	BOQ < 1	BOQ < 1
Arsenic	BOQ < 0.3	BOQ < 0.3
Lead	BOQ < 0.1	BOQ < 0.1

Preliminary phytochemical analysis

The preliminary phytochemical screening showed the availability of plant chemical in the AK listed in Table 5. The retention factors (R_f) of methanol extract of AK in two different solvent systems observed under 254 nm UV light are shown in Table 6. The methanol extract of AK produced two fractions under toluene: ethyl acetate solvent and three fractions under toluene: ethyl acetate: formic acid solvent systems. The bands could not observe under 366 nm wavelength of UV light.

Table 6: R_f value of Avarai Kudineer in two different solvent, bands observed under 254 nm wavelength

R _f value toluene: ethyl acetate (9:1)	R _f value toluene: ethyl acetate: formic acid (9:1:1)
0.16	0.23
0.26	0.32
	0.41

Total bacterial and fungal count of precursor mixture of AK were 3×10^4 and 1×10^3 cfu/gm respectively, whereas no microorganisms observed at aqueous extract of AK. *Staphylococcus aureus* and specially enterobacteriaceae pathogens as *E. coli* and *Salmonella* spp. not reported. The screening study of microorganisms listed at Table 7.

Table 7: Test for organisms in Avarai Kudineer.

Organisms	Precursor mixture of AK	Aqueous extract of AK
Total bacterial count	3×10^4 cfu/gm	Absent
<i>E. coli</i>	Absent	Absent
<i>Salmonella</i> spp.	Absent	Absent
<i>Staphylococcus aureus</i>	Absent	Absent
<i>Pseudomonas aeruginosa</i>	Absent	Absent
Total fungal count	1×10^3 cfu/gm	Absent

Discussion

Pharmacognostical study of AK has not reported previously. A number of literature reviews [4, 5, 6] and *in vivo* pharmacological study [8] available regarding AK, even though the constituents plants and the parts used to prepare the AK were differed, literature to literature.

The macroscopic and microscopic analysis could be useful in the identification of constituent plants of AK and preparation of AK. Microscopic examination and phytochemical analysis helps to distinct the original plants from adulterants.

Ash Value indicates the availability of inorganic materials as carbonates, silicates, oxalates and phosphates [10]. Ash value help to detect the extent of adulteration, quality and purity of a drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash indicating the contamination of earthy materials. The study documented less amount of ash value (10.21%) and very less acid insoluble ash (1.24%) indicating unavailability of adulteration of AK.

Most of the bioactive compounds dissolved in alcohol and water and generally used to detect extractive index. The extractive value in water > the extractive value in alcohol of AK indicates the bioactive compounds highly dissolve in water than alcohol, it is added advantage factor for drugs.

The heavy metal analysis indicated that all the heavy metals tested as mercury, arsenic and lead below the level of ingestion (BOQ < 1), is another of positive result for AK.

The screening of microorganism revealed that aqueous extract of AK is free from microbial contamination. The toxicological studies will help to confirm the possibility to presence of exotoxins.

The preliminary phytochemical study confirmed the presence of alkaloids, flavonoids, sterols, saponins and tannin whereas

absence of sugar, is a positive factor and confirms this formulation can be used in the condition of diabetes. TLC profiling showed the number of phytochemicals available in the extract. The TLC method is one choice for identification of secondary metabolites present in the plants. The different R_f values indicate the presence of different phytoconstituents in the extract. In addition different R_f values of the extract shows the availability of different polarity of the compounds [18]. The current study will help to get an idea for select appropriate solvent system for further separation of plant chemicals from AK.

The parameters used to conduct the current study, could be used as significant parameters for plant identification and poly herbal drugs. Preliminary phytochemical analysis could useful to get an idea regarding pharmacologically active phytochemicals. Advance phytochemical analysis will be needed to confirm the standard markers for AK. The current study will serve as a preliminary study to develop a standardization of AK.

Conclusion

The study could be use as diagnostic keys for identification and preparation of aqueous extract of *Avarai Kudineer*. The developed parameters and technique will be useful for standardization of *Avarai Kudineer* and other different formulations.

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