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Evaluation of some physicochemical properties as quality control parameters of an ayurvedic preparation "Khadirarishta"

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Abstract: Traditional or alternative medicines such as Ayurvedic drugs are getting importance and reputation day by day in the treatment of various diseases. This is because it has fewer side effects and gives necessary pharmacological action. But prolonged and excess usage may lead to harmful effects such as damage of the heart muscle leading to various types of arrhythmia and coronary artery diseases. "Khadirarista", an Ayurvedic drug, was traditionally used for heart diseases along with jaundice, anemia, abdominal tumor and leprosy. The physicochemical properties such as pH, density, viscosity, conductivity, loss on drying (LOD), chromatographic and spectral study, and presence of metal ions the drug "Khadirarista" were studied. It was found that pH values were 4.53, 4.21 and 4.31 at 1%, 5% and 10% of test solution, respectively. It indicated that "Khadirarishta" was an acidic preparation. Density, average viscosity and residue (LOD) of the drug was 1.0076 g/mL, 25.22 pascal/second and 9.33 g/100 mL, respectively. The spectral study of the test sample was performed and peaks were obtained at the wavelengths of 275, 254, 274.80, 254.60, 275.60, 273.60, 211, 253.60 nm in UV spectrophotometer with 276.60 nm being the λ_{max}. HPLC analysis of the sample was performed and retention time (R_t) of the possible active components were found to be 4.12, 4.22 and 4.83 min. R_t at 4.12 min was the most sharp peak. Applying TLC, the R_f values of the active compounds were found to be 0.6 and 0.7. The level of various metal ions in the sample was measured by Flame Photometry and Na, K, Ca levels were found to be 75 ppm, 5 ppm, 12 ppm, respectively.

Keywords: Khadirarishta, HPLC, Flame Photometry, Physicochemical Parameters

1. Introduction

Plant and man are, were and will be inseparable, because plants not only provide us with food, shelter and medicine but also the life sustaining oxygen gas. Since, disease, decay and death have always co-existed with human civilization; the early man had to think about disease and its treatment at the dawn of intellect. Thus the race started using plants as a means of treatment of diseases and injuries. Human race has successfully used plants and products thereof as effective therapeutic tools for fighting against disease and various health hazards[1]. Medicinal plants are the principal healthcare resources for the majority of people all over the world. Pharmaceutical importance of plants has led to the discovery and adoption of plant extracts which were commonly used in traditional medicine as alternative source of remedy[2]. Herbal medicines refer to the use of any plant seed, berries, roots, leaves, bark or flower for medicinal purpose[3]. The economic significance of medicinal plants stems from the fact that the number of patients suffering from chronic ailments is on the rise and drugs from medicinal plants are proving to be more

effective in treating such disorders[4,5,6]. Medicinal and aromatic plants (MAPs) are produced and offered in a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal remedies, teas, spirits, cosmetics, sweets, dietary supplements, varnishes and insecticides[7-9]. Ayurvedic medicine is still the mainstay of the world's populations for primary health care because of better cultural acceptability, better compatibility with the human body and few side effects. Now a day's many Ayurvedic preparation are used for the different disease purpose, but have not proven scientific evidence about their proper action and lethal dose.

Ayurveda is used to restore the physical, mental and emotional balance in patients, thereby improving health, preventing disease and also treating any current illness[11]. It is a Sanskrit term, made up of the words "*ayus*" and "*veda*". "*Ayus*" means life and "*Veda*" means knowledge or science. The term "*Ayurveda*" thus means 'the knowledge of life' or 'the science of life'[12]. The quality control of herbal drugs is repeatedly asked question that bewildered the scientist community. Strict quality control of traditional medicine is based largely on the empirical measurement of few non-specific physical properties such as pH, viscosity, density etc. But quality control by specific methods still faces authenticity. This problem lies in the process of manufacture of the traditional drugs. However, we have taken this research work to evaluate the quality control parameters of marketed preparation "Khadirarishta". 'Khadirarishta" is an Ayurvedic preparation and manufactured by Sree Kundeswari Aushadhalaya Ltd., Chittagong, Bangladesh as liquid by arista process.[16]

2. Methods and Materials

2.1. Study of Physical Properties of Khadirarishta

2.1.1. Determination of pH

The pH of the drug was determined at 1% solution, 5% solution and 10% solution by using pH meter (pH-211 Microprocessor, HANNA, Japan).

2.1.2. Determination of Conductivity

The conductivity of the drug was determined at 1% solution, 5% solution and 10% solution by using conductivity meter (EC-215 Conductivity Meter, HANNA, Japan).

2.1.3. Determination of Density

The density, ρ, is elementary physical property of matter. For a homogeneous object, it is defined as the ratio of its mass m to its volume V.

$$\rho = m/V \quad \text{(i)}$$

Density was determined by pycnometer. The weight of blank pycnometer was taken and then filled the pycnometer with distilled water and weighed it again. After then pycnometer was filled with Khadirarishta and weighed. Then weight of Khadirarishta with pycnometer minus blank pycnometer divided by volume of pycnometer[17].

Density = [(Weight of pycnometer with test sample – weight of blank pycnometer)/Volume of liquid in pycnometer]…(ii)

2.1.4. Determination of Viscosity

The viscosity of 1%, 5% and 10% drug solutions was determined by using Viscosity tube. At first used 1% solution which was kept in viscosity tube up to the mark and start to time count. When the solution reached the finish mark then it was stopped. It was done for 5% and 10% solution in the same way.

2.1.5. Determination of Loss on Drying

At first 100 mL Khadirarishta was taken in a beaker and was weighed. Then Khadirarishta with beaker was put in a water bath for completing evaporation. The residue was weighed after cooling.

LOD = Weight test sample before evaporation - weight test sample after evaporation.

2.2. UV Spectral Study

2.2.1. Apparatus

UV-Visible Spectroscopy: A PC based UV-Visible recording spectrometer-1700 Series (Shimadzu, Japan), with slit width 1.0 nm, light source change wavelength at 340.8 nm was employed for all measurements.

2.2.2. Preparation of Test Sample

For UV study 0.1%, 0.25%, 0.5% solutions of Khadirarishta were prepared and the solutions were scanned at 200-800 nm.

2.3. Thin Layer Chromatographic Analysis

Khadirarishta comprises active and inactive compounds, therefore, to identify these compounds thin layer chromatographic (TLC) technique was used with chloroform and ethyl acetate at different ratios as solvent systems. The following characteristics (Table 1) were used to determine the number of compounds present in the Khadirarishta.

Table 1. *Characteristics of TLC plate.*

Item	Characteristics
Plate size	8 cm × 3 cm
Stationary phase	Thin film of silica gel GF
Film thickness	0.5 mm
Activation	at 110 °C for one hour
Type of development	Ascending and one-dimensional

2.3.1. Detection of Compound by R_f Value

There was no specific reagent for easily detection of antimicrobial compound(s). Therefore, simple method like spraying with a characteristic reagent on the TLC plate could be used to detect antibiotics. However, following processes (Table 2) were utilized in order to ascertain the number of compounds present in Khadirarishta.

Table 2. *Processes for of TLC plate.*

Item	Processes
i) UV light	The developed and dried TLC plates were visualized under UV light (254 nm) to locate UV absorbing or quenching compounds.
ii) Vanillin-sulfuric acid spray reagent	The plate sprayed with 1% (W/V) vanillin in H_2SO_4 reagent and then heated at 110 °C for 10-15 minutes. The resolved compounds were detected with the development of a specific color[19-20].

2.4. Study by Using HPLC

2.4.1. Column

Analytical reversed phase C-18 column [Luna C-18, 5µ. 250×4.6 mm, Phenomenex, Inc] was used to analyze the samples.

2.4.2. Mobile Phase

Nano pure water was sonicated for 10 min and then it was filtered through a 0.22 µm Millipore filter and degassed. HPLC grade 50% methanol and 50% water were sonicated and filtered through 0.22 µm Millipore filter and degassed before using.

2.4.3. Chromatographic Conditions

All analysis was done at ambient temperature under isocratic condition. The mobile phase comprised of 50% aqueous methanol at the flow rate 0.7 mL/min. The injection volume was 20 µL. Before analysis, every sample was filtered through 0.45 µm filter tips. The column eluate was monitored with UV detection at 254 nm.

2.4.4. Preparation of Test Sample

1 mL drug were weighted and dissolved in 9 mL water and then sonicated for 10 min and then filtered through 0.45 µm filter tips and filled the sample in vial.

2.5. Detection of Metal (Sodium, Potassium, Calcium)

To prepare standard solution of sodium, potassium, calcium solution the concentration was 60 ppm, 50 ppm, 40 ppm, 30 ppm, 20 ppm were selected.

2.5.1. Preparation of Test Sample

To prepare the test sample 5 mL of Khadirarishta in 50 mL volumetric flask was collected and made up to 50 mL for sodium and calcium ion analysis. In this experiment potassium ion was to be large and for that 500 times dilution of Khadirarishta was used for determination of potassium ion.

3. Results and Discussion

3.1. Physiochemical Parameters

Physicochemical tests of Khadirarishta was performed to measured the physicochemical parameters and it was found that pH of the solution was 4.53, 4.21, 4.31 for 1%, 5% and 10 solutions of test drug which indicated that Khadirarishta is an acidic preparation. Density of drug was found to be as 1.0076 g/mL, the average viscosity was 25.22 pascal/ second and the residue (LOD) of Khadirarishta was 9.33 g/100 mL. The conductivity test was also carried out and results were shown in the Table 1.

In the experiment of viscosity determination of Khadirarishta in viscosity tube, the viscosity of time was 25.59 pascle/second, 25.14 pascle/second, 24.89 pascle/second and average time was 25.22 pascle/second.

Table 3. *Conductivity determination in different solutions.*

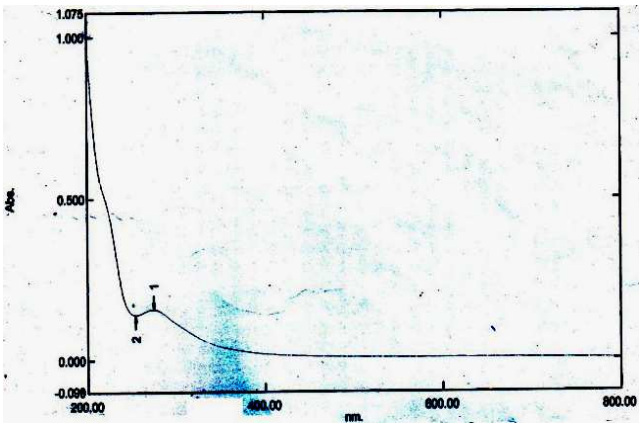
Percent of Solution	Conductivity (scale = 19.99 ms)	Conductivity (scale = 1999 µs)
1%	0.15	164
5%	0.52	520
10%	0.97	961

3.2. UV Spectral Study

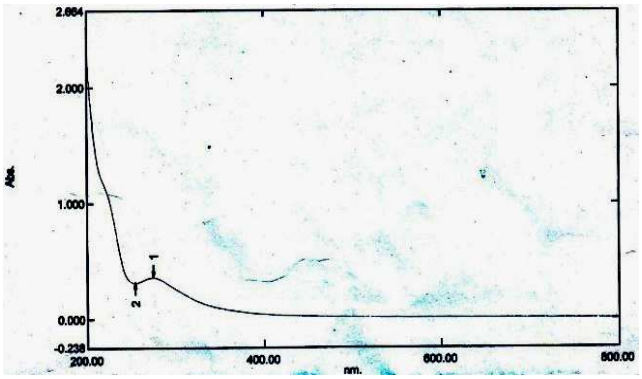
The UV Spectrum of Khadirarishta contained various compounds which were detected by UV spectrophotometer as shown in Table 4 and Figure 1.

Table 4. *Absorptive values*

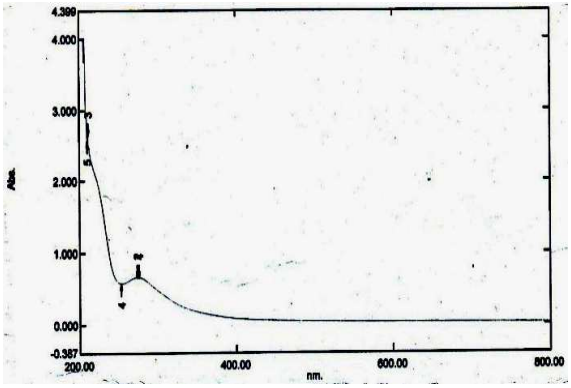
Concentration (Percent of Solution)	Wavelength	Absorbance
0.1	275.00	0.156
	254.20	0.139
0.25	274.80	0.362
	254.60	0.316
	275.60	0.667
	273.60	0.667
0.5	211.00	2.637
	253.60	0.579
	210.20	2.572



A



B



C

Figure 1. *UV spectra of Khadirarishta (A= 0.1% solution, B = 0.25% solution, C = 0.5% solution)*

3.3. TLC Study

Table 5. *Results of TLC analysis (Observation under UV light and after spraying).*

Compounds	Observation under UV light	R_f value	Observation after Spraying
Compound-1	Glowing deep (254nm) pink	0.6	----------
Compound-2	Glowing deep (254nm) violet	0.7	Glowing deep pink

To determine the R_f values of the Khadirarishta compounds a spot for each of compounds were given on a TLC plate and it was run using Chloroform **:** Ethyl acetate (EtoAC **:** $CHCl_3$) solvent systems. The spot was found after observation under UV light and after spray of Vanillin-sulfuric acid spray reagent on the TLC plate. Many compounds were found on this plate which were overlapping to each other. But one was compound in spraying method and two compounds were found in UV light clearly observed which were of the R_f values as 0.6 and 0.7 visualized under UV light and R_f 0.7 after spraying (Table 5).

3.4. HPLC Study

HPLC chromatograms at 254 nm showed that Khadirarishta contained various compounds HPLC various compounds having retention time (R_t) at 4.12, 4.22, and 4.83 minute (Figure 2).

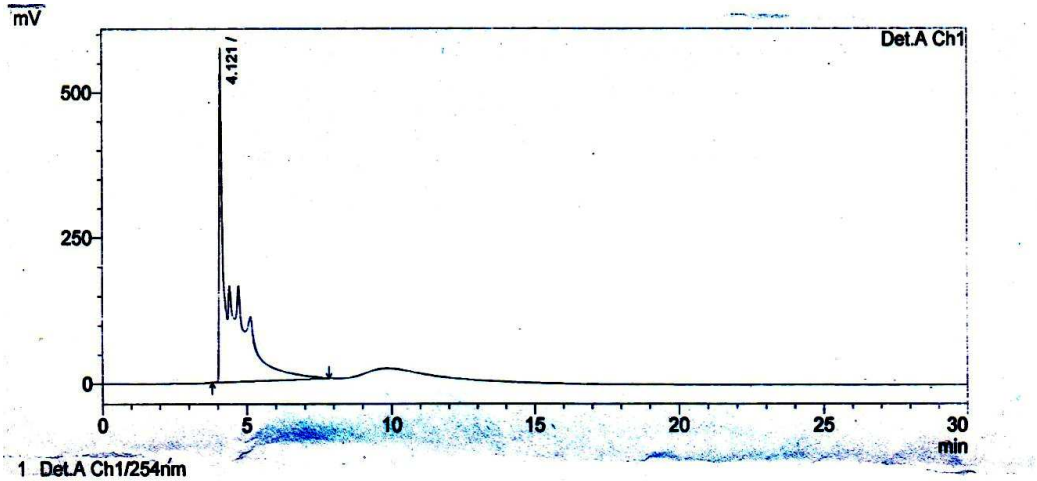


Figure 2. *HPLC analysis of khadirarishta*

3.5. Determination of Metal

In the detection of metal analysis we found too much sodium present in Khadirarishta with minimum amount of calcium. Na, K, Ca levels were found to be 75 ppm, 5 ppm, 12 ppm.

4. Conclusion

The preparations from different batches showed similar results.

Limitation of Our Study

The present study was conducted only on marketed preparations. We could not use any standard preparation of our own or from other sources.

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