

## Pharmaceutical Standardization

# Analytical study of *Kuberaksha/Kantaki Karanja Patra Churna* [*Caesalpinia Bonduc* (L.) Roxb. leaf powder]

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### Abstract

*Caesalpinia bonduc* (L.) Roxb. (*Kuberaksha*) is an Ayurvedic herb used in the management of malaria, liver disorders, worms, edematous conditions, etc. Based on classical Ayurvedic textual indications and recent pharmacological studies, its leaf powder was selected for studying its effect clinically on filaria. Before conducting the clinical trails, this leaf powder was subjected to certain chemical studies to find the pH, ash value, extractive values, High Performance Thin Layer Chromatography (HPTLC), etc. for standardization of the drug.

**Key words:** Acid insoluble ash, analytical study, *Caesalpinia bonduc*, *Kuberaksa* leaf powder, loss on drying, pH value, total ash.

## Introduction

The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. In those days, the physician himself identifies, checks the drugs based on habitat, morphology, taste, color, texture and uses as medicine. The nomenclature of many Ayurvedic herbs denotes their physical and certain chemical characteristic features which are considered as primitive standardization tools. For example, the name *Kuberaksha* indicates an eye-shaped seed; the drug name *Kiratatikta* (*Swertia chirata* Buch. Ham.) denotes its extreme bitter taste and its habitat (*Kirata desa*). Similarly, the word *Aswagandha* [*Withania somnifera* (L.) Dunal] means that its root smells like horse.

But in modern times, these tests and tools are not sufficient to control the quality, and hence, there is a need to standardize the herbal drugs according to the newly available standardization methods for the acceptance of herbal drugs at international levels. In recent years, the plant materials, especially the Ayurvedic herbs, are gaining a sustained proportion of global market, due to the cost effectiveness and lesser side effects. Hence, the World Health Assembly (WHA42.43-1989) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards. Assessment of complete and accurate physicochemical value of Ayurvedic

herbs not only provides scientific basis of its quality but also helps in globalization of *Ayurveda*. Hence, to provide standard parameters for the quality control of *Kuberaksha* leaf powder, the present study was carried out.

### Botanical description

*Caesalpinia bonduc* (L.) Roxb. is a scrambling, prickly, woody liana. Leaves are of 4–7 pairs, and rachis is prickly. Leaflets are of seven to nine pairs, ovate-elliptic, pubescent below, entire and mucronate. Flowers are yellow in axillary and terminal racemes. Pods are oblong and dehiscent. Seeds are one or two, globose or ovoid, gray.<sup>[1,2]</sup>

### Distribution

It grows along the field hedges, outskirts of villages and near sand beds throughout India.<sup>[1,2]</sup>

### Therapeutic efficacy in *Ayurveda*

The leaves are anthelmintic, emmenagogue and febrifuge, and are useful in mitigating *Kapha* and *Vata* (types of body humors), piles, intestinal worms, elephantiasis, splenomegaly, hepatomegaly, amenorrhea, dysmenorrhea, fevers and pharyngodynia.<sup>[2,3]</sup>

### Phytochemical profile

A bitter substance named bonducin, phytosterinin, fatty acids, caesalpins ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\psi$ ), a new dieterpine caesalpin, a new homoisoflavone (bonducellin) and citrulline are the main phytochemicals.<sup>[1]</sup>

Seeds contain bonducin, saponin, a bitter substance (phytosterinin), a thick-yellow fatty oil (20–24%) having a disagreeable odor of the following fatty acid composition: palmitic 4.5%; stearic 7.5%; oleic 29.0%; linoleic 59.0% and lignoceric in traces. The defatted kernels contain a, b-, g-, d- and e- caesalpins,

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caesalpin F, homoisoflavone (bonducillin), and an amorphous glycoside (bonducin). In addition, the seeds also contain starch, sucrose, 2-phytosterols and proteins (25.3%). The amino acid composition of the seed proteins is as follows: arginine 0.2%; cystine 0.9%; histidine 3.4%; leucine and isoleucine 15.4%; lysine 6.8%; methionine 0.9%; phenylalanine 5.2%; threonine 8.2%; tryptophan 0.4% and valine 8.5 g/16 g N.<sup>[4]</sup>

### Review of previous works

Standardization work on seed was conducted by Central Council for Research in Homeopathy<sup>[4]</sup> and the results were as follows.

Seed color and appearance: Powder is brown colored, somewhat sticky and grainy.

In water: Powder produces a faint straw colored turbid solution.

In 5% KOH solution: Brown colored turbid solution.

In 5% H<sub>2</sub>SO<sub>4</sub> solution: Faint straw colored turbid solution.

In petroleum ether and NH<sub>3</sub>: No significant results with the powder.

In FeCl<sub>3</sub> solution: Powder settles at the bottom and a few particles remain suspended giving a dark brown solution.

In Dragendorff's solution: Powder settles at the bottom and turns black giving a clear orange brown solution.

In KI + I<sub>2</sub> solution: Powder settles at the bottom and turns black giving a clear brown solution

### Thin layer chromatography

Evaporate 20 ml of the homoeopathic mother tincture on a water bath to remove alcohol. Extract the residue with 3 × 20 ml petroleum ether and concentrate the extract to 2 ml. Carry out the thin layer chromatography (TLC) of petroleum ether concentrate using petroleum ether:diethyl ether (9:1 v/v) as solvent system. In UV light, one spot appeared at R<sub>f</sub> 0.13 (blue). After spraying with antimony trichloride reagent, the following spots appeared at R<sub>f</sub> 0.09 (violet) and 0.15 (violet).<sup>[4]</sup>

## Materials and Methods

The present study was undertaken at Regional Research Institute (Ay), Vijayawada, as a part of PhD study under Dr. NTR University of Health Sciences. The analytical study was conducted at Laila Impex R&D Center, Vijayawada.

### Collection and authentication of plant material

*C. bonduc* (L.) Roxb. was collected from village outskirts and field hedges of Nuziveedu area of Krishna district, Andhra Pradesh. The plants were identified and authenticated at Plant Taxonomy Division, Laila Impex R&D Centre, Vijayawada, and the voucher specimen was deposited at that Institute (Voucher Specimen sample No. LIH 6268 & LIRD 754).

The *Kuberaksha* [*C. bonduc* (L.) Roxb.] leaf powder was studied for organoleptic characters and physicochemical properties, viz., color, texture, odor, taste, pH value, loss on drying, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives, heavy metal toxicity, High Performance Thin Layer Chromatography (HPTLC) finger prints.

## Observations and Results

### Description

The description of the plant is given below.<sup>[1,2,5-10]</sup>

Color: Green color dry powder

Texture: Smooth and powdery

Odor: Strong and pungent

Taste: Bitter

### Determination of pH value

The drug, *Kuberaksha* leaf powder was made as a 10% solution in water, and the pH of the liquid was determined with the help of pH meter and electrode system. The pH value of this drug was 5.97.<sup>[5,9,11,12]</sup>

### Loss on drying

Two grams of the drug was accurately weighed and put in a porcelain crucible. It was heated on a hot plate at 110°C for 3 hours. After considerable heating, the crucible was allowed to cool in desiccators. It was then weighed. Heating, cooling and weighing was continued till a constant weight was achieved. The difference in the weight of the porcelain crucible was calculated for loss on drying. The loss on drying of the sample was 4.92%.<sup>[5,9,11]</sup>

### Total ash

About 5 g of the ground air-dried material was placed in a previously ignited and weighed crucible (usually of platinum or silica). The material was spread in an even layer and ignited by gradually increasing the heat to 500–600°C, until it is white, indicating the absence of carbon. The heating, cooling and weighing were repeated until the weight of the crucible becomes constant. The content of total ash was calculated in milligrams per gram in comparison to air-dried material. Thus, the total ash was found to be 8.61%.<sup>[5,9,11]</sup>

### Acid insoluble ash

The total ash was collected and boiled with 25 ml of dilute HCl for 5 minutes. This solution was filtered with the Whatman (No.40) filter paper. Along with the insoluble ash, the filter paper was burnt in a Gooch crucible. Heating, cooling and weighing of the crucible was done until the weight of the crucible comes constant. The percentage of acid insoluble ash was calculated with reference to the air-dried drug (5 g) and it was 2.33%.<sup>[5,7,9,11,12]</sup>

### Extraction values

#### Water soluble extractive

Exactly 5 g of sample was mixed with 100 ml of chloroform water, shaken frequently for 6 hours and kept for 18 hours without disturbing and filtered rapidly taking precautions against loss of solvent. Twenty-five milliliters of filtrate was taken using a pipette, and evaporated in a tared shallow bottom dish and dried on a water bath up to constant weight. The percentage of water soluble extractive<sup>[5,7,9,11]</sup> was calculated with reference to the moisture free drug. The water soluble extractive value was observed to be 27.87%.

#### Alcohol soluble extractive

Exactly 5 g of sample was mixed with 100 ml of 90% alcohol

and shaken frequently for 6 hours and kept for 18 hours without disturbing. The above procedure was followed and the percentage of alcohol soluble extractive with reference to the moisture free drug was calculated. The alcohol soluble extractive<sup>[5,7,9,11]</sup> value so obtained was 22.63%.

### Limit tests for heavy metals

The test for heavy metals<sup>[5,11,12]</sup> is designed to determine the content of metallic impurities that are colored by sulfide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of the heavy metal per million parts of the substance (by weight), as determined by visual comparison of the color produced by the substance with that of a control prepared from a heavy metal standard solution.

### Limit test for arsenic

The glass tube was lightly packed with cotton wool, previously moistened with lead acetate solution and dried, so that the upper surface of the cotton wool was not less than 25 mm below the top of the tube. The upper end of the tube was then inserted into the narrow end of one of the pair of rubber bungs to a depth of about 10 mm. A piece of mercuric chloride paper was placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band in such a manner that the borings of the two bungs (or the upper bung and the glass tube) met to form a true tube, 6.5 mm in diameter, interrupted by a diaphragm of mercuric chloride paper.

The test solution was prepared as per the norms and placed in a wide-mouthed bottle. Then, 1 g of potassium iodide AsT and 10 g of zinc AsT were added and the prepared glass tube was placed quickly in position. The action was allowed to proceed for 40 minutes. The yellow stain which was produced on the mercuric chloride paper was compared by day light with the standard stains produced by operating in a similar manner with known quantities of dilute arsenic solution AsT. The comparison of the stains was made immediately at the completion of the test. By matching the depth of color with that of the standard stains, the proportion of arsenic in the substance was determined. A stain equivalent to the 1-ml standard stain, produced by operating on 10 g of substance, indicates that the proportion of arsenic is 1 part per million. Here, in this test, the drug was studied in comparison with 2 ppm standard strain and we found that the arsenic content was below the normal value (<2 ppm).<sup>[5,11,12]</sup>

### Limit test for lead

The prepared sample was added to 6 ml of ammonium citrate solution Sp (special solutions prepared as per method specified in API for Limit test for Lead), and 2 ml hydroxylamine hydrochloride solution Sp. Two drops of phenol red solution were added and the solution was made just alkaline (red in color) by the addition of strong ammonia solution. The solution was cooled and added to 2 ml of potassium cyanide solution Sp. Immediately, the solution was extracted with several quantities each of 5 ml of dithizone extraction solution, draining off each extract into another separating funnel, until the dithizone extraction solution retained its green color. The combined dithizone solutions were shaken for 30 seconds with 30 ml of a 1%

w/v solution of nitric acid and the chloroform layer was discarded. The solution was added to exactly 5 ml of standard dithizone solution and 4 ml of ammonia cyanide solution Sp. and shaken for 30 seconds; By this a chloroform layer is developed. The colour of this chloroform layer is compared to the control one which is made with a volume of dilute standard lead solution equivalent to the amount of lead permitted in the sample under examination. The colour of chloroform layer of tested one is not in deeper shade of violet than that of control one. The control made with a volume of dilute standard lead solution equivalent to the amount of lead permitted in the sample under examination.

Here, in this test, the drug was studied in comparison with 20 ppm standard stain and it was found that the lead content below the normal value (<20 ppm).<sup>[5,11,12]</sup>

### Thin layer chromatography/high performance thin layer chromatography

#### Sample preparation

Exactly 3 g of sample was refluxed with 3 × 50 methanol for 1 hour, filtered and concentrated to form a residue and made to 10 ml. With methanol, 5 µl was spotted using LINOMAT IV(CAMAG, Sonnenmattstrasse, 11, Switzerland)

#### Stationary phase (application)

The prepared sample was applied over the pre-coated silica gel 60 F<sub>254</sub> plate of 0.2 mm thickness (Merck, Germany).

#### Development (mobile phase)

The sample was developed with the help of mobile phase, i.e., ethyl acetate:methanol:water (100:13.5:10).

#### Visualization (scanning)

For visualization, the plate was dried at 100°C and scanned at 254 nm UV.

#### Observations

The sample plate scanned under UV wavelength 254 nm showed two peaks (spots) and the observed R<sub>f</sub> values were 0.33 and 0.37. [Figure 1]

### Discussion

The sample, *Kuberaksha* leaf powder, was studied for

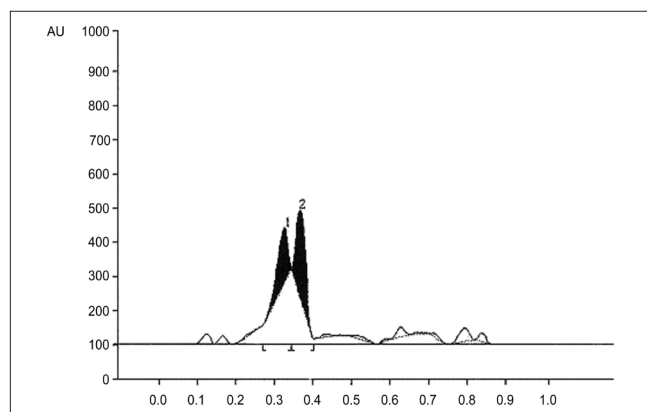


Figure 1: HPTLC finger prints of *Kuberaksha* (*Caesalpinia crista* Linn.) leaf powder (UV 254 nm)

organoleptic characters and subjected to physicochemical analysis to standardize for further studies and utility. Though there are many techniques for standardization of herbal drugs, suitable and available techniques were selected for present study.

It became a difficult task to collect leaves alone from the plant having hard yellowish recurved thorns. To study leaves alone, large quantity of the raw drug was collected with all probable precautions and dried in shade. Lot of weight variation was observed (20:1) between fresh and dry raw drug samples, after shade drying. The leaflets were separated from primary and secondary rachis and ultimately these leaflets were only ground to powder. This may be the probable reason for getting the total ash value as 8.61% and acid insoluble ash value as 2.33%. The nonphysiological ash (2.33%) which denotes inorganic contents like sand and siliceous matter represents this large quantity of fresh drug collection. The pH value of drug suggests its little acidic nature. The drug is soluble both in water and alcohol. In heavy metal toxicities, arsenic and lead were observed to be present below the normal levels.<sup>[1,5,6,2,10,11]</sup>

The developed HPTLC finger prints of the drug are useful to verify the quality and purity and also to confirm the same drug in compound formulations.

## Conclusion

It can be concluded that the complete and accurate physicochemical values of the present study are useful in identification and authentication of *Kuberaksha* leaf powder.

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## हिंदी सारांश

### कुबेराक्ष/कण्टकी करञ्ज के पत्र चूर्ण का विश्लेषणात्मक अध्ययन

गोलि पंचल प्रसाद, जी. त्रिमूर्तुलु, के. एन. रेड्डी, एम. एल. नायडु

आयुर्वेद वैद्यकशास्त्र में कुबेराक्ष/कण्टकी करञ्ज के पत्र चूर्ण का प्रयोग शोथ, ज्वर, क्रिमिरोग, श्लीपद इत्यादि व्याधियों में प्राचीन काल से किया जाता रहा है। श्लीपद के रोगियों की चिकित्सा में इस औषधी पत्रचूर्ण की महत्वपूर्ण भूमिका जानने के पहले आधुनिक विज्ञान की दृष्टि से मानकीकरण के लिए इस पत्र चूर्ण का विश्लेषणात्मक अध्ययन किया गया। इस अध्ययन की परिणाम में कुबेराक्ष पत्र चूर्ण की पी.एच. ५.९७, लॉस ऑन ड्राइन्ग ४.९२%, टोटल एशा ८.६१%, एसिड इन्सालुबुल एशा २.३३%, जलघुलनशीलसत्व २७.८७%, मद्य घुलनशीलसत्व २२.६३% पाया गया। भारी धातु जैसे आर्सिनिक एवं लेड के लिमिट टेस्ट में ये अपने लिमिट लेवल के अंतर्गत पाये गये। एच.पी.टी.एल.सी. के अध्ययन में ०.३३ एवं ०.३७ आर.एफ़. वेल्यु पर दो बार उच्चतम स्तर पर पाया गया। इस अध्ययन से इस चूर्ण का मानकीकरण सम्भव हुआ है।