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STANDERDIZATION OF MARKETED HERBAL EXTRACT

* G. Palshikar [1], B. Dixit[2]

1.Department of Pharmacognosy JSPM's Jayawantrao sawant College of Pharmacy & Research, Hadapsar, Pune.
2. Department of Microbiology, Pratap College, Amalner, Jalgaon.

ABSTRACT

Plants are known to produce and store many Biochemical products; lot of them can be extracted and used for research work. Medicinal plants are commonly called as *"Chemical Goldmines"* as they have natural chemicals; which are acceptable to Animals system and human also, many commercially important secondary metabolites are used in number of Pharmaceutical compounds. Different type of Herbal extract is commercially available in market. Become a medical use the potency of these extracts should be compatible & as per the standards prescribed in Pharmacopeia. Our aim is to evaluate the marketed extract sample and to check its authenticity and purity as per the standard parameter.

Keywords: Chemical Goldmines, Pharmaceutical compound, Herbal extract, Crude extract, Standardization, Evaluation.

INTRODUCTION

Ashwagandha consists of dried mature roots and steam bases of *Withania somnifera* (Linn.) Dunal.
Belonging to the family *Solanaceae*

Synonym-

- **Marathi** - Askagandha
- **Hindi** - Asgandh
- **Sanskrit** - Vijigandha

English - Winter Cherry

History

Historically, Ashwagandha was widely used throughout India as a tonic, especially for emaciation in people of all ages, including infants, and for enhancing reproductive function in both men and women. In one text, it was stated that Ashwagandha taken for a fortnight with milk, ghee, oil, or warm water promotes development in an emaciated body "as rains do for younger crops". It is classed among the "rasayanas" (rejuvenative tonics), the most highly regarded of all medicinal substances in ayurveda. The Ayurvedic scholar Charaka (100 BC) wrote of rasayanas, "One obtains longevity, regains youth, gets a sharp memory and intellect and freedom from diseases, and gets a lustrous complexion and strength of a horse". Charaka described various uses for Ashwagandha, including its

effectiveness for treating hiccups and female disorders.[1]

Extraction procedure

The extract was taken from Shamantak Enterprises, Pune.
The extraction of secondary metabolites was carried out following a modified method of Bandhoria et al 8. The dried and powdered. Material (20 g) was extracted with 800ml 50% ethanol by sonication for 20 mins at room temperature. The ethanolic extracts were evaporated in a water bath at 40°C. The aqueous layer from the ethanolic extracts was subjected to sequential extraction with chloroform, ethyl acetate and n!butanol. The extracted fractions were evaporated to dryness in a flash evaporator (Roteva ! Equitron, Make). The residues obtained were redissolved in HPLC grade methanol.
Chloroform fraction was used for silica gel column chromatography. Silica gel (100/200 mesh, size) was used for packing the column with chloroform as packing solvent and eluted with chloroform and methanol at different ratios.

Macroscopic characters

The roots show buff to grey yellow outer color with longitudinal wrinkles. They are unbranched, straight, and conical and some of them bear a crown. The root crown possesses a number of bud scars. Roots are bitter in taste and fresh roots smell similar to urine of horse (hence Ashwagandha). The fracture is smooth and powder

Fig. 1. Morphology of Ashwagandha root.

Microscopic characters

The transverse section of root shows exfoliated cork which is no lignified with 2-4 layers of phellogen and about 15-20 rows of phelloderm. It prominently shows parts of vascular tissue like cambium, consisting of 3 – 5 layers of tangentially elongated cells, phloem region with parenchyma, sieve tubes and companion cells. Secondary xylem is hard which forms a continuous vascular ring interrupted by medullary rays. The transverse section of stem base shows pith, pericyclic fibres, xylem with tracheids, fibres, and starch grains.

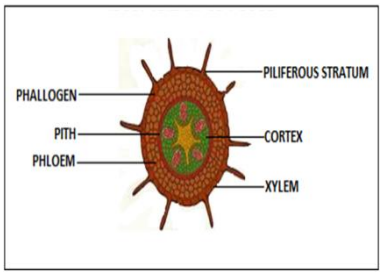

Fig. 2 CROSS SECTION OF A ROOT

Chemical constituents

The main constituents of Ashwagandha are alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine , somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo tropine, 3- α -gloyloxytropane, chiline, cuscohygrne, DI-isopelletierine, anaferin and anahydrine. Two acyl steryl glucosides viz. Sitoindoside VII and sitoindoside VIII have been isolated from roots.

Chemistry

The chemistry of Ashwagandha has been extensively studied. More than 35 chemical constituents have been identified, extracted and isolated. Pharmacological activity was mainly due to alkaloids, With anolides and sitoindosides.

Alkaloids: Roots of Ashwagandha is considered to have a significant. The alkaloidal constituent of *Withania somnifera* was studied and six alkaloids were isolated and confirmed as somniferine, somniferinine, withanine, withanonine and withaninine38. About 0.2% of total alkaloids are present.

Withanolides: are compounds with C-28 steroids on an ergostane-type skeleton in which C-22 and C-26 are appropriately oxidized to form a lactone ring. They are characterized by 9C atoms side chain with a 6-membered ring lactone occurring in plants of Solanaceae family. About 35 withanolides have been isolated till now.

Three new withanolides were isolated from Withania somnifera (Solanaceae) of which the major steroidal components were Withaferin A and Withanolide D. Structures were assigned based on spectral evidence (NMR, IR and UV), analysis of their fragmentation under electron impact, as well as on chemical degradation of known compounds.

Pharmacological activity of Ashwagandha is mainly due to the presence of steroidal lactones like Withaferin A and alkaloids. The total alkaloidal content in the plant is found to vary widely. Total alkaloids were reported to be present in the range of 0.1 – 0.33%. Reports have shown that the amount of Total Withanolides was present inthe range of 0.5-1.5% and glycowithanolides were present in the range of 0.3-2.5% in roots of Ashwagandha. Standardization of Ashwagandha and its extracts have beenreported in many standard books. Withaferin A is used as one of the marker compounds to standardize Ashwagandha.

Standards

(A)Foreign matter:- Not more than 02%
(B)Total Ash:- Not more than 07%
(C)Acid-insoluble ash:- Not morethan01%
(D)Alcohol-soluble extractive:- Not less than 15%
(E)Moisture Content:- Not more than 10 %
(F)Alkaloids:- Not less than 0.2 %

Adulterants

It has been reported that Ashwagandha is often substituted or adulterated with W. coagulans.

Ayurvedic Preparation

- **Powder [Churna]**

Mix with equal parts of ghee and honey or with milk.

- **Decoction [Kwatha]**

1 part fresh herb per 16 parts water or 1 part dried herb per 8 parts water boiled slowly until reduced to
1/4 and 1/16, respectively.

- **Medicated Wine [Arishta]**

950 mL of decoction, 350 g of cane sugar (jaggery), 190 mL honey per 35 g of herb. Allow to ferment for 7-15 days .

- **Medicated Ghee [Ghrita]**

1 part decoction of herbs, 10 parts milk, 1 part ghee, Simmer slowly to paste (to be taken with meals).

- **Medicated Oil [Narayana taila]**

Decoction of herbs. Add 40 parts sesame oil per 2 parts herb paste. Boil together 1 hour.

USES

1. Traditional and modern use

Ashwagandha holds a prominent place in Ayurveda and Unani. The plant finds its use as a rejuvenative herb. It is used as health care food supplement39. The fresh berries are used as emetic, sedative and anti-asthmatic. Dried fruits and roots are sedative, carminative, diuretic, anti-inflammatory and used for curing general and sexual weakness in human beings, goiter, fainting, blood disorders, leucoderma, chronic liver complications, skin diseases, bronchitis and ulcers. In Rajasthan, roots are used for curing rheumatism and dyspepsia; in Punjab they are used to relieve loin pain and in Sind for abortion. Leaves are used as anthelmintic, insecticide, febrifuge and tonic. It is used to cure inflammation of tubercular glands, piles, sore eyes, boils and swelling of hand and foot. In some areas, warm leaves are used to provide comfort during eye diseases. The plant is used as abortifacient, antiasthmatic, bactericide, contraceptive, diuretic, sedative, tonic, anti-inflammatory and in treatment of cold, dropsy, headaches, convulsions, sleeplessness, anemia, fever, hypertension.

2. Antihypertensive activity

An extract induced significant decrease in the arterial and diastolic blood pressure in normotensive pentobarbital anaesthetized dogs.

3. Immunomodulatory activity

Withania somnifera exhibited non-specific immunostimulatory activity in various models. The Immunomodulatory activity of sitoindosides IX and X was studied in rats and mice. A stastically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes secreted by the activated macrophages were observed.

4. Psychological Effects of Stress

Studies suggest that the inability to adapt to stress is associated with the onset of depression or anxiety. Certainly, on a more obvious level, stress diminishes the quality of life by reducing feelings of pleasure and accomplishment, and relationships are often threatened.

5. Antiviral activity

An extract showed a dose-dependent inhibition of spinach mosaic virus.

6. Gastrointestinal Problems

Prolonged stress can disrupt the digestive system, irritating the large intestine and causing diarrhea, constipation, cramping and bloating. Irritable bowel syndrome (or spastic colon) is strongly related to stress.

7. Anti-inflammatory activity

Muscular and Joint Pain. Chronic pain caused by arthritis and other conditions may be intensified by stress.
Headaches. Tension-type headache episodes are highly associated with stress events
Withania somnifera extract exhibited significant anti inflammatory activity against carrageenan-induced paw oedema in rats.

MATERIALS AND METHODS

I) Phytochemical Investigation
i) Test for Carbohydrate
a) Molisch's test:-To the test solution add few drops of alcoholic α-Naphthanol, and then add few drops of conc. Sulphuric acid (H_2SO_4) through sides of test tube, A purple to violet colour ring appears at the junction.
b) Barfoerd's test :-1ml of test solution is heated with 1ml of Barfoed's reagent on water bath, if red cupric oxide is formed, mono-saccharide is present.
c) Selivanoff's test (Test for Ketones) :-To the test solution add crystals of resorcinol and equal volume of conc. Hydrochloric acid (HCI) and heat on water bath rose colour is produced. (E.g. Fructose, honey).
d) Test for pentoses :-To the test solution add equal volume of Hydrochloric acid (HCI) containing of small amount of Phloroglucinol and heat, a red colour is produced.

ii) Test For Alkaloids :-
a) Dragondorff's reagent test :-Alkaloids give reddish brown precipitate with Dragondorff's reagent. (Potassium Bismuth Iodide solution).
b) Mayer's reagent test:-Alkaloids give cream colour precipitate with Mayer's reagent. (Potassium Mercuric Iodide).
c) Wagner's reagent test:-Alkaloids give reddish brown precipitate with Wagner's reagent. (Iodine-Potassium Iodide).
c) Tannic acid test:-Alkaloids give buff colour precipitate with Tannic acid solution.

iii) Test for Glycosides :-
a) Modified Borntrager's test:-Boil 200mg of the test material with 2ml of dilute Sulphuric acid (H_2SO_4). Treat it with 2ml of 5% aqueous Ferric chloride solution (Freshly prepared) for 5 minutes, Shake it with equal volume of Chloroform & continue the test as above. As some plants contains Anthracene Aglycone in a reduced form, if Ferric chloride is used during the extraction, Oxidation to Antraquinones takes-place, which shows response to Borntrager's test.
b) Test for Hydroxy- Antraquinones :-Treat the sample with Potassium Hydroxide (KOH) solution A red colour is produced.
c) Keller-Killiani test (Test for Deoxysugars) :-Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of Glacial-acetic acid containing trace amount of Ferric chloride. Transfer to a small test tube, add carefully 0.5ml of conc. Sulphuric acid (H_2SO_4) by the side of the

test tube. Acetic acid layer shows blue colour.

d) Baljet's test:-Treat the test solution with Picric acid or Sodium picrate, orange colour is formed.

iv) Test for Flavonoids :-

a) Shinoda test:-To the test solution add few Magnesium turnings and conc. Hydrochloric acid (HCl) drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

b) Alkaline reagent test :-To the test solution add few drops of Sodium hydroxide (NaOH) solution, intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicate presence of Flavonoids.

d) Zinc Hydrochloride test:-To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid (HCl). It gives red colour after few minutes.

v) Test for Starch:-

a) To the test solution add water, mix. it well by heating it on water bath, add weak aqueous iodine solution, blue colour indicates presence of starch.

b) Cool the above solution blue colour disappears.

c) Heat the above solution blue colour reappears.

vi) Test for Protein:-

a) Xanthoproteic test: - To the 5ml of test solution, add 1ml of conc .Nitric acid and boil, yellow precipitate is formed. After cooling it, add 40% Sodium hydroxide solution, orange colour is formed.

b) Biuret test:-To the 2ml of test solution add 2ml Biuret reagent, violet colour indicates presence of protein.

c) Heat test:-Heat the test solution in boiling water bath, protein get coagulated.

vii) Test for Amino acid:-

a) Million test: - Heat 3ml of sample and 3 drops of Millionreagent Solution shows dark red colour.

b) Ninhydrin test: - Heat 3 ml sample and 3 drops of Ninhydrin solution in boiling water bath 10 min. Solution shows purple or bluish colour.

viii) Test for Tannins:-

a) Goldbeater skin test:-Add 2% Hydrochloric acid to a small piece of Goldbeater skin, rinse it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a1% ferrous sulphate solution. A brown or black colour on the skin indicates presence of tannins. (Goldbeater skin is a membrane obtained from intestine of the Ox)

b) Ferric chloride test:-Treat the extract with Ferric chloride solution, blue colour appears if hydrolysable tannins are present and green appears if condensed tannins are present.

c) Test for Catechin:-Dip a matchstick in the test solution, dry it and lastly moisten with conc. hydrochloric acid (HCl). Then warm the stick near the flame. The colour of the wood changes to pink due to Phloroglucinol. (Phloroglucinol is formed when Catechin are treated with acids)

ix) Test for Steroids &Triterpenoids:-

a) Liberman – Burchard test:- Treat the extract with few drops of Acetic anhydride, boil and cool .Then add conc.Sulphuric acid (H_2 SO_4) from the side of the test tube ,brown ring is formed at the junction two layers and upper layer turns green which shows presence of Steroids and formation of deep red colour indicates presence of Triterpenoids.

b) Salkowski Test :-Treat the extract with few drops of conc.Sulphuric acid (H_2 SO_4), red colour at lower layer indicates presence of Steroids and formation of yellow coloured lower layer indicates presence of Triterpenoids.

c) Sulfur powder test :-Add small amount of sulfur powder to the test solution, it sinks at the bottom .

II) Authentication

1) Parts of plants:-The extraction of crude drug is obtained from roots of plant

2) Biological source:-It contains of dried mature roots and steam bases of *Withania somnifera* (Linn.) Dunal.

3) Family:-It is belonging to the family of a *Solanaceae*.

4) Chemical constituents:-The main constituents of drug are Alkaloids and Steroidal lactone.

III) Organoleptic evaluation

1. **Colour:-**White to a creamy- yellow colour
2. **Odour:-**Characteristic, Horse-like.
3. **Taste:-**Drug is bitter in taste

V) Physical Evaluation

1. **Total Ash**

- **Definition-**Total ash is the amount of the residue remains after complete incineration of sample.

2. **Acid Insoluble-Ash**

- **Definition** – Acid-insoluble ash, which is a part total ash insoluble in dilute Hydrochloric acid, is also recommended for certain drugs.

soluble Extractive Value

- **Definition** –It the amount the chemicals which are extracted by using water from the crude drug sample**.**

3. **Alcohol soluble Extractive Value**

- **Definition** –It the amount the chemicals which are extracted by using alcohol from the crude drug sample**.**

4. **Moisture content**

Definition –It the amount moisture or water present in crude drug sample.

RESULTS AND DISCUSSION

Table 1. Phytochemical investigation

Sr. no	Chemical Test	Observation	Result
	A) Test for Carbohydrate		
1.	Molish's test	No violet ring produces	-
2.	Barfoed's test	No red ppt observed	-
3.	Selivanoff's test	No rose colour produces	-
4.	Test for Pentoses	No red colour developed	-
	B) Test for Alkaloids		
1.	Dragendroff's test	Reddish ppt observed	+
2.	Mayer's test	Cream colour ppt	+
3.	Hager's test	Yellow colour ppt	+
4.	Wagner's test	Buff colour ppt	+
	C) Test for Glycosides		
1.	Modified Borntrager's test	Oxidation taken place	+
2.	Test for Hydroxy-Antraquinones	Red colour developed	+
3.	Keller-Killiani	Blue layer	+

	test	observed	
4.	Baljet's test	Orange colour produced	+
	D) Test for Flavonoids		
1.	Shinoda test	No pink colour observed	-
2.	Alkaline reagent test	No yellow colour	-
3.	Zinc Hydrochloride test	NO red colour produced	-
	E) Test for Starch		
1.	Test sample + water & Heat	No blue colour	-
2.	Cool the above solution	Colour not disappear	-
3.	Heat the above solution	Colour not reappear	-
	F) Test for Protein		
1.	Xanthoproteic test	No white ppt	-
2.	Biuret test	No violet colour	-
3.	Heat test	Protein not coagulated	-
	G) Test for Amino acid		
1.	Ninhydrin test	No purpul colour	-
2.	Million test	No violet ppt	-
	H) Test for Tannins		
1.	Goldbeater skin test	No black ppt	-
2.	Ferric chloride test	No green colour	-
3.	Test for Catechin	No white ppt	-
	I) Test for Steroids&Triter		
	penoids		
1.	Liberman – Burchard test	Brown ring formed at the lower junction	+
2.	Salkowski Test	Red colour formed at bottom of test tube	+
3.	Sulfur powder test	Powder sinck at bottom of test tube	+
4.			

Table 2. Authentication

Sr. no	Chemical Test	Observation	Result
1.	**Parts of plants**	**Roots**	+
2.	**Biological source**	***Withania somnifera***	+
3.	**Family**	***Solanaceae.***	+
4.	**Chemical constituents**	**Alkaloids and Steroids**	+

Table 3. Organoleptic evaluation

Sr. no	Chemical Test	Observation	Result
1.	**Colour**	**White to a creamy**	+
2.	**Odour**	**Characteristic, Horse-like**	+
3.	**Taste**	**bitter**	+

Table 4. Physical Evaluation

Sr. no	Chemical Test	Observation	Result

1.	**Total Ash**	**6 %**	+
2.	**Acid Insoluble-Ash**	**0.8 %**	+
3.	**Water-soluble Extractive Value**	**60.6%**	+
4.	**Alcohol soluble Extractive Value**	**56.2 %**	+
5.	**Moisture content**	**5.54 %**	+

CONCLUSION

The present study on physicochemical parameters, preliminary phytochemical analysis, phytochemical analysis provides important information which may be help in authentication and quality of marketted crude drug extract of roots of *Withania somnifera*. The present study adds to the existing knowledge of standards of the drug and useful for development of a standards for the extract. Current study indicates that marketted sample of Ashwagandha extract has passed through all Evaluation & Standardization test. This shows presence of active constituent in adequate amount.

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