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Standardization of an herbal preparation – *Vṛddhadaruka mula curṇa* [*Argyreia nervosa* (Burm.f.) Boj.] root powder

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Vṛddhadaru (*Argyreia nervosa* (Burm.f.)Boj.) is an Ayurvedic herb used in the management of vitiated conditions of *Kapha,* inflammations, arthritis, synovitis, seminal weakness, etc. Based on classical Ayurvedic textual indications and recent pharmacological studies root powder was selected to study on Filarial patients. Before conducting the clinical trials this herbal powder was subjected to certain chemical studies to find out the *p*H, ash value, extractive values, HPTLC, etc. for standardization of the drug.

Keywords: - *Vṛddhadaru, Argyreia nervosa* (Burm.f.) Boj, Root powder, Standardization
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Traditional medicine, Ayurveda is flourishing by the total knowledge, skills and practices based on the theories and experiences indigenous to our country. In many cases, the theory and applications of this ancient system of medicine differs from conventional medicine. Researchers in India always tried to corraborate ancient wisdom in modern parameters. The standardization of medicinal plants started in those days by the physician himself by identifying and checking the drugs based on the knowledge on various parameters transmitted from generation to generation in terms of the habitat, morphology, taste, colour and texture of medicinal plants and was ultimately confirmed by using 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 drug *Kiratatikta* (*Swertia chirata* Buch- Ham.) indicates its extreme bitter taste and its habitat (*Kirata desa*); But in modern times these tests and tools are not sufficient for quality control and ultimately there is a need of standard methods and standard derivatives to use 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. A complete and accurate physico- chemical value of Ayurvedic herbs not only provide scientific basis but also help in globalization of Ayurveda.

Materials

Argyreia nervosa (Burm.f.) Boj., a large woody climber with a white wooly tomentose stems. It grows on bushes of open forests especially near the moist areas and also grown as an ornament through out India. Leaves are 7.5-30.0 cm, acute, ovate, glabrous above, persistently white-tomentose beneath, base cordate; petioles 5-15 cm, long, white-tomentose; flowers in subcapitate cymes; penduncles 7.5-15 cm, white-tomentose; fruits 2cm diameter, globose, apiculate; roots are brownish in colour, texture moderate smooth with fibrous in nature and some dark reddish brown exudate present on cortex (Figs.1-4)[2,11].

In the present study *Argyreia nervosa* (Burm.f.) Boj. was collected from hilly area of *Kondapalli* RF, Krishna district, Andhra Pradesh. The plants was identified and authenticated at Plant Taxonomy Division, Laila Impex R&D Centre, Vijayawada and

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the voucher specimens was deposited at that Institute (Voucher Specimen sample No. LIH 6853 & LIRD 717.)

LIH: Laila Impex Herbarium Number
LIRD: Laila Impex Raw drug Number

Methodology

The *Vṛddhadaru* (*Argyreia nervosa* (Burm.f.) Boj.) root powder (Fig. 5) was studied for organoleptic characters and physicochemical properties, viz. colour, texture, odour, taste, *p*H 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[1-8].

Results

Colour of the sample is brown; texture is smooth (powdery) with pungent odour and astringent and slight bitter taste. The drug *Vṛddhadaru* (*Argyreia nervosa* (Burm. f.) Boj.) root powder was made as a 10% solution in water, and the *p*H of the liquid was determined with the help of *p*H meter and electrode system. The *p*H value of this drug is 5.01[1,7,9].

Two gram of drug was accurately weighed and put in a porcelain crucible. It was heated on a hot plate at 110º C for 3 hrs. 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 1.76%[1,7,9].

About 5 gm 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 500-600º C until it is white, indicating the absence of carbon. The heating, cooling and weighing were repeated until the weight of the crucible comes constant. The content of total ash was calculated in mg per gm in comparison to air dried material. Thus the total ash found was 3.85%[1,4,7,9].

The total ash was collected and boiled with 25ml of diluted HCl for 5 minutes. This solution was filtered with the Whatmans (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

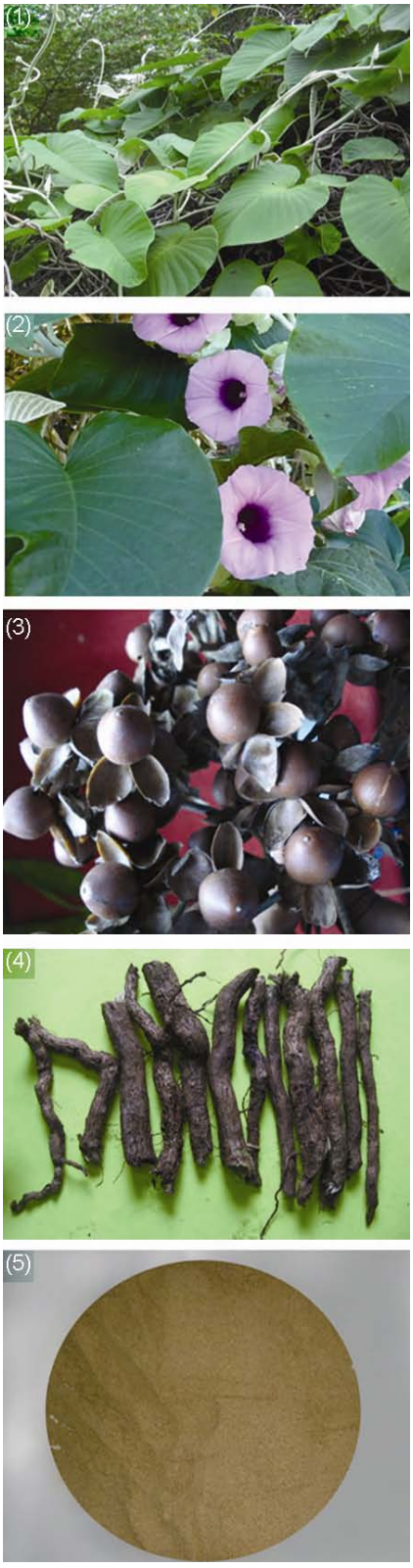

Fig. 1—5: 1 *A. nervosa* (Burm.f.) Boj whole plant; 2 *A. nervosa* (Burm.f.) Boj flowering; 3 *A. nervosa* (Burm.f.) Boj fruiting; 4 *A. nervosa* (Burm.f.) Boj root; 5 *A. nervosa* (Burm.f.) Boj root powder

comes constant. The percentage of acid insoluble ash was calculated with reference to the air dried drug (5gm) is known as Acid insoluble ash and it was 0.58%[1,4,7].

Extraction values

A. Water soluble extractive

Five gm of sample was mixed with 100 ml of chloroform water, shaken frequently for 6 hrs and kept for 18 hrs without disturbing and filtered rapidly taking precautions against loss of solvent. Twenty five ml filtrate was taken with pipette, and evaporated in a tarred shallow bottom dish and dried on water bath up to constant weight. The percentage of water soluble extractive calculated with reference to the moisture free drug was calculated. The water soluble extractive value (7.73%) was observed[1,4,7,9].

B. Alcohol soluble extractive

Five gm of sample was mixed with 100 ml of 90% alcohol and shaken frequently for 6 hrs and kept for 18 hrs without disturbing. Followed the above procedure and calculated the percentage of alcohol soluble extractive with reference to the moisture free drug. The alcohol soluble extractive value obtained was 7.82%[1,4,7,9].

Limit tests for heavy metals

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide 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 colour produced by the substance with that of a control prepared from a heavy metal standard solution[1,9].

Limit test for Arsenic and Lead

The glass tube is lightly packed with cotton wool, previously moistened with lead acetate solution and dried, so that, the upper surface of the cotton wool would not be 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) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of mercuric chloride paper[1,9].

The test solution was prepared as per norms and placed in the wide-mouthed bottle, 1 gm of potassium iodide as T and 10 gm of zinc as T 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 as T. The comparison of the stains was made immediately at the completion of the test. By matching the depth of colour with standard stains, the proportion of arsenic in the substance was determined. A stain equivalent to the 1ml standard stain, produced by operating on 10 gm 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 found that the arsenic content below the normal value (<2ppm)[1,9].

The prepared sample was added with 6 ml of ammonium citrate solution Sp, and 2 ml hydroxylamine hydrochloride solution Sp. Two drops of phenol red solution was added and the solution was made just alkaline (red in colour) by the addition of strong ammonnia solution. The solution was cooled and added with 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 retains its green colour. The combine dithizone solutions were shaken for 30 seconds with 30 ml of a 1% w/v solution of nitric acid and discard the chloroform layer. The solution was added with exactly 5 ml of standard dithizone solution and 4 ml of ammonia-cyanide solution Sp. and shaken for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of dilute standard lead solution equivalent to the amount of lead permitted in the sample under examination[1,9].

Here, in this test, the drug was studied in comparison with 20 ppm standard strain and found that the Lead content below the normal value (<20 ppm).

TLC/HPTLC

Three gm of sample was refluxed with 3 × 50 methanol for 1 hr, filtered and concentrated to fine a residue and made to 10ml. With methanol, 5 ul is

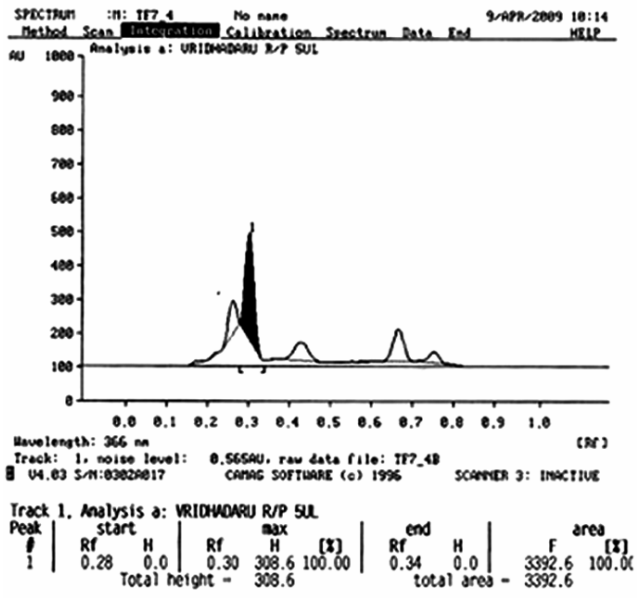

Fig. 6—HPTLC finger print of *A. nervosa* (Burm.f.) Boj root powder(UV 254nm)

spotted using LINOMAT IV (CAMAG, SWITZERLAND).

The prepared sample was applied over the pre-coated silica gel 60 F_{254} plate, 0.2 mm thickness (Merk, Germany).

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

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

Observations

The sample plate scanned under UV, wavelength 254nm showed 1 peak (spot) and the observed Rf. values was 0.30 (Fig. 6)[1,4,6,7].

The sample is brown; texture is smooth (powdery) with pungent odour and astringent and slight bitter taste. The *p*H value was 5.01, the loss on drying of the sample was 1.76%, total ash was 3.85%, Acid insoluble ash was 0.58%, water soluble extractive value was 7.73% and alcohol soluble extractive value so obtained was 7.82%. In the limit tests arsenic and lead were found below the normal value, i.e. <2 ppm and <20 ppm, respectively. Under HPTLC the sample plate scanned under UV at the wavelength 254nm showed 1 peak (spot) and the observed Rf. values was 0.30.

Discussion and conclusion

Now a days many herbal raw drugs were adulterated in the market. To confirm the identity of a sample Standardization became a must. The standardization of the single and compound formulations became compulsory to attain good results and to prevent adverse actions. Repeated standardization studies on a particular drug and particular part of drug provides the authenticity in fixing the referral values for future identification and utility. In this regard *Vṛddhadaru* [*Argyreia nervosa* (Burm.f.) Boj] root powder, very useful drug of Ayurvedic system of medicine, was studied for organoleptic characters and subjected to physico-chemical analysis to standardize for further clinical studies and utility. Though, there are many techniques for standardization of herbal drugs, the suitable and available techniques were selected for present study. On comparison of the results with the standard works and books it is established that results are similar to that of the earlier studies and the genuineness of the drug was proved[10,12,13]. The results are further useful for the scholars working in the field of medicinal plants, as referral values of standardization. The developed HPTLC finger prints of the drug are also useful in identifying the drug. The Limit tests of Arsenic and Lead were found below the normal level and this result is needed to conduct clinical trail.

By the present study, it can be concluded that results of the study proved the genuineness of the drug by the comparison with the earlier studies and at the same time the study will be considered useful to the scientific fraternity as referral tool in identifying and testifying the drug.

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