



ASSESSMENT OF QUALITY OF *WITHANIA SOMNIFERA* DUNAL (SOLANACEAE) - PHARMACOGNOSTICAL AND PHYTO PHYSICOCHEMICAL PROFILE

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ABSTRACT

Pharmacognostical standardization of dried, matured roots of *Withania somnifera* Dunal (Solonaceae) has been carried out in the present study. The study includes macroscopical and microscopical evaluation along with estimation of its physicochemical parameters such as ash and extractive values and preliminary phytochemical screening. It also includes quantification of some of the active constituents like alkaloids. The present study reveals standardization profile for drug like *Withania somnifera* Dunal., which would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

Keywords: *Withania somnifera*, Standardization, Pharmacognosy, Physicochemical standards.

INTRODUCTION

During the past decade, the therapeutic use of herbal medicine is gaining considerable momentum in the world. The use of herbal medicine due to toxicity and side effects of allopathic medicines, has led to sudden increase in the number of herbal drug manufactures. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health. Therefore, reproducible standards of each plant are necessary for effective quality control to prevent adulteration.

Standardization is a system to ensure that every packet of medicine that is sold has the correct amount and will induce its therapeutic effect¹. Standardization serves number of purposes including: Batch to batch consistency, Confirmation of correct amount of dosage or extract per dosage unit, positive control to indicate possible loss or degradation during manufacturing.

Thus, the present study deals with standardization of medicinal plant i.e. *Withania somnifera* Dunal.(Solanaceae)^{2, 3}, widely distributed in north-western India. It shows the presence of alkaloids and steroidal lactones. The alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, cuscohygrine, anferine and anhydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root⁴. The drug known to be the great adaptogenic⁵ and rejuvenating herb⁶ is used as aphrodisiac for sexual health, anti – cancer , anti-inflammatory⁷, anti-depressant, anti-stress⁸ , anxiolytic, anti –oxidant, antimicrobial, hepatoprotective, diuretic, hypoglycemic, hypocholesterolemic⁹ and cardioprotective agent¹⁰. It is also used in rheumatism, general debility, insomnia, cough and cold, joint and nerve pain, impotency, infertility, skin infections etc. Due to its wide therapeutic importance it is worthwhile to obtain various qualitative and quantitative standards of drug to prevent its adulteration.

MATERIALS AND METHODS

Plant material authentication

The identity of the plant was confirmed by Dr.P.Jayaraman, Botanist, PARC, Chennai. Reg.No.PARC/2010/657. The plant was compared with voucher specimen in the institute. For further confirmation, the microscopic characteristics of this plant were studied and compared with available literature. The fresh plant material collected was thoroughly cleaned and air-dried. It was then homogenized to fine powder and stored in air-tight bottles for further studies.

Foreign matter

The term "Foreign Matter" is used to designate any matter, which does not form a part of the drug as defined in the monograph.100g of the powdered drug is taken and spread out in a thin layer. Plant material collected should be free from foreign matters like soil, insect parts or animal excreta. They are separated and weighed and the percentage is calculated.

Pharmacognostic studies

Macroscopic study

Macroscopic observation of roots of *Withania somnifera* was done. It comprised of shape, size, surface characteristics, texture, color, consistency, odour, taste, etc

Microscopic study

Transverse sections of *Withania somnifera* roots were taken by using a microtome. Permanent mount of stem was prepared using saffranin fast green stain by double staining technique¹¹.

Physicochemical studies

Physicochemical parameters were determined as per guidelines of WHO. Total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water soluble extractive value were determined^{12,13,14}.

Preliminary phytochemical studies

The roots of *Withania somnifera* Dunal. were coarsely powdered and extracted with methanol and water (6:4) using cold maceration technique. The extract is filtered and concentrated and dried in a Rota evaporator initially and then in vacuum desiccator. Preliminary phytochemical screening of extract was done for the presence of various phytoconstituents by using standard procedure¹⁵.

Pesticide residue¹⁶

Pesticide residue is done by TLC by using Benzene: methanol as mobile phase and Precoated silica gel 60 F254 TLC plate of 0.2mm thickness. Detection by UV from 200 to 300 nm.

Microbial contamination¹⁷

For the safe use of the plant drug, microbial count was done and checked whether total aerobic count, total yeast and mould count are within the prescribed WHO limits.

Quantitative estimations

Total alkaloid content was estimated by gravimetric method according to the method described by Edeoga¹⁸.

RESULTS

Foreign matter

Foreign matter was found to be 0.2%. The permissible limit as per standards is not more than 2%.

Pharmacognostic studies

The pharmacognostic study is the major and reliable criteria for identification of plant drugs. The pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of the crude drug. The detailed and systematic pharmacognostic evaluation would give valuable information for future studies.

Macroscopic studies

Roots straight, unbranched, thickness varying with age. roots bear fibre-like secondary roots, outer surface buff to grey-yellow with longitudinal wrinkles, crown consists of 2-6 remains of stem base, stem bases

variously thickened, nodes prominent only on the side from where petiole arises, cylindrical, green with longitudinal wrinkles, fracture, short and uneven, odour, characteristic, taste, bitter and acrid.

Microscopic studies

The microscopic studies of root showed following tissue systems:

Cork exfoliated or crushed, when present isodiametric and non-lignified, Cork cambium of 2-4 diffused rows of cells, Secondary cortex about twenty layers of compact parenchymatous cells, Phloem consists of sieve tubes, companion cells, phloem parenchyma, Cambium 4-5 rows of tangentially elongated cells, Secondary xylem hard forming a closed vascular ring separated by multiseriate medullary rays, a few xylem parenchyma, Vessels with bordered pits and horizontal perforations. Fibres aseptate with pointed ends. Starch grains abundant, simple, mostly spherical, reniform - oval with central hilum. Microcrystals in parenchyma cells.



Fig. 1,2: Roots of *Withania somnifera*

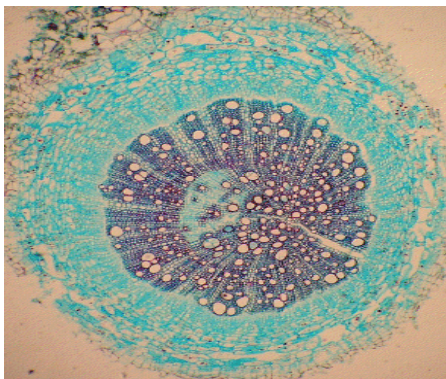


Fig 3.

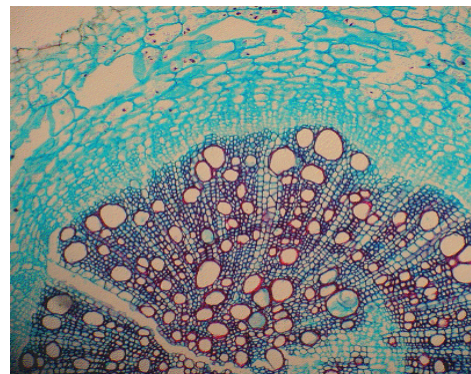


Fig 4.

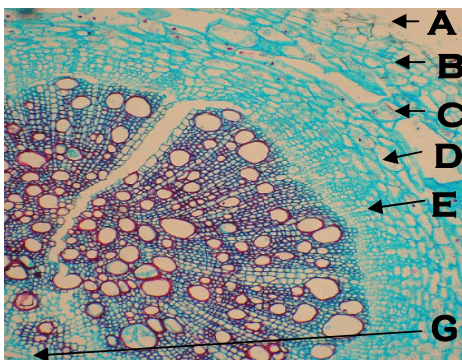


Fig 5.

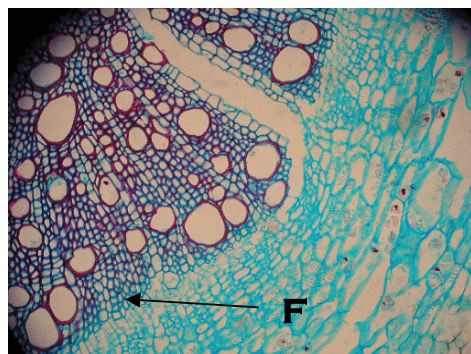


Fig 6.

A - Cork; B - Cortex; C - Endodermis; D - Pericycle; E - Phloem; F - Medullary ray; G - Pith

Photomicrographs of the specific characteristics determined from the powder study of *Withania somnifera* root



Fig. 7: Cortex

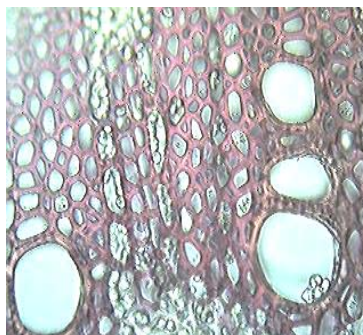


Fig. 8: Xylem

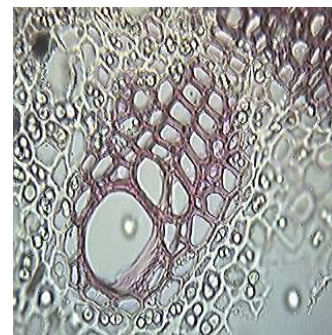


Fig. 9: Starch grains

Photomicrographs of the microscopic characteristic of roots of *Withania somnifera* DUNAL. in different views

Physicochemical studies

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica.

Table 1: Determination of proximate parameters of crude powder of *Withania somnifera* root

S.No	Physicochemical standards	Results % w/w	Standard value % w/w
1.	Total Ash	5.1	NMT 7 %
2.	Acid Insoluble ash	0.24	NMT 1%
3.	Water soluble extractive value	15.53	NLT 15%
4.	Alcohol soluble extractive value	16.8	NLT 15%
5.	Loss on drying	2.31	NMT 8%

The results of physicochemical parameter analysis of crude powder of *Withania somnifera* root are shown in Table 1. The average values are expressed as percentage of air-dried material.

The crude powder of *Withania somnifera* root was analyzed for the presence of heavy metals¹⁹. The results (Table No. 2) showed that arsenic, mercury and cadmium were not present in any of the samples; The presence of lead was found only to be less than 5 ppm. Although, there was minor presence of some heavy metals but the sample did not exceed the limit given according to the WHO guidelines. Therefore, the samples investigated were free from heavy metal contamination.

Table 2: Determination of heavy metals

S.No.	Heavy metal	Result (PPM)
1.	Arsenic	Nil
2.	Lead	Less than 5ppm
3.	Cadmium	Nil
4.	Mercury	Nil

Preliminary phytochemical studies

Preliminary phytochemical screening showed the presence of terpenoids, alkaloids, saponins, carbohydrates, glycosides, flavonoids, tannins, steroids, etc. (Table No.3)

Table 3: Phytochemical studies

S.No.	Constituents	Results
1.	Steroids	+
2.	Flavones	+
3.	Alkaloids	+
4.	Carbohydrates	+
5.	Glycosides	+
6.	Bitters	-
7.	Phenols	-
8.	Proteins	-
9.	Resins	-
10.	Saponins	+
11.	Tannins	+
12.	Terpenoids	+
13.	Anthraquinones	-
14.	Gums	-

+ = present, - = absent

Pesticide residue

No pesticide was found to present (Table 4).

Table 4: Pesticide residue

S.no	Pesticide residue	Extract
1.	DDT	ND
2.	Benzene Hexachloride	ND
3.	Aldrin	ND
4.	Dieldrin	ND
5.	Lindane	ND
6.	Chloropyrophos	ND
7.	Enosulphan	ND

ND - not detected (Concentration less than the minimum detection limit even in ng/l units). From the results it can be concluded that the plant material is totally safe and there is no traceable limit of pesticide in them

Microbiological studies

Results are given in table 5.

Table 5: Microbial contamination

S.No	Microorganism	Values obtained	WHO limit
1.	Total bacterial count	5 cfu/gm	NMT 1000cfu/g
2.	Yeast and moulds	Nil	NMT 100cfu/g
3.	<i>Escheria coli</i>	Negative	Absent
4.	<i>Salmonella</i>	Negative	Absent
5.	<i>S.aureus</i>	Negative	Absent

As per the WHO standards, the plant material is free from microbial load and safe for further use in formulation.

Quantification

The total alkaloid content was found to be 0.9818mg/100g of sample.

DISCUSSION

The primary steps for establishing the quality control profile of any plant drug is the macroscopic and microscopic evaluation and according to WHO, botanical standards should be proposed as a protocol for the diagnosis of the herbal drug. The histochemical studies give a preliminary idea about the type of compounds and their accumulation in the plant tissues. This is of great interest for quality control in basic research and drug production, especially for imported items and for raw material sold by traditional herbalists.

Physicochemical standards such as total ash value helps us in determining both physiological ash (plant tissue) and non-physiological ash (extraneous matter like sand and soil), whereas acid insoluble ash gives an idea about the amount of silica present, especially as sand and siliceous earth. Extractive values help us in determining the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists. The phytoconstituents quantified in the present study exhibit great deal of medicinal importance like alkaloids acts as anti-oxidant and immunomodulatory agent and steroidal glycosides having anti-inflammatory property. The quantified values of the above phytoconstituents can be used as a major tool for obtaining a quality control profile for a drug.

CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research and its use in Ayurvedic system of medicine.

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