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INVESTIGATION OF PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL PARAMETERS OF *ALSTONIA SCHOLARIS* LEAF AND DEVELOPMENT OF AN AQUEOUS EXTRACT–BASED FORMULATION

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ABSTRACT

Objective: The current study focused on establishing the standardization of the physicochemical and phytochemical parameters of the *Alstonia scholaris* leaf and its aqueous formulation. **Methods**: *Alstonia scholaris* leaves are gathered, then dried in the shade, and finally extracted with demineralized water. Standardization parameters such as identification, organoleptic evaluation, ash values, loss on drying, extractive values, pH, viscosity, specific gravity, solubility, TLC profiling, phytochemical screening, microbial analysis, and total dissolved solids were assessed. To maintain reproducibility and authenticity, all experiments were performed thrice. **Results**: The aqueous extract was dark brown in colour and bitter in taste. The presence of alkaloids, glycosides, steroids, tannins, terpenoids, carbohydrates, saponins, and reducing sugars was indicated by TLC & was confirmed Phytochemical Screening. The total ash value was around 0.1%, pH was 7. The physicochemical and phytochemical properties of the drug were similar to those of the drug with improved Formulation (palatability). **Conclusion**: The analytical parameters generated may be used as the first quality control benchmarks of *Alstonia scholaris* s leaf and its aqueous formulation. The use of leaves is in line with the concept of ecological conservation and at the same time, phytochemical integrity is maintained. More pharmacological and clinical investigations are advised, especially for metabolic disorders.

KEYWORDS: *Alstonia scholaris*, Ayurveda, standardization, physicochemical evaluation, phytochemical screening, TLC.

INTRODUCTION

Ayurveda is a holistic medical system that has been in practice for over 5000 years and places great emphasis on the prevention of diseases and the provision of individualized therapy. Medicinal plants continue to be the mainstay of Ayurvedic therapeutics [1][2] and thus require scientific standardization to maintain their quality, safety, and effectiveness.

Alstonia scholaris [3][4][5] (Family: Apocynaceae) is a well-known plant from the Saptaparna group, which, according to the anti-inflammatory, astringent, antipyretic, antidiabetic, and antimalarial activity, has been conventionally used. It is the stem bark that is mostly utilized. However, the indiscriminate cutting down of the stem is threatening [6][7] the survival of the plant. The leaves come back quickly and contain biologically active compounds that are a safe and renewable source.

Standardization [8] of herbal drugs is the bridge between traditional knowledge and modern pharmaceutical needs. Physical, chemical, and phytochemical characterization provides quality parameters that are reproducible and, thus, necessary for the development of formulations and registration purposes.

MATERIALS AND METHODS

Collection and Authentication

Leaves of *Alstonia scholaris* were collected fresh from Purba Bardhhaman (West Bengal) in October 2023 and authenticated by the Botanical Survey of India, Howrah.

Preparation of Extract

Leaves that had been dried in the shade were powdered coarsely. 250 grams of the powder was mixed with 9 L of demineralized water and kept for 48 hours. After that, the mixture was filtered and the filtrate was concentrated to one-fourth of the original volume at a temperature below 65°C.

Formulation

The aqueous formulation was made by adding two to three drops of edible peppermint oil per 250 mL of the extract to improve the taste. The formulation was kept in an amber-colored glass bottle at room temperature.

Table 1: Composition of Aqueous Extract–Based Formulation of *Alstonia scholaris.*

S.L No.	Ingredient	Quantity (for 250 mL)	Function
1	*Alstonia scholaris* leaf aqueous extract	250 mL	Active herbal ingredient
2	Xanthan gum	1.0 g	Thickening and suspending agent (base)
3	Madhu (Honey)	12.5 g	Sweetening agent, bio-enhancer, palatability improver
4	Sodium benzoate	0.25 g	Preservative
5	Edible peppermint oil	0.10 g (2–3 drops)	Flavouring agent
6	Purified water	q.s.	Volume adjustment
7	Container	Amber glass bottle	Light protection
8	Storage condition	Room temperature	Stability maintenance

Physicochemical Evaluation

1. **Description (Organoleptic Properties):**

   The properties experienced by human sense organ (sight-eyes, hearing-ears, touch-skin, taste and odour) are known as organoleptic properties. In pharmaceutical field Colour, odour and Taste are considered as the organoleptic properties.

2. **Total Ash** [9]**:**

   Total ash is designed to measure the total amount of inorganic material produced after complete incineration of the drug material at as low temperature as possible (about 450°C) to remove all the carbons. Total ash usually consists of carbonates, phosphates, silicates and silica.

Material, Apparatus and Instrument Required -

Crucible with Lid, Tong, Muffle Furnace, Hot Air Oven, Plant material, Desiccator, Acetone.

Procedure –

Take a crucible and rinse with Acetone and keep it to Hot Air Oven for 15 minutes at 105˚C for drying. After drying, put the crucible to the desiccator for cooling. After cooling, take the weight of the empty crucible. Add 5 gm of crushed, shaded dried leaf in crucible and placed it to the muffle furnace and set it to 450˚C to 550˚C. Incinerate the leaf until it became carbon free. Cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug.

3. Acid Insoluble Ash [9]:

The term acid insoluble ash (AIA) refers to the inorganic residues that remain after a sample is burned in order to determine its mineral content. Acid insoluble ash is used to measure the amount of minerals, such as silica, that are present in a sample.

Material, Apparatus and Instrument Required -

Crucible with lid, Total Ash, Ashless Filter Paper, Funnel, Conical Flask, Measuring Cylinder, Muffle furnace, dil. HCl.

Procedure –

Take a crucible and rinse with Acetone and keep it to Hot Air Oven for 15 minutes at 105°C for drying. After drying, put the crucible to the desiccator for cooling. After cooling, take the weight of the empty crucible. Boil the ash obtained in Total Ash for 5 minutes with 25 ml of dilute hydrochloric acid. Collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and put it on Muffle Furnace until the matter becomes complete white ash. Calculate the percentage of acid-insoluble ash with reference to the air-dried drug.

4. Moisture Content (Loss on Drying) [10]:

Material, Apparatus and Instrument Required -:

Shaded dried and crushed leaf, LOD bottle with Lid, Hot Air Oven, Tong, Weight Machine, Desiccator.

Procedure:

Take a LOD bottle with lid and rinse with Acetone and keep it to Hot Air Oven for 15 minutes at 105°C for drying. After drying, put the LOD bottle to the desiccator for cooling. After cooling take the weight of the empty LOD bottle with the lid. Put the shaded dried and crushed leaf in a tare LOD bottle. Note the weight of LOD bottle with shaded dried and crushed leaf and placed it to the Hot Air Oven at 105°C. Continue the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

5. Extractive value [11][12][13]:

Definition: Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The solvent used Menstruum and the inert insoluble material that remains after extraction is called Marc.

The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

A. Water Soluble Extractive [14]:

Material, Apparatus and Instrument Required -

Shaded dried and crushed *Alstonia Scholaris* leaf, Chloroform water, Conical Flask, China Dish, Funnel, Filter Paper, Water Bath, Weighing Machine, Hot Air Oven, Desiccator, Tong, Aluminium Foil.

Procedure –

Macerate 5 g of the air-dried drug, coarsely powdered *Alstonia Scholaris* leaf, with 100 ml of Chloroform Water in a closed conical flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, take 25 ml of the filtrate and evaporate to dryness in a tared flat bottomed shallow China dish, and dry at 105º, to constant weight and weight. Calculate the percentage of alcohol-soluble extract with reference to the air-dried drug.

B. Hot Continuous Extraction (Soxhlet) [15]:

Material, Apparatus and Instrument Required –

Soxhlet apparatus, Thimble, Round Bottom Flask, Condenser, Heating Mantle, China Dish, Water bath, Hot Air Oven, Desiccator, Weighing Machine, air-dried drug, coarsely powdered *Alstonia Scholaris* leaf.

Procedure –

Took a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with Solvent ether (or petroleum ether, b.p. 40º to 60º) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105ºC to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

6. pH Value [16]:

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g, per litre. Although this definition provides a useful practical means for the quantitative indication of the acidity or alkalinity of a solution, it is less satisfactory from a strictly theoretical point of view. No definition of pH as a measurable quantity can have a simple meaning, which is also fundamental and exact.

The pH value of a liquid is determined potentiometrically by means of the glass, electrode and a suitable pH meter.

Material, Apparatus and Instrument Required –

pH paper, pH meter, Extracts of *Alstonia Scholaris* leaf, Beaker.

Procedure –

(For pH paper)

Took about 20-30 ml extract and insert a pH paper to it and note the colour and compare with the standard given.

(For pH Meter)

Calibrate pH meter with pH 7,4,10 buffer. Took 30-40 ml of extract in a beaker and inserted the electrode on it. Note the reading.

7. Specific Gravity (Wt./ml) [17]:

Material, Apparatus and Instrument Required –

Pycnometer, DM water, Extract.

Procedure –

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled Water at 25º and weighing the contents. Assuming that the weight of 1 ml of water at 25º when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20º and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25º, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per millilitre dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Specific gravity – The specific gravity of a liquid is the weight of a given volume of the liquid at 25ºC (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. Method Proceed as described under Wt. Per ml. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 25ºC unless otherwise directed in the individual monograph.

8. Viscosity [18]**:**

Material, Apparatus and Instrument Required –

Ostwald viscometer, DM Water, Extract, Stopwatch.

Definition: Viscosity is a property of a liquid, which is closely related to the resistance to flow. In C.G.S. system, the dynamic viscosity (n) of a liquid is the tangential force in dryness per square centimetre exerted in either of the two parallel planes placed, 1 cm apart when the space between them is filled with the fluid and one of the planes is moving in its own plane with a velocity of 1 cm per second relatively to the other. The unit of dynamic viscosity is the poise (abbreviated p). The centi poise (abbreviated cp) is 1/100th of one poise.

Procedure –

The liquid under test is filled in an Ostwald viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified weight of the viscometer and the time taken for the meniscus to pass the two specified marks is measured. The kinematic viscosity in centistokes is calculated from the following equation: Kinematic viscosity = kt Where k = the constant of the viscometer tube determined by observation on liquids of known kinematic viscosity. T = time in seconds for meniscus to pass through the two specified marks.

9. Solubility Test [19]**:**

Material, Apparatus and Instrument Required –

Test tube, test tube Rack, Extract, Measuring cylinder, Hexane, Pet ether, Chloroform, Acetone, Methanol, Ethyl acetate, DM Water.

Procedure –

5ml each solution to each 7 test tubes separately and add 2-3 ml of the sample (*Alstonia scholaris* water extract) to each test tube separately and vortex it. Note the observation.

10. TLC (Thin Layer Chromatography) [20]:

Material, Apparatus and Instrument Required –

Pre-coated TLC plates, Capillary tubes, UV cabinet, Extract, TLC chamber, Iodine Chamber.

Procedure -

Pre-coated TLC plates are used for the whole procedure. Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow it to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray. When the method prescribed in the monograph specifies ‘protected from light’ or ‘in subdued light’ it is intended that the entire procedure is carried out under these conditions.

Sample preparation: Took water extract as well as alcohol extract of *Alstonia scholaris leaf.* Took Extracts with the help of capillary tube and spotted over precoated TLC plate.

Mobile phase:

Ethyl acetate: Methanol (1:1)

Visualisation:

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

R_f value [21][22]:

It can be defined as it is a physical parameter and definite for a definite compound with respect to the definite solvent system. R_f value doesn't have any unit because it is a ratio.

R_f= distance travelled by the solute/ distance travelled by solvent.

11. Phytochemical screening [23]**:**

Phytochemical screening is identification of different classes of phytoconstituents present in various parts of a plant. Phytochemicals are the chemicals that are present naturally in plants. Plants consist of various kinds of chemical constituents that includes phenol, flavonoids, alkaloids, terpenoids, saponins, among many others. The botanical nutraceuticals are not only rich in phytochemicals but also enhance wellness and reduce health risk factor. Their use is gradually increasing due to their effectiveness against several physiological threats. Phytochemical screening not only helps to reveal the constituents of the plant extracts and the one that predominates over the others but also is helpful in searching for bioactive agents that can be used as dietary supplement. The plant contains secondary metabolites that show many pharmacological activities. These secondary metabolites are nothing but some chemical group having a particular structure. The test is available to detect the presence of a particular group in the extracts. These are based on colour reaction or precipitation response to a particular chemical reagent. Chemical screening is mainly performed to identify or detect the phytoconstituents present in the extract of plant material.

Sample Solution Preparation - Took water and alcoholic extract of *Alstonia scholaris*.

1. **Test for Carbohydrate** [24][25] _

a. **Iodine test**: Took 2 ml of iodine solution mixed with crude plant extract. Purple or dark blue colours prove the presence of the carbohydrate.

b. **Molisch's test:** Took the extracts and the solution and a few drops of Molisch's reagent (alcoholic a-naphthol). Then added a few drops of concentrated H_2SO_4 through the side of the test tube. Purple to violet colour ring form in case of positive response.

2. **Test for Protein** [24][25]**_**

Ninhydrin Test – Took 2-3 ml of extracts and added Ninhydrin reagent. Violet colour is formed in a positive response.

3. **Test for Fat** [24][25]**_** Take a filter paper and drop a few drops of extract to it. The filter paper becomes translucent in positive response.

4. Test for Steroids [24][25]_

Libermann-Burchad test -

To these extracts 1 ml of acetic anhydride was added and then 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

5. Test for Terpenoid [24][25]_

Libermann-Burchad test -

Take 2-3 ml of Extract and add acetic anhydride then 10ml H_2SO_4 under cooling ethanol is added. The appearance of blue green colour indicates the presence of Terpenoid.

6. Test for Phenol [24][25]_

a. Ammonia Test –

Add 1-2 drops of extract on filter paper, and exposed to Ammonia vapour. Yellow colour appeared in positive response.

b. Shinoda test – Add extracts to the test tube and add 2ml dil. HCl. Red/magenta colour appeared in positive response.

7. Test for Alkaloids [24][25]_

a. Mayer's test –

Add extracts to the test tube then add Mayer's reagent to it. Cream red colour formed in positive response.

b. Dragenroff test -

Each extract 2 ml was acidified with a few drops of dilute hydrochloric acid. Then 1 ml of Dragendroff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

c. Wagner's test –

Took 2-4 ml of extract to the test tube and add Wagner's Reagent. Reddish brown colour appears in positive response.

d. Hager's test –

Took 2-4 ml of extract to the test tube and add Hager's Yellow precipitation form in positive response.

e. Murexide test -

Took 2-4 ml of extract to the test tube and added a small amount of $KClO_3$ and 1 drop of HCl and heated it then exposed to NH_3. Purple colour forms in positive response.

8. Test for Glycosides [24][25]_

a. Baljet's test –

Add extracts to the test tube then add sodium picrate to it. Yellow to orange colour appeared in positive response.

b. Ferric chloride test

Took 1 ml of each extract, a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

9. Test for Tannins [24][25]_

a. Lead Acetate –

Took 2 ml of each extract and a few drops of 10 % lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

b. Ferric chloride –

Took 2 ml of each extract and a few drops of Ferric chloride. Dark blue colour appears for hydrolysable tannin in positive response. Green colour appeared for condensed tannin in positive response.

10. Test for Fixed oil [24][25]**-**

Spot test –

Add a filter paper and drop a few drops of extract to it. The filter paper become translucent in positive response.

11. Test for Saponins [24][25] _

Froth Test –

Took 1 ml of each extract taken in a measuring jar, 9 ml of distilled water was added and shaken vigorously for 15 seconds and extract were allowed to stand for 10 min. Formation of stable foam (1 cm) indicates the presence of saponins.

12. Test for Flavonoid [24][25]_ Took 1 ml of each extract and was shaken with 10 ml petroleum ether to remove the fatty materials (lipid layer). The defatted residue was

dissolved in 20 ml of 80 % ethanol and filtered. The filtrate was used for the following tests:

5 ml of the dilute ammonia solution was added to the portion of the aqueous filtrate of each plant extract followed by the addition of concentrated Sulphuric acid. The appearance of the yellow colouration indicated the presence of flavonoids.

13. Test for Reducing Sugar [24][25]**–**

a. Fehling test –

A few ml of extract is taken in the test tube and a few drops of Fehling's reagent is added.

b. Benedict's test –

A few ml of sample is taken in the test tube and a few drops of Benedict's reagent is added.

12. Total Dissolve Solid [26]**:**

Material, Apparatus and Instrument Required –

Alstonia scholaris extract, Petri dish, Boiling chip, Water bath, Hot Air Oven, Desiccator, Weighing Machine.

Procedure –

Take a Petri dish and rinse with Acetone and keep it to Hot Air Oven for 15 minutes at 105˚C for drying. After drying, put the Petri dish to the desiccator for cooling. After cooling, take the weight of the empty Petri dish. Add extract to the Petri dish and put it in the Water bath. After complete evaporation took the Petri dish to the Hot Air Oven at 105˚C for complete drying. Put it on a desiccator for cooling and weigh it. Calculate the percentage.

13. Microbial Analysis [27]**-**

Microbiological analysis covers the use of biological, biochemical or chemical methods for the detection, identification or enumeration of microorganisms. It is often applied to disease causing and spoilage microorganisms.

Procedure –

Sterilize all the instruments, glassware & distilled water. Prepare peptone water & inoculate sample for growth & incubate for 24 hours.

Prepare five plates with nutrients for antimicrobial testing. Swap for growth, put antibiotic disc of Klebsiella, *pseudomonas, proteas* as a standard & cut filter paper into small circle pieces to dip into the sample and put in the plate. Incubate for 24 hours. I took the observation.

RESULTS

Analytical Result of *Alstonia scholaris* Leaf

Table:2 (Analytical Result of *Alstonia scholaris* Leaf).

SL No.	Parameters		Set 1	Set 2	Set 3
1	Identification		This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.	This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.	This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.
2	Description (Organoleptic Properties)	Colour	Dark brown	Dark brown	Dark brown
		Odour	Characteristic	Characteristic	Characteristic
		Taste	Bitter	Bitter	Bitter
3	Total Ash		0.1 %	0.3 %	0.19 %
4	Acid Insoluble Ash		0.5 %	0.6 %	0.6 %
5	Loss on Drying		16.19 %	16.2 %	16.19 %
6	Extractive values:	water extractive value	12.6 %	13 %	12.6 %
		continuous heat extraction	15.487 %	15.700 %	15.732 %
7	pH		7.04	7.00	7.04
8	Specific gravity (wt./ml)		1.007 g/ml	1.007 g/ml	1.006 g/m,
9	viscosity		1.22 $Kg.m^{-1}. s^{-1}$	1.23 $Kg.m^{-1}. s^{-1}$	1.23 $Kg.m^{-1}. s^{-1}$
10	Solubility		Table 2(A)		
11	TLC		Table 2(B)		
12	Phytochemical Screening		2(C)		
13	Microbial Analysis		Table 2(D)		

Table:2(A) Result of Solubility of *Alstonia scholaris* leaf.

Solution	Set 1	Set 2	Set 3
Hexane	Practically insoluble	Practically insoluble	Practically insoluble
Pet ether	Practically insoluble	Practically insoluble	Practically insoluble
Chloroform	Practically insoluble	Practically insoluble	Practically insoluble
Acetone	Freely soluble	Freely soluble	Freely soluble
Methanol	Very soluble	Very soluble	Very soluble
Ethyl acetate	Very soluble	Very soluble	Very soluble
Water	Very soluble	Very soluble	Very soluble

Table:2(B) Result of TLC (Rf) of *Alstonia scholaris* leaf.

Set of experiment	Set 1	Set 2	Set 3
R_{f1}	5/5 = 1	5/5 = 1	5/5 = 1
R_{f2}	4.5/5 = 0.9	4.5/5 = 0.9	4.5/5 = 0.9
R_{f3}	4/5 = 0.8	4/5 = 0.8	4/5 = 0.8
R_{f4}	3.9/5 = 0.78	3.9/5 = 0.78	3.9/5 = 0.78
R_{f5}	3/5 = 0.6	3/5 = 0.6	3/5 = 0.6

Table: 2(C) Result of Phytochemical Screening of *Alstonia scholaris* leaf.

SL No.	Phytochemicals	Tests	Set 1	Set 2	Set 3
1	**Carbohydrate**	Iodine	+	+	+
		Molisch	+	+	+
2	**Protein**	Ninhydrin	-	-	-
3	**Fat**	Spot test	-	-	-
4	**Steroid**	Libermann-Burchad test	-	-	-
5	**Terpenoid**	Libermann-Burchad test	+	+	+
6	**Phenolic Compound**	Ammonia Test	+	+	+
		Shinoda Test	+	+	+
7	**Alkaloid**	Mayer's Test	+	+	+
		Dragendroff's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
		Murexide Test	+	+	+
8	**Glycoside**	Baljet's Test	+	+	+
		Ferric Chloride Test	+	+	+
9	**Tannin**	Lead Acetate Test	+	+	+
		Ferric Chloride Test	+	+	+
10	**Fixed Oil**	Spot Test	-	-	-
11	**Saponin**	Froth Test	+	+	+
12	**Reducing Sugar**	Fehling's Test	+	+	+
		Benedict's Test	+	+	+

Table: 2(C) Result of Microbial Analysis of *Alstonia scholaris* leaf.

Sl. No.	Microbial Analysis	Set 1	Set 2	Set 3
1	*Proteus*	-	-	-
2	*Escherichia coli*	-	-	-
3	*Staphylococcus aureus*	-	-	-
4	*Klebsiella*	-	-	-

Analytical Result of the formulation using water extract of *Alstonia scholaris* Leaf

Table:3 (Analytical Result of *Alstonia scholaris* Leaf).

SL No.	Parameters		Set 1	Set 2	Set 3
1	Identification		This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.	This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.	This specific plant sample is identified by the Botanical Survey of India, Central National Herbarium, Howrah, Pin 711103.
2	Description (Organoleptic Properties)	Colour	Dark brown	Dark brown	Dark brown
		Odour	Characteristic	Characteristic	Characteristic
		Taste	Bitter	Bitter	Bitter
3	Total Ash		0.1 %	0.3 %	0.19 %
4	Acid Insoluble Ash		0.5 %	0.6 %	0.6 %
5	Loss on Drying		16.19 %	16.2 %	16.19 %
6	Extractive values:	water extractive value	12.6 %	13 %	12.6 %
		continuous heat extraction	15.487 %	15.700 %	15.732 %
7	pH		7.04	7.00	7.04
8	Specific gravity (wt./ml)		1.007 g/ml	1.007 g/ml	1.006 g/m,
9	viscosity		1.22 $Kg.m^{-1}. s^{-1}$	1.23 $Kg.m^{-1}. s^{-1}$	1.23 $Kg.m^{-1}. s^{-1}$
10	Solubility		Table 3(A)		
11	TLC		Table 3(B)		
12	Phytochemical Screening		3(C)		
13	Total Dissolved Solid (TDS)		ó	ó	ó
14	Microbial Analysis		Table 3(D)		

Table:3(A) Result of Solubility of *Alstonia scholaris* leaf.

Solution	Set 1	Set 2	Set 3
Hexane	Practically insoluble	Practically insoluble	Practically insoluble
Pet ether	Practically insoluble	Practically insoluble	Practically insoluble
Chloroform	Practically insoluble	Practically insoluble	Practically insoluble
Acetone	Freely soluble	Freely soluble	Freely soluble
Methanol	Very soluble	Very soluble	Very soluble
Ethyl acetate	Very soluble	Very soluble	Very soluble
Water	Very soluble	Very soluble	Very soluble

Table: 3(B) Result of TLC (Rf) of *Alstonia scholaris* leaf

Set of experiment	Set 1	Set 2	Set 3
R_{f1}	5/5 = 1	5/5 = 1	5/5 = 1
R_{f2}	4.5/5 = 0.9	4.5/5 = 0.9	4.5/5 = 0.9
R_{f3}	4/5 = 0.8	4/5 = 0.8	4/5 = 0.8
R_{f4}	3.9/5 = 0.78	3.9/5 = 0.78	3.9/5 = 0.78
R_{f5}	3/5 = 0.6	3/5 = 0.6	3/5 = 0.6
R_{f6}	2.2/5 = 0.4	2.2/5 = 0.4	2.2/5 = 0.4
R_{f7}	1/5 = 0.2	1/5 = 0.2	1/5 = 0.2

Table: 3(C) Result of Phytochemical Screening of *Alstonia scholaris* leaf.

SL No.	Phytochemicals	Tests	Set 1	Set 2	Set 3
1	**Carbohydrate**	Iodine	+	+	+
		Molisch	+	+	+
2	**Protein**	Ninhydrin	-	-	-
3	**Fat**	Spot test	-	-	-
4	**Steroid**	Libermann-Burchad test	-	-	-
5	**Terpenoid**	Libermann-Burchad test	+	+	+
6	**Phenolic Compound**	Ammonia Test	+	+	+
		Shinoda Test	+	+	+
7	**Alkaloid**	Mayer's Test	+	+	+
		Dragendroff's Test	+	+	+
		Wagner's Test	+	+	+
		Hager's Test	+	+	+
		Murexide Test	+	+	+
8	**Glycoside**	Baljet's Test	+	+	+
		Ferric Chloride Test	+	+	+
9	**Tannin**	Lead Acetate Test	+	+	+
		Ferric Chloride Test	+	+	+
10	**Fixed Oil**	Spot Test	-	-	-
11	**Saponin**	Froth Test	+	+	+
12	**Reducing Sugar**	Fehling's Test	+	+	+
		Benedict's Test	+	+	+

Table: 2(C) Result of Microbial Analysis of *Alstonia scholaris* leaf.

Sl. No.	Microbial Analysis	Set 1	Set 2	Set 3
1	*Proteus*	-	-	-
2	*Escherichia coli*	-	-	-
3	*Staphylococcus aureus*	-	-	-
4	*Klebsiella*	-	-	-

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CONFLICT OF INTEREST

The Authors have no Conflict of Interest.

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