



STANDARDIZATION, QUALITY CONTROL AND ANTIMICROBIAL STUDY OF ARKA TAILA: AN AYURVEDA FORMULATION

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ABSTRACT

Herbal medicines have a huge therapeutic history for a long period of time and still serving many more health requirements in the worldwide. Although there is a still very huge challenge in its quality control and quality assurance; because of the high variability of chemical compounds present in it. Herbal drugs, singularly and in combinations, contain numerous complex matrices in which not a single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards and standardization of finished herbal drugs. Many drugs in *Ayurveda* describe regarding treatment of *vicharchika* (Atopic dermatitis). *Arka taila* is one of them. In this study *Arka taila* was subjected to study organoleptic analysis, phytochemical analysis, and qualitative analysis to detect the presence of various functional groups, and thin layer chromatography (TLC) examination by optimizing the solvent systems. The investigation revealed the density, Refractive index, Saponification value, Rancidity, Total fatty matter and Iodine value mainly and also subjected to in vitro antibacterial assay against human pathogens MTCC No.39-*Klebsiella aerogenes*, MTCC No.10239-*Escherichia coli*, MTCC No.1034-*Pseudomonas aeruginosa*, MTCC No.6908-*Staphylococcus aureus* by Mueller hinton agar well diffusion method. The results of this study suggest that *Arka taila* can be used as an antibacterial agent against infections caused by *Klebsiella aerogenes*, *Escherichia coli*, *Staphylococcus aureus*.

Keywords: Arka taila, quality control, standardization, Antimicrobial activity

INTRODUCTION

Over a longer period of time, humans have relied on the plants for the basic needs such as food, clothes and shelter. In addition, plants have also formed the basis of effective traditional medicine that has been used for thousands of years. Many references of their usages as drugs can be traced from *Atharvaveda* which is the base of *Ayurveda* medicines in India.

Plants have an important role in drug discovery and chemical biology. Some therapeutic benefits can be seen in specific compound of plants. Many plants contain more than one active compound; together they give proper therapeutic value to that particular plant. In last few decades researchers have been much more interested in herbal drugs as a new antimicrobial agent. Development of microbial resistance to the available antimicrobial drugs has led this condition. India is rich in medicinal diversity. Mostly all types of agro-climatic and ecological conditions available within India.

For desirable therapeutics effect drug concentration at the site of action, one has to introduce large quantity of drug, which may cause gastric discomfort and unwanted adverse effect and even if drug is relatively safe, frequent administration is not practical way.

Looking to above discussion one can say that only oral administration is not enough for elimination of disease. Therefore, local application is essential part in the management of skin disease (*Kushth*¹).

Here *Arka taila*² is a combination of mainly three drugs – *arka*³ (*Calotropis procera*), *haridra*⁴ (*Curcuma longa*) and *sarsapa*⁵ (*Brassica campestris*). Pharmacologically leaf of *arka*, tuber of *haridra* and oil of *sarsapa* are mainly used. Alone above three drugs are used as *kushthaghna*, *kandughna*, *krimihara*, *varnya*, *vamaka* and *vidahi*. And together as per *Ayurveda* classical texts that drug is used in *kushtha roga* specially *pama*, *kandu* and *Vicharchika*⁶. Thus, present study was done to study organoleptic analysis, phytochemical analysis, and qualitative analysis to detect the presence of various functional groups, and thin layer chromatography (TLC) examination by optimizing the solvent systems and also to evaluate the antimicrobial potency of *Arka taila* against *Klebsiella aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHOD

Macroscopic study - The manufactured sample of *Arka taila* studied organoleptic with naked eye & magnifying lens, with the help of Pharmacognostic procedure i.e. Taste, Odor and color findings were recorded⁷.

Preliminary Phytochemical Analysis

For the Preliminary Phytochemical analysis were detected by usual prescribed methods⁸. They were-

Density

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled Water at

25°C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C when weighed in air of density 0.0012 gram per ml, is 0.99602 gram. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20°C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in gram, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Refractive Index

The refractive index (η) was measured with Abbe's refractometer in sunlight at 25°C. Opened the prism of refractometer and clean with soft cotton. Calibrated the apparatus against distilled water which had a refractive index of 1.3325 at 25°C. Placed a drop of the sample to be tested on the lower part of the prism and close the refractometer. Observed through eyepiece and turn the dispersion correction compensator knob until the colored indistinct boundary seen between the light and dark field becomes a sharp line. Adjust the knurled knob until the sharp line exactly intersects the midpoint of the cross wires in the image. Read the refractive index from the magnifier in the pointer and record the reading. Cleaned the prism with cotton wool wetted with hexane or acetone. Closed the prism and keep the refractometer in a box and place it at identified location.

Saponification Value

Melt the sample if it is not already liquid and filter through a filter paper to remove any impurities and the last traces of moisture. Make sure that the sample is completely dry. Weigh about 2-gram sample in 250ml Erlenmeyer flask. Added 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser for 1 hr. on water bath; boil gently but steadily until Saponification is complete, as indicated by absence of any oily matter and appearance of clear solution. Cool it down and Shake frequently. Add 1 ml Phenolphthalein solution as an indicator. The excess potassium hydroxide with 0.5N hydrochloric acid was titrated. Observed Pink color at end point, Noted the number of ml of HCL Required (A). Repeated the same procedure without taking sample for Blank Titration, Note the number of ml of HCL required (B).

Calculation:

Saponification Value = (B-A) x Normality of HCL x 56.10 / Wt. of Sample (gm)

Unsaponifiable Matter

Melt the sample and filtered through a filter paper to remove any impurities and the last traces of moisture. The sample was completely dry. Weigh about 2gm sample in 250ml Erlenmeyer flask. Added 25ml of 0.5N alcoholic KOH and mix well, attach this to a reflux condenser for 1 hr. On water bath, it boiled gently and steadily till Saponification was achieved, it was indicated that there was absence of any oily matter and its appearance was clear. Add 1 ml Phenolphthalein solution as an indicator. The excess potassium hydroxide with 0.5N hydrochloric acid was titrated. Then observed Pink color at end point, noted down the number of ml of HCL Required (A). Repeat the same procedure without

taking sample for Blank Titration, Noted the number of ml of HCL required (B). Saponification value or number of fat = mg of KOH consumed by 1g of fat.

Calculation

Saponification Value = (B-A) x Normality of HCL x 56.10 / Wt. of Sample (gm)

Rancidity

First one should take 1 ml of melted sample in a test tube. Added 1ml Concentrated Hydrochloric Acid & Mixed it well. Then add 1ml of 1% solution of Phloroglucinol in diethyl ether. Again, mix above combined mixture very well. Pink Color formation indicated that fat is slightly oxidized. Red Color formation indicates that fat is completely oxidized.

Total Fatty Matter

For this Procedure, took accurately weighed air dried plant material (W_1) and extract with petroleum ether (40-60°C) in Soxhlet apparatus. Filtered it and remove the solvent under vacuum at 40°C. Dried the extract on tarred evaporating dish (W_2) and weighted up to constant weight (W_3). Calculate the percentage with reference to the plant material.

Calculation

Percentage of fatty matter (w/w): $(W_3 - W_2) \times 100 / W_1$

Where, W_1 = Weight of drug taken, W_2 = Weight of empty dish, W_3 = Weight of dish + dried residue.

Iodine Value

Placed accurately weighed sample in dry iodine flask. Added 10 ml of carbon tetrachloride and dissolved it. Added 20 ml of iodine mono-chloride solution and inserted it in the stopper, previously moistened with solution of potassium iodine. Allowed that solution to stand in a dark place at a temperature of 17°C for 30 minutes. Add 15 ml of solution of potassium iodide and 100 ml water. Shake it well and titrate with 0.1N sodium thio-sulphate using solution of starch as indicator. Note down the number of ml of 0.1N sodium thio-sulphate required. At the same time carry out the operation in exactly the same manner but without the substance being tested. Note the number of ml 0.1N sodium thio-sulphate required.

Calculation

Calculate the iodine value from the formula: -

Iodine value = $(b-a) \times 0.01269 \times 100 /$ Weighed of sample in gm

Where, b = Weight of dish with residue, a = Weight of dish.

Thin Layer Chromatography

Thin layer Chromatography is a tool for separation and identification of chemical constituent. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or

plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be affected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F₂₅₄

Plates were dried in hot oven at 105^o C for one and half hour.

Preparation of mobile solution

Chloroform: Hexane (9.5:0.5)

Preparation of test solution

4 gram powdered drugs were extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

Sample application

Samples were applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

Visualization: Iodine Vapours

R_f Value-

Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

Calculation of R_f Value

$R_f = \text{Distance travelled by solute from origin line} / \text{Distance travelled by solvent from origin line}$

Antimicrobial Study⁹

Pathogens, distilled water, ethyl alcohol, Povidone-iodine, Cetyl alcohol, Stearic acid, tri-ethylamine, coconut oil, beaker, glass rod, hot plate, Mueller-Hinton agar medium, discs, loops, conical flasks, cotton swab, borer, spirit lamp, hot air oven, BOD incubator, masks, gloves, vials for preserving extracts, 1ml, 2ml & 5ml syringes, 10 μ l pipette, weighing balance.

Methods

1. Selection and collection of pathogens
2. Preparation of Test sample
3. Preparation of media & media plate and antibacterial activity using well diffusion method
4. Recording and interpreting results

Selection and collection of pathogens

For this research work, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Escherichia coli* were taken as they are most responsible for causing skin infection.

The pathogenic strains of different species of bacteria were procured from 'Institute of Microbial Technology' (IMTECH), Chandigarh and the stock cultures maintenance & antibacterial study were done at 'Analytical Division of Bilwal Medchem and Research Laboratory Pvt Ltd, SKS Reengus Industrial Area, Reengus, Jaipur, Rajasthan, India.

MTCC No. 39- *Klebsiella aerogenes*

MTCC No. 10239- *Escherichia coli*

MTCC No. 1034- *Pseudomonas aeruginosa*

MTCC No. 6908- *Staphylococcus aureus*

Preparation of test sample

Test sample (*Arka taila*) had prepared in NIA Pharmacy.

Preparation of media & media plate and antibacterial activity using well diffusion method

Preparation of media & media plates

Mueller-Hinton agar medium was taken for all pathogens.

Agar- 38 gram

Distilled water- 1 liter

pH- 7

Heated the agar with water at 100^oC till it becomes transparent, and then kept it in hot air oven for 15 minutes. The sterilized media were poured in sterile Petri dishes aseptically in a Laminar flow cabinet.

The Agar (solidifying agent), which was added in a broth medium, hardens at it cools. After solidifying of agar plates (nearly about 15 to 20 minutes), they were kept inverted in incubator at 37^oC for overnight for checking any contamination. The ready Agar plates were then transferred in zip seal plastic cover and kept in a cold room. The media & media plates were prepared time to time as per requirement and used for streaking purpose and also for Antibacterial evaluation.

Revival of microbial cultures

Like all other living forms, micro-organisms need suitable nutrients and favorable environments for growth. A simple way to obtain bacteria is to grow them in a flask in broth medium.

100 ml Nutrient broth medium were transferred in conical flasks (of quantity 100ml) 20ml each. The flasks were capped with cotton plug and autoclaved at 121^oC for 20 minutes at 15 lb pressure per square inch.

Inoculation

To start a bacterial culture, a number of cells (the inoculums) are to be transferred (inoculated) into a sterilized broth media. The lapful of freeze-dried cultures was transferred. In this inoculation procedure, the loop that was used to transfer micro-organisms has been heated to redness by flaming immediately before and after the transfer. Flaming destroys living forms on the surface of the loop. During transfer, the flask was held in the left hand and the cotton plug between the fingers of the right hand. The mouths of the flasks into which cultures were transferred, were also passed through the burner flame immediately before and after the loop was introduced and removed. In addition to destroying organisms on the mouths of the flasks, flaming creates outward convection currents, which decreases the chance of contamination. This inoculation procedure was done in a laminar flow chamber. After inoculation, the bacterial cultures were incubated at room

temperature overnight in a shaker for their growth. Growth, in this case, means the development of a population of cells from one or few cells. Next day, the mass of daughter cells became visible to the naked eye as cloudiness (turbidity) in all flasks.

Streaking

Bacteria grow very well in fluid media i.e. nutrient broth. Hence, they are used as enriched media before plating on solid media. Solid media is essential for isolation of organism in pure form. So, for isolation of micro-organisms in pure form without contamination, streaking was done on solid media plates. Applied a microbial culture to the surface in a Petri plate and spread them with cotton swab sticks. The prepared plates were then incubated in inverted position at 37°C for 24 hours. After incubation, we got the pure cultures. This procedure is termed as 'Sub culturing'. In this way, frequent sub-culturing was done whenever required during antibacterial study.

Applying a microbial culture to the surface of Agar in a Petri plate and spread them with a loop or a bent needle or cotton swab.

Preparation of concentrations of the extracts and antibiotic (positive control)

The concentrations 5 % w/v of solvent was prepared in sterile Eppendorf tubes.

Well Diffusion Method

Wells (of about 5mm diameter) were made on the plates with the help of sterile stainless-steel borer. About 5, 10 And 15 % concentrations of test sample were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 hours. Control experiments comprising inoculums without test sample were set up. The plates were incubated at 37°C for 48 hours for bacterial pathogens.

Groups design

Negative Control

- DMSO Solution

Positive control

- 5 % Vancomycin

Test groups

- *Arka taila*

Recording and interpreting results

After the disks were placed on the plate, inverted the plate and incubate at 35°C for 48 hours. After incubation, measured the diameter of the zones of complete inhibition (including the diameter of the disk) and recorded it in millimeters. The measurement was made with a ruler on the under surface of the plate without opening the lid. In the instance, slight growth (80% inhibition) should be ignored and the zone diameter should be measured to the margin of heavy growth. The zones of growth inhibition were compared and recorded. Colonies growths within the clear zone of inhibition may represent resistant variants or mixed inoculums. The distance from the colonies closest to the disk to the centre of the disk should be measured and then doubled to obtain a diameter. The diameter of the outer clear zone should be recorded as well, and an interpretation recorded for each diameter. The presence of colonies within a zone of inhibition may predict eventual resistance to that agent.

OBSERVATION AND RESULT

Pharmacognosy and Phytochemical Analysis are tabulated below-

Table 1: Macroscopic study

Macroscopic study	<i>Arka taila</i>
Color	Yellow Green
Odor	Characteristic
Taste	Characteristic

Table 2: Quality Control Analysis of *Arka taila*

Tests	<i>Arka taila</i>
Density	0.91 gm/ml
Refractive Index	1.4745
Saponification Value	192 mg KOH/ gm
Unsaponifiable Matter	19.64 gm
Rancidity	Absent
Total Fatty Matter	41 %
Iodine Value	110 gm/100gm

Thin Layer Chromatography

Arka taila



Iodine

R_f Value: 0.24, 0.56, 0.72

Antimicrobial Study

Table 3: Zone of Inhibition (mm)

Microorganism	-ve Control (DMSO Solution)	Test sample <i>Arka Taila</i>	+ve control (5% Vancomycin)
<i>Klebsiella aerogenes</i>	4	13	19
<i>Escherichia coli</i>	4	15	22
<i>Pseudomonas aeruginosa</i>	4	11	24
<i>Staphylococcus aureus</i>	4	14	21

Table 4: Activity Index

Microorganism	Test sample (%)
<i>Klebsiella aerogenes</i>	0.68
<i>Escherichia coli</i>	0.68
<i>Pseudomonas aeruginosa</i>	0.46
<i>Staphylococcus aureus</i>	0.67

DISCUSSION

Pharmacognosy and Phytochemical analysis of *Arka taila* suggest that the oil has yellow green color and odor and taste are characteristics while in quality control analysis suggest that density is 0.91gm/ml, refractive index 1.4745, Saponification value 192mg KOH/gm, Unsaponifiable matter 19.64gm, Rancidity absent, total fatty matter 41%, Iodine value is 110gm/100gm and TLC is R_f Value: 0.24, 0.56, 0.72. Antimicrobial activity of test sample *arka taila* had found positive response against pathogens and Inhibition of *arka taila* found greater than 0.5 so this sample found biological active against *Klebsiella aerogenes*, *Escherichia coli*, *Staphylococcus aureus*.

CONCLUSION

The study reveals that sufficient quality control parameters were followed during the preparation of formulation. Organoleptic parameters and physicochemical analysis were carried out as per the norms of WHO guidelines and the absence of heavy metals and microbes in the finished product indicates the genuineness of final product. TLC profile generated in this particular study can be considered as a preliminary tool. Antimicrobial activity of this study suggests that *Arka taila* can be used as an antibacterial agent against infections caused by *Klebsiella aerogenes*, *Escherichia coli*, *Staphylococcus aureus*.

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