

## RESEARCH ARTICLE

The Development of Standardization Parameters of *Alstonia scholaris*Sudhir Dhangar<sup>1</sup>, Swapnil Goyal<sup>2\*</sup>, Naveen Choudhary<sup>3</sup><sup>1</sup>Mandsaur Institute of Pharmacy, Mandsaur – 458001 (M.P), India<sup>2</sup>Head, Department of Pharmacology, Mandsaur Institute of Pharmacy, Mandsaur – 458001 (M.P), India<sup>3</sup>Department of Pharmacognosy, Mandsaur Institute of Pharmacy, Mandsaur – 458001 (M.P), India

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## ABSTRACT

**Background:** *Alstonia scholaris* is an important herbal plant. It is commonly used as astringent, rheumatism, antiseptics, anti leprotic, ulcer, and to diverse inflammatory chronic skin diseases, rheumatic pain. general tonic, aphrodisiac, anticholinergic, antidysentery, antipyretic, emmenagogue, vulnerary agents, fatigue, irregular menses, liver disease, dysentery, malaria, diabetes, anthelmintic, Stomachache, skin diseases, swelling, malaria fever, bone fracture, urinary infections. **Objective-** Present study deals the development of standardization parameters of *Alstonia scholaris*. **Material and method:** In the present study, a coarse powder of *Alstonia scholaris* leaves was prepared by grinding method and standardization parameter were performed in the laboratory like LOD (Loss of drying), Ash value, Acid insoluble ash, water soluble ash, Extractive value, Solubility test, Phytochemical screening test, U.V. spectrum. **Results:** The value of physiochemical parameter was calculated. **Conclusion:** the use of herbal drug are also interrupted due to use of various adulterants so various quantitative and qualitative parameter are use to set the standardization parameter of herbal drugs. This standardization parameters can help to identify and choose appropriate species of selected plants. This parameter also provides information related to purity and efficacy.

**Keyword:** -U.V. spectrum, *Alstonias chloaris*, standardization parameters, LOD (Loss of drying), Extractive value.

## INTRODUCTION

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the wide spread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants.<sup>[1]</sup>

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such

as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total.<sup>[2]</sup> Chemical compounds in plants mediate their effect on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.<sup>[3]</sup> Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans.

These plants are either “wild plant species” those growing spontaneously in self maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human

actions or the contrasting “Domesticated plants species” those that have arisen through human actions such as selection or breeding and depend on management for their existence. For example Aloe Barbadensis.<sup>[4]</sup>

Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As mentioned in the introduction only a certain percentage of plants are used in traditional medicines.<sup>[5]</sup>

## MATERIALS AND METHODS

### Collection of plant

Plant leaves were collected from the Herbal Garden of Mandsaur Institute of Pharmacy, Mandsaur & then it is placed for drying in hot air oven.

### Identification of Plant

Plant was identified by Mr. S.N. Mishra (senior scientist) of KNK Horticulture college of Mandsaur. HerbariumNo.MIP /Pharmacognosy /2015/508.

### Physiochemical parameter

#### Loss on drying

The determination of moisture content, determines the amount of volatile components i.e. H<sub>2</sub>O drying of the drug. The drug leaves (115g) was placed in a Hot Air oven. The leaves was dried at 50<sup>0</sup>C for 6 hours and weighted. The drying of leaves was continues until constant weight of dried was found.<sup>[6]</sup>

#### Ash value:-

The determination of ash value is useful for detecting low grade product, exhausted drugs and excess of sand or earthy matter. Different types of ash values are used in detection of crude drug like, total ash, acid insoluble ash, water soluble ash, and sulphatedash.<sup>[7]</sup>

This parameter can be used for the determination of inorganic materials, such as carbonates, oxalates and phosphates. Heating causes the loss of material in the form of CO<sub>2</sub> leaving behind the inorganic components.<sup>[6]</sup>

#### Acid insoluble Ash:-

The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy material.<sup>[6]</sup>

#### Water soluble ash:

Water soluble ash that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drug.<sup>[8]</sup>

#### Extraction Method: Hot Extraction Method:-

In crude drugs sometimes the active chemical consistent cannot be determined and thus the water, alcohol or ether soluble extractive values are determined for evaluation of such drug.<sup>[9]</sup>

In this method, the material to be extracted is placed in a ‘thimble’ made of cellulose or cloth in a central compartment with a siphoning device and side-arm both connected to a lower compartment. The solvent is placed in a lower compartment and a reflux condenser is attached above the central sample compartment. Note that each component of the setup is a separate item of glass was which assembled together with the appropriate contents, to make the complete apparatus.

The solvent in the lower container (usually a round bottomed flask) is heated to boiling, and the vapor passes through the side arm up into the reflux condenser. Here the vapor liquefies and drip into the thimble containing the material to be extracted. The warm solvent percolate through the material and wall theof thimble and the extract gradually collects in the central compartment. One the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this and back into the lower solvent container.<sup>[8]</sup>

#### Solubility test:-

Firstly take the different solvent in definite volume (10ml) in beaker example; Chloroform, water, Petroleum ether and butanol.1g Petroleum ether extract was dissolved in solvents and observed the solubility. Similar process was performed with the Chloroform, Ethanol and Water extract.<sup>[11]</sup>

#### Phytochemical screening tests:

##### Test for Steroids and Triterpenoids:

• **Salkowski test:** -Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

• **Libermann-Burchard test:** - Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid 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.<sup>[12]</sup>

#### Test for fat and oil

- To ethanolic solution, add few drops of CuSO<sub>4</sub> and NaOH solutions. Clear blue solution is observed.

#### Test for Triterpenoids:

- **Libermann-Burchard test:** - Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid 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.

#### Test for Flavonoids:

- Addition of increasing amount of sodium hydroxide to the reduce shows yellow colouration, which decolourises after addition of acid.

#### Test for Alkaloids:-

Alkaloid containing plants constitute an extremely varied group both taxonomically and chemically, a basic nitrogen being the only unifying factor for the various classes. For this reason, question of the physiological role of alkaloids plant, their importance in taxonomy and biogenesis are often most satisfactory discussed at the level of a particular class of alkaloid.<sup>[10]</sup>

#### Test for Alkaloids:-

- **Dragendorff's test:** - To the extract add Dragendorff's reagent, reddish brown precipitate indicates presence of alkaloids.

#### Test for Anthraquinone glycosides: -

- **Borntrager's test:** - Boil the test material with 1ml of sulphuric acid in a test tube for 5minutes. Filter while hot. Cool the filtrate and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammonical layer.

#### Test for Flavonoid: -

- Addition of increasing amount of sodium hydroxide to the reduce shows yellow colouration, which decolourises after addition of acid.

#### Test for Triterpenoids:-

- **Libermann-Burchard test:** - Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid 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.

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#### Ultraviolet spectrophotometer:

If it is not already on, turn on the Spectrophotometer using the power On/Off switch (Left hand side of instrument). Turn on the computer and click on the UV Probe software (available on the desktop). Click the "Connect" button to initiate communication between the computer and the Spectrophotometer. The instrument will now begin its initialisation procedures and begin to warm up. The machine should be allowed at least ½ an hour to warm up fully before proceeding with the remainder of this procedure. Select the "Spectrum Mode" using the button on the toolbar. Click on the "Spectrum Method" button on the toolbar. A tabbed dialogue button will now open to allow you to set the parameters for the measurement.

In the "Measurement" tab:

Enter the wavelength range via the "Start" and "End" fields. Always enter the longer wavelength into the "Start" field and the shorter wavelength into the "End" field. NOTE the operational region of the spectrophotometer is between 200-800nm. Select the scan speed to be Medium via the drop down menu. Medium is fine for the broad absorption bands typical of organic materials; if you have samples with sharp absorption features,

it may be better to run a slower scan. Select the sampling interval as 1nm from the drop down menu. The “Scan Mode” should be set to “Single”. In the “Instrument Parameters” tab:

Select Absorbance from the “Measuring Mode” drop down menu. (Transmittance is also possible) Select a value of 1nm from the “Slit Width” drop down menu.

Ensure that the “Light Source Change Wavelength” is set to 360nm and the “S/RExchange” is set to “Normal”. Set up the sample mounts in the configuration that you will use to carry out measurements without any sample or reference in place. Close the lid of the sample compartment. Run a baseline calibration by clicking on the “Baseline” button at the bottom of the screen. Ensure that the baseline range matches that which you have previously entered in the “Spectrum Method” dialogue. Click “OK” to run the baseline calibration. To obtain a spectrum, click on “Start” and the machine will begin collecting data with the parameters you have specified. Ensure that the baseline is good by first running a scan with no sample in place. You should obtain a flat baseline with noise distributed about zero. Now insert your sample and reference into the sample mounts in the sample compartment (Ensure that the lid is closed before taking measurements) and take a spectrum as before. When the scan is finished, enter a label into the dialogue box which appears. NOTE: This does NOT save your data. To save a data set, select “Save As” from the “File” menu and enter a filename. Select “Data Print Table (\*.txt)” from the “Save As Type” drop down menu. This will save the data in an ASCII text format which you can access using other software. Please only save data in your directory under the “Users Data” folder.

It is good practice to take another absorption spectrum with your sample oriented at 90° in the sample mount. This will give you two data sets for the same sample and will allow you to assess the reliability of your data, which may be affected by the uniformity of the film. To shut the instrument down press the “Disconnect” button at the bottom of the screen and turn the instrument off using the Power On/Off button. Close the software and shut the computer down when you are finished with it. [14]

## RESULTS

**Table 1: Determination of Moisture content (LOD) of *Alstonia scholaris***

S. No	Particular	Weight of Drug
1	Weight of leaves	115g
2	First day	75g
3	Second day	50g
4	Third day	45g
5	Fourth day	45g
6	Constant weight of dry leaves	45g
7	LOD	70g

**Table 2: Determination of Ash value of *Alstonia scholaris***

S. No	Particular	%yield
1	Ash value	14.16
2	Acid insoluble ash value	2
3	Water soluble Ash value	37.54

**Table 3: Determination Extractive Value of *Alstonia scholaris***

S. No	Plant Name	Extract	Colour & Nature of Extract	% Yield
1	<i>Alstonia scholaris</i>	Petroleum extract	Black and sticky	6.6
2	<i>Alstonia scholaris</i>	Chloroform extract	Greenish and dry	4.2
3	<i>Alstonia scholaris</i>	Ethanol extract	Black and Dry	8.95
4	<i>Alstonia scholaris</i>	Water extract	Black and Sticky	24.87

**Table 4: Determination Solubility Test of Petroleum ether Extract of *Alstonia scholaris***

S. No	Solvent name	Solubility
1	Petroleum ether	Completely Soluble
2	Chloroform	Partially soluble
3	Butanol	Sparingly soluble
4	Water	Poor soluble

**Table 5: Determination Solubility Test of Chloroform Extract of *Alstonia scholaris***

S. No	Solvent name	Solubility
1	Petroleum ether	Partially Soluble
2	Chloroform	Completely soluble
3	Butanol	Sparingly soluble
4	Water	Poor soluble

**Table 6: Determination Solubility Test of Ethanol Extract of *Alstonia scholaris***

S. No	Solvent name	Solubility
1	Petroleum ether	Poor Soluble
2	Chloroform	sparingly soluble
3	Ethanol	Completely soluble
4	Water	Partially soluble

**Table 7: Determination Solubility Test of Water Extract of *Alstonia scholaris***

S. No	Solvent name	Solubility
1.	Petroleum ether	Poor Soluble
2.	Chloroform	sparingly soluble
3.	Ethanol	Partially soluble
4.	Water	Completely soluble

**Table No.8 Phytochemical screening test of Petroleum ether extract of *Alstonia scholaris*.**

S. No	Test Name	Observation	Result
	<b>Test for steroid</b>		
1	Salkowski reaction	Appear greenish yellow fluorescence	+
	<b>Test for fat and oil</b>		
2	Ethanol solution + few drops of CuSO <sub>4</sub> and NaOH solution	Blue colour observed	+
	<b>Test for terpenoids</b>		
3	Liebermann-Burchard reaction	Blue colour observed	+

**Table 9: Phytochemical screening test of Chloroform extract of *Alstonia scholaris***

S. No	Test Name	Observation	Result
	<b>Test for terpenoid</b>		
1	Liebermann-Burchard reaction	Blue colour observed	+
	<b>Test for flavanoid</b>		
2	Extract solution + addition of increasing amount of sodium hydroxide.	Yellow colour observed	+
	<b>Test for alkaloid</b>		
3	Dragendorff's test	-	-

**Table 10: Phytochemical screening test of Ethanol extract of *Alstonia scholaris***

S. No	Test Name	Observation	Result
	<b>Test for alkaloid</b>		
1	Dragendorff's test	Orange greenish ppt observed	+
	<b>Test for Glycoside</b>		
2	Brontrager Test	-	-
	<b>Test for flavonoid</b>		
3	Extract solution + addition of increasing amount of sodium hydroxide.	Yellow colour observed	+
	<b>Test for terpenoid</b>		
4	Liebermann-Burchard reaction	-	-

**Table 11: Phytochemical screening test of water extract of *Alstonia scholaris* leaves**

S. No	Test Name	Observation	Result
	<b>Test for Glycoside</b>		
1	Brontrager Test	Pink colour observed	+

### UV Spectrum:

**Table 12: U.V. Spectrum**

S. No	Wavelength	Absorbance
1	770.50	0.12
2	687.00	0.05
3	642.00	0.04
4	613.50	0.05
5	565.50	0.04
6	553.00	0.04
7	502.00	0.04
8	331.00	1.64
9	248.50	0.02

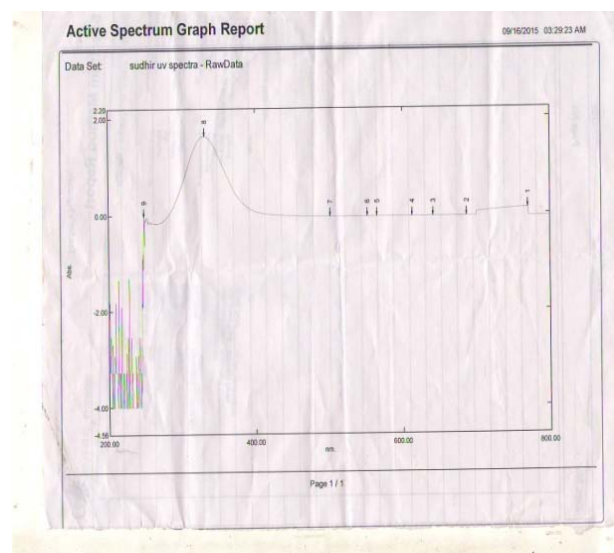
### DISCUSSION

Standardization is the process of implementing and developing technical standards. Standardization can help to maximize compatibility, interoperability, safety, repeatability, or quality. Various physiochemical parameters i.e. LOD, ash value, extractive value are helpful in determining

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the quality of herbals. U.V. Spectrum is help ful in the detection of number of compound in herbal plant extract on the basis of absorbance at the particular wavelength. U.V. Spectrum of chloroform extract has been detected the nine compound by dilution of chloroform extract was run in U.V. Spectrum between 200-800nm. Among all the compounds, compound no. 8 are highly available in Chloroform extract because there absorbency are high (1.64) at the wavelength 331.00nm.


**Figure 1: U.V Spectrum of Chloroform Extract**

### CONCLUSION

The use of herbal drug are also interrupted due to use of various adulterants so various quantitative and qualitative parameter are use to set the standardization parameter of herbal drugs. This standardization parameters can help to identify and choose appropriate species of selected plants. This parameter also provides information related to purity and efficacy.

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