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ORIGINAL RESEARCH ARTICLE

Phytochemical Study of *Shorea robusta* (SHALA)

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ABSTRACT

Ayurveda is a complete self being system, which dominantly stresses on living a good and healthy life style that does not have any imbalance in the harmony and system of the body.

Since the time immemorial the society relay on plants not only for dietic purpose but also for medicinal purpose. Also in the present scenario human being relay in 75% of herbal remedies. Lots of description regarding plants available from the Vedic eras to Nighantu period.

Modern scientific progress has gifted us new methods, techniques & relevant precision instruments for verification of drugs. But today we are standardizing the action of any drug in terms of modern parameter which will enrich the store of modern system and will provide new drugs to them. So it is ideal that Ayurvedic drug research & standardization should be based on Ayurvedic principles.

As single plants having ability to cure multiple diseases that mentioned in our classical Ayurvedic texts now need to be scientifically authentication in order to make the society believe that what had ever been written by our Acharyas are right.

To establish the same above said by taking a scientific study of Shala (*Shorea robusta)* in order to establish its Phytochemical study with the clue that mentioned in different texts.

Key words: Ayurveda, modern system, *Shorea robusta*, Phytochemical study.

INTRODUCTION

Phytochemistry is the branch of natural product chemistry in which qualitative and quantitative of herbal drugs take place. Phytochemistry is in the strict sense of the word, the study of phytochemicals. These are chemicals derived from plants. In a narrower sense, the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers.

Standardization starts right from the collection of raw materials to the extreme clinical application. In case of Ayurvedic medicines, the therapeutic efficacy is a total effect of its chemical constituents. So, the quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardizing an Ayurvedic drug. This multidimensional approach should cover every minute aspect of Ayurvedic drug specifically the name, botanical source, geographical source, organo-leptic, morphological, anatomical, physical, chemical, biological and relevant references from the classical texts are to be considered in detail as mentioned –

In Charak Vimansthana 8/87 very scientifically, parameters at drug. Standardization or evaluations are described in details.

Drug Review

Shorea robusta order Dipterocarpaceae is a large, deciduous tree up to 50 m tall.

As the drug having Kashaya rasa, Ruksha guna, Sheeta virya, Katu vipaka and it pacifies Pitta and Kapha, so prevent the formation and growth of Krimis.

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In Charaka Samhita the Shala has been described in 'Vedanasthapana Mahakashya'and in 'Kashaya skandha'also mentioned in 'Aasavyonivriksha'.

In Sushruta Samhita the Shala has been described in Eladi Gana, Shalsaradi Gana, Rodhradi Gana and in Shiro Virechana Dravya.

Phytochemical Analysis

The systematic investigations of plant materials for its phytochemical behavior involves for different stages.

- The procurement of drug material and quality control.
- Examination, Purification and Characterization of the constituents for pharmaceutical interest and in process of quality control.
- Investigation of Bio-synthetic pathways of a particular compound.
- Quantitative evaluations.

Aims and Objectives of the Study

1. Phytochemical analysis in order to determine the different active constituent of plant
2. To assess the drug on Ayurvedic parameter as described in Dravya Guna (Namarupa Vigyana).

MATERIALS AND METHODS

In the plants, two types of phytochemicals are present,

(i) Inorganic matters (ii) Organic matters.

Inorganic Matters are those which are free from carbon i.e. all electrolytes comes under inorganic matter. **Organic Matters** are secondary metabolite products in the plants. Role of these components for medicinal purposes are important. Examinations of both Organic and Inorganic matters are done both qualitatively and quantitatively.

(1) *Qualitative examination of Inorganic matters*

It involves qualitative examinations of electrolytes, which are present in ash of sample. Higher plants, for growth and reproduction require sixteen or Seventeen elements. Elements required in relatively large quantities are termed as macronutrients, where as nutrients required in small amounts are termed as micronutrients. All such elements are used up in various metabolic processes. Variety of organic compounds synthesized by the plants incorporates these elements in their chemical constitutions. Therefore, their presence can be detected by simple chemical analysis.

- **Preparation of Test Sample**

Take 5 gm of drug samples in crucibles. The crucibles are kept in muffle furnace at 550°C for nearly 6 hours. After 6 hours, the ash is removed from the muffle furnace. Dissolve the ash in 5ml of slightly acidic distilled water and use the solution for detecting the presence of mineral elements.

- **Calcium:** Take 0.5ml of test sample and 2 drops of conc.H_2SO_4. Formation of white precipitate indicates presence of Calcium.
- **Iron:** Take 0.5 ml of test sample and 3 drops KSCN reagent. Formation of red colour indicates presence of Iron.
- **Manganese:** Take 0.5 ml of test solution and add 1ml of 1% KOH solution then 5 drops of Benzedine reagent. Formation of blue colour shows presence of Mn.
- **Phosphorus:** Take 0.5 ml of test solution and two drops of Ammonium Molybdate reagent. Formation of yellow colour indicates presence of Phosphorus.
- **Potassium:** Take 0.5 ml of test solution and 2 drops of 15% $HClO_4$ soln. Formation of $KClO_4$ crystals indicate presence of K.
- **Sulphur:** Take 0.5 ml of test solution and 2 drops of $BaCl_2$ Formation of white ppt. of $BaSO_4$ indicate presence of Sulphur.

(2) *Determination of Heavy Metals*

- **Preparation of Test Sample**

Take 5 gm of drug samples in crucibles. The crucibles are kept in muffle furnace at 550°C for nearly 6 hours. After 6 hours, the ash is removed from the muffle furnace and uses the ash for detecting the presence of heavy metals.

- **Cobalt Compounds**

  Dissolve 20 mg of the ash of the drug in about 0.5 mL of distilled water, and acidify with a few drops of dil hydrochloric acid. Add a few drops of dil solution of sodium hydroxide. A blue ppt is formed which turns pink on warming.

- **Copper Compounds**

  Dissolves 20 to 25 mg of the drug in 1 mL of distilled water and add dil ammonia solution, dropwise until a clear blue solution is obtained. Heat to boiling and add dropwise 2% W/V alcoholic solution of α-benzoinoxime. A green ppt is formed.

- **Mercury Compounds**
  Dissolve 20 to 25 mg of the ash of the drug in 1 mL of distilled water, and add 2 M sodium hydroxide until solution becomes strongly alkaline. A dense yellow ppt is formed.
  Dissolve 20 to 25 mg of the ash of the drug in 1 mL of distilled water, and add potassium iodide solution. A red ppt is formed that dissolves in an excess of the reagent.
- **Nickel Compounds**
  Dissolve 20 mg of the ash of the drug in about 0.5 mL of water, acidify with a few drops of dil hydrochloric acid, and then add drop by drop a dil solution of sodium hydroxide. A blue ppt is formed which turns green on warming.
- **Silver Compounds**
  Dissolves 20 to 25 mg of the ash of the drug in 2-3 mL of distilled water and add 0.2 mL of 7 M hydrochloric acid. A curdy white ppt is formed that is soluble in 3 mL of 6 M ammonia. Add a few drops of a 10% W/V aq soln of potassium iodide a yellow ppt is developed.
- **Zinc Compounds**
  Dissolve 20 to 25 mg of the ash in 2 to 3 mL of distilled water, and add 0.2 mL of 10 M sodium hydroxide. A white ppt is formed which dissolves in 2 mL of 10 M sodium hydroxide solution. Add about 5 mL of 2 M ammonium chloride followed by 0.1 mL of sodium sulphide solution. A flocculent, white ppt is produced.

(3)*Quantitative Examination of Inorganic Matter*

The quantitative Examination include the following examinations

a. Loss On Drying or Moisture Content

The drug samples were shredded to give parts of about 1-2 mm in size. 5 grams of each sample was weighed to constant in the digital balance and kept in a pre-weighed Petri dish. The Petri dish was then kept in the oven at 105°C for 5 hours and weighed. The weighing was repeated once in every hour to get two successive weights varying less than 0.25%. The Petri dish was allowed to cool in a desiccator and subsequently weighed. Calculations:

- Weight of the Empty Petri dish = W_1 gm
- Weight of the Drug Sample = X gm
- Wt. of the Petri dish with drug before drying = $W_3 = (W_1+X)$
- Weight of Petri dish after Drying = W_2 gm
- Loss on Drying in % $= \frac{W_3 - W_2 \times 100}{X}$

b. Determination Of Total Ash

Three Silica Crucibles were cleaned, dried well and then weighed to constant weight and labeling was made A1, B1, and C1. 3gm and 5gm of the drug sample were then weighed accurately and placed in the Silica Crucibles respectively. These crucibles were placed in a muffle furnace at a temperature of 450°C ±5°C till were become totally free from Carbon. The time taken for this process was about 6 hrs. The crucibles containing the ash were allowed to be cooled in a desiccator and subsequently weighed to constant weight.

Calculation

- Wt. of Empty Silica Crucible = A_1, B_1, C_1 gm
- Wt. of Sample (X) = X gm
- Wt. of the Crucible with Ash = A_2, B_2, C_2 gm
- Percentage of Total Ash = A_2- A_1/X ×100

The process was repeated three times for each drug sample and the Average Total Ash value was calculated.

c. Determination Of Acid Insoluble Ash

The ash obtained from above procedure was added to 100 ml of diluted HCl (2N) in a beaker. The mixture was then heated at temperature of 70 to 80 °C for 5 minutes. The mixture was filtered into a pre-weighed Gooch's Crucible fitted with a Whatman's filter paper No 1, transfixed to a beaker that was attached to vaccum pump. The glass beaker containing Ash and HCl mixture was then washed with boiling water three times and the water was also poured to the Gooch's Crucible.

The mixture was then allowed to be filtered by using the vaccum pump. The Gooch's Crucible with residual Ash was then dried in the oven at 50°C till completely dried. It was then allowed to be cooled in a desiccator and subsequently weighed and the Acid Insoluble Ash was calculated.

Calculation

- Wt. of Drug sample = X gm
- Wt. of Empty Gooch's Crucible with filter paper = G_1gm
- Wt. of the Gooch's Crucible with residual Ash = G_2 gm
- Percentage of Acid Insoluble Ash = G_2- G_1/X ×100

The procedure was repeated three times for each sample and the average value was calculated.

d. Determination Of Water Soluble Ash

The Ash obtained from above procedure (Ash of 3 or 5 gm drug) was mixed with 100 ml of distilled Water in a beaker and the mixture was heated at 70-80°C for 5 minutes. This mixture was poured into a pre-weighed Gooch's Crucible fitted with a Whatman's filter paper transfixed in a beaker attached to a vaccum pump. The mixture was filtered into the beaker. The Gooch's Crucible with residual insoluble Ash was dried at 50°C in oven and then allowed to be cooled in desiccator and subsequently weighed and the Water Soluble Ash was then calculated.

Calculation

- Wt. of the Empty Gooch's Crucible with filter paper = G_1 gm
- Wt. of Drug Sample = X gm
- Wt. of the Gooch's Crucible with Water Insoluble Ash = G_2 gm
- Wt. of Total Ash = A gm
- Percentage of Water Soluble Ash = $A-(G_2-G_1)/X\times100$

The procedure was repeated three times for each sample and average value was calculated.

(4) *Qualitative Examination of Organic matter*

S. No	Organic substances	Test Applied
1	Carbohydrates	Molisch's reagent
2	Starch	Iodine solution
3	Tannin	Vanillin solution
4	Protein	Ninhydrine solution
5	Saponin	Shaking with water
6	Phenol	$FeCl_3$ solution
7	Glycoside	Killer killani test
8	Alkaloids	Dragondroff's reagent

(5) *Quantitative Examination for Organic matter*

a. Determination of Extractive values

The organic substances of the different parts of *Shorea robusta* show their solubility in various, solvents in different quantities. So for this purpose of determination of extractive values seven main solvents were selected according to their polarity.

1. Hexane 2.Chloroform 3.Ethyl acetate 4.Acetone 5.Methanol 6.Water.

- **Method of Determination of Extractive values of Resin**

  Three conical flasks (200ml) were thoroughly cleaned. Then 5 gm. resin powder weighed and poured into each flask then 100 ml of solvent was poured into flask.

  All flasks were fitted with corks and fixed them firmly to the shaker machine. They were shaken continuously for minimum 6 hours. This time allow complete extraction of drug sample.

  After then they were filtered and transfered into different beaker and kept them inside the oven at 50℃ temp and allow to dry. After drying each sample transferred into individual sterilized test tube and made them airtight by cork.

- **Method of Determination of Extractive values of L*eaf, Bark & Root powder***

  Some conical flasks (500ml) were thoroughly cleaned then drug material (leaf, bark and root powder) weighed and poured into each flask then solvent was poured into flask.

  Flask is attached to a reflux condenser and heated for 2hrs, on water bath. After 2hrs, the flask is allowed to cool and the content is filtered through filter paper. The filtrate is transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then the dish is kept in oven for six hours for the contents to get dried fully. The Dish is cooled by keeping in a desiccator for 30 minutes and weighed without delay.

  The residual mass remained in filter paper is dried as such and is collected fully. This mass in again put into the conical flask and added with next solvent according to polarity, and fitted with reflux condenser, and extract is prepared in the same method used above. This procedure is repeated with all the solvents. .

Calculations:

- Weight of the drug material = Xgm
- Weight of the empty petridish = W_1gm
- Weight of the pertidish with dried extract = W_2gm
- Percentage of extractive value $=\frac{(W_2-W_1)}{X}\times 100$

The procedure was carried out with same drug sample with different solvent taken in the order of polarity.

(6) *Chromatography*

a. Thin Layer Chromatography (T.L.C.)

Thin layer chromatography is a technique to separate the compounds from a mixture based on adsorption principle. It has the advantage of faster runs, better separations, and the choice between different adsorbents. Different compounds in the sample mixture travel different distances according to how strongly they interact with the adsorbent. This allows the calculation of an Rf value and can be compared to standard compounds to aid in the identification of an unknown substance.

Calculation of R_f Value

$$R_f = \frac{\text{Distance traveled by solute from origin line}}{\text{Distance traveled by solvent from origin line}}$$

(7) Density

Density is a physical property of matter that describes the degree of compactness of a substance- in other word how closely packed together the atoms of an element or molecules of a compound are mass per unit volume.

(8) Determination of pH of different parts of *Shorea robusta*

- Acidic solution have a pH between 1 and 6.9
- Alkaline solution have a pH between 7.1 and 14
- Neutral solution is neither acidic nor alkaline so their pH is 7.

pH= -log (H^+)

RESULTS AND DISCUSSIONS

1. *Qualitative examination of Inorganic matters*

S. No	Parts of the Drug	Name of the Minerals / Electrolytes					
		Calcium	Iron	Manganese	Phosphorus	Potassium	Sulphur
1	Resin	-	+	-	-	-	-
2	Leaf	+	+	+	+	+	+
3	Root	-	+	+	+	-	+
4	Bark	+	+	+	-	-	-

2. *Determination of Heavy Metals*

S. No	Parts of the Drug	Name of the Heavy Metal					
		Cobalt	Copper	Mercury	Nickel	Zinc	Silver
1	Resin	-	-	-	-	-	-
2	Leaf	-	-	-	-	-	+
3	Root	-	-	-	-	-	+
4	Bark	-	-	-	-	-	-

3. *Quantitative Examination of Inorganic Matter*

The quantitative Examination include the following examinations

a. Loss On Drying Or Moisture Content

- ✓ Loss on drying of resin = 8.29 %.
- ✓ Loss on drying of bark = 9.86 %.
- ✓ Loss on drying of root= 6.72 %.
- ✓ Loss on drying of leaf= 7.40 %.

b. Determination Of Total Ash

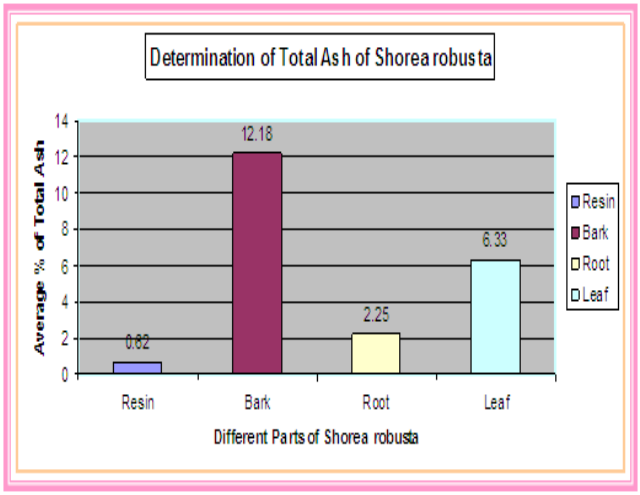
- ✓ The Average Percentage of Total ash of resin = 0.6108%.
- ✓ The Average Percentage of Total ash of leaf= 6.3337%.
- ✓ The Average Percentage of Total ash of root = 2.2460%.
- ✓ The Average Percentage of Total ash of bark= 12.2486%.

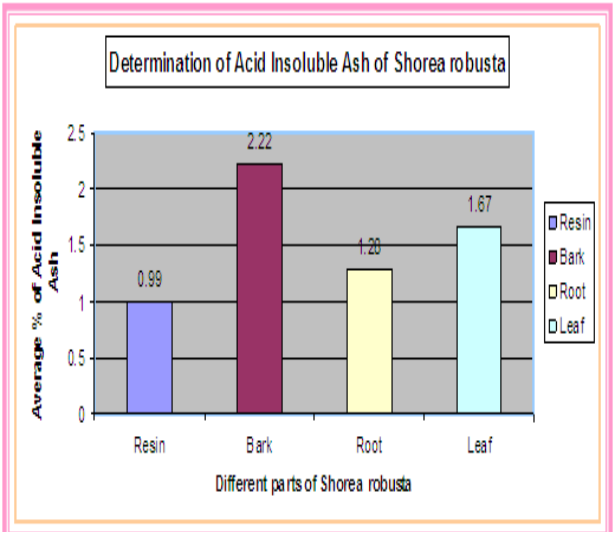
c. Determination Of Acid Insoluble Ash

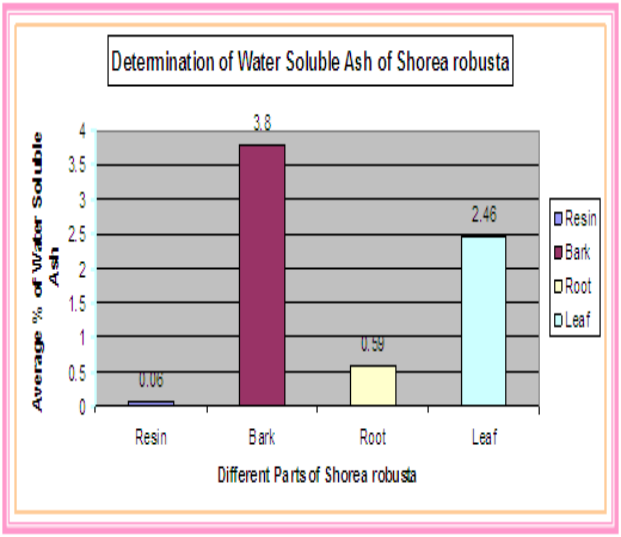
- ✓ The average percentage of acid insoluble ash of resin = 0.9820%
- ✓ The average percentage of acid insoluble ash of leaf = 1.6650%
- ✓ The average percentage of acid insoluble ash of root = 1.2720%
- ✓ The average percentage of acid insoluble ash of bark= 2.22%

d. Determination Of Water Soluble Ash

- ✓ The average percentage of water soluble ash of resin = 0.0580 %
- ✓ The average percentage of water soluble ash of leaf = 2.4660%
- ✓ The average percentage of water soluble ash of root = 0.5870%
- ✓ The average percentage of water soluble ash of bark = 3.886%







4. *Qualitative Examination of Organic matter*

Observation of Qualitative analysis of Organic matter

S. No	Organic Substances	Result			
		Resin	Leaf	Root	Bark
1	Carbohydrates	**	+	+	+
2	Starch	**	+	+	-
3	Tannin	**	+	+	-
4	Protein	**	+	+	-
5	Saponin	**	+	+	-
6	Phenol	**	+	+	+
7	Glycoside	-	+	+	+
8	Alkaloids	-	+	-	+

****Not soluble in water**

5.*Quantitative Examination for Organic matter*

a. Determination of Extractive values

Determination of Extractive values

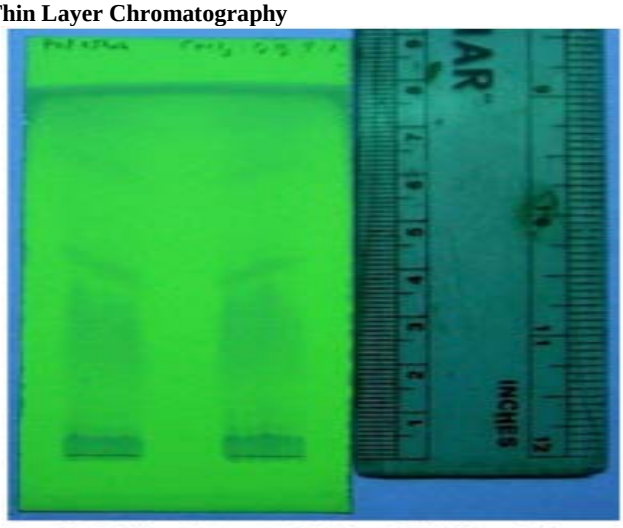
S. No	Solvent	Extractive values of			
		Leaf	Resin	Root	Bark
1	Hexane	1.40 %	-	0.68 %	.422 %
2	Chloroform	1.19 %	-	0.94 %	.801 %
3	Ethyl acetate	0.75 %	-	3.04 %	.767 %
4	Acetone	1.50 %		1.80 %	1.076 %
5	Methanol	7.11 %	75.95 %	5.42 %	1.047 %
6	Water	7.79 %	-	4.25 %	1.156 %
7	Ehanol	-	75.5 %	-	-
8	Toluene	-	66.48 %	-	-

(6) *Chromatography*

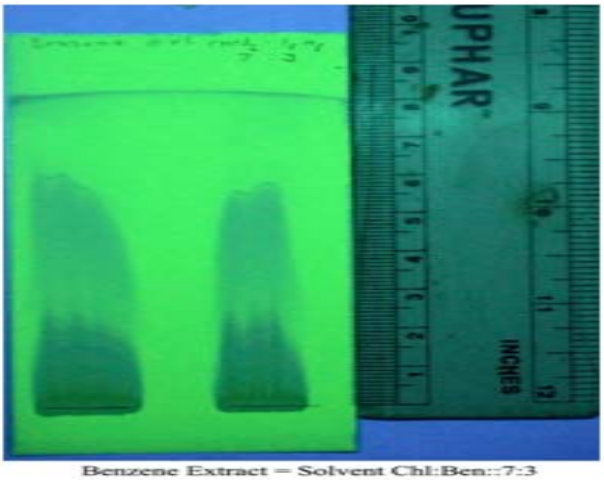
a. Thin Layer Chromatography (T.L.C.)

S. No	Extract of Resin	R_f Value
e	**Pet ether Extract** Mobile phase-Solvent Chloroform:Benzene::9:1 Visualization: Short Wave (254 nm) UV	0.08, 0.13, 0.16, 0.38, 0.51, 0.60 0.78, 0.84 0.92
f	**Pet ether Extract** Mobile phase-Solvent Chloroform:Benzene::9:1 Visualization:Vanilline+Sulphuric acid	0.08, 0.14, 0.38, 0.51, 0.57, 0.82 0.89, 0.95
g	**Benzene Extract** Mobile phase-Solvent Chloroform:Benzene::7:3 Visualization: Short Wave (254 nm) UV	0.13, 0.24, 0.27 0.73
h	**Toluene Extract** Mobile phase-Solvent Chloroform::100% Visualization: Short Wave (254 nm) UV	0.26, 0.41, 0.85
i	**Chloroform Extract** Mobile phase-Solvent Chloroform:Methanol::10:1drop Visualization: Short Wave (254 nm) UV	0.14, 0.21,0.25, 0.30, 0.37, 0.45 0.71
j	**Ethanol Extract-1** Mobile phase-Solvent Chloroform:Benzene::7:3 Visualization: Short Wave (254 nm) UV	0.16, 0.56
k	**Ethanol Extract-2** Mobile phase-Solvent Chloroform:Methanol::10:1drop Visualization: Short Wave (254 nm) UV	0.23, 0.46, 0.65 0.85
l	**Ethanol Extract-3** Mobile phase-Solvent Chloroform:Benzene::9:1 Visualization: Short Wave (254 nm) UV	0.16, 0.21, 0.65
m	**Methanol Extract-1** Mobile phase-Solvent Chloroform:Benzene::7:3 Visualization: Short Wave (254 nm) UV	0.13, 0.16, 0.58
n	**Methanol Extract-2** Mobile phase-Solvent Chloroform:Benzene::9:1 Visualization: Short Wave (254 nm) UV	0.16, 0.21, 0.67
o	**Methanol Extract-3** Mobilephase-Solvent Chloroform:Methanol::10:1drop Visualization: Short Wave (254 nm) UV	0.26, 0.51, 0.70 0.90

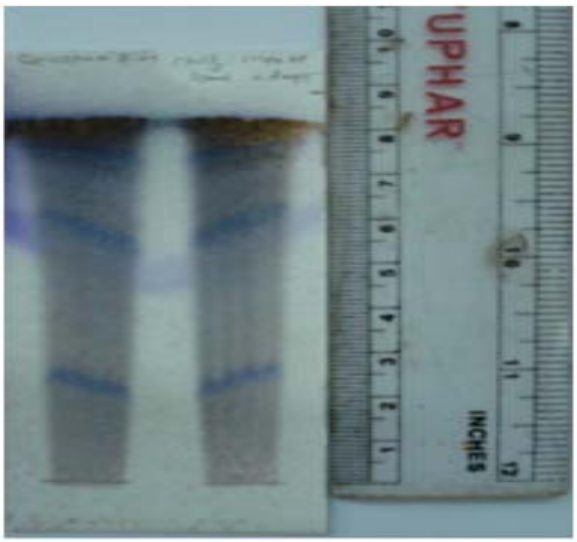
Thin Layer Chromatography



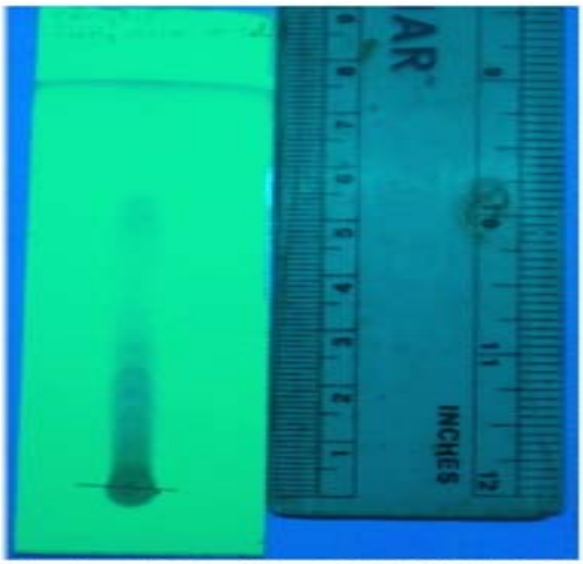
Pet Ether Extract – Solvent Chl:Ben::9:1
Visualization:: Short Wave (254 nm) UV



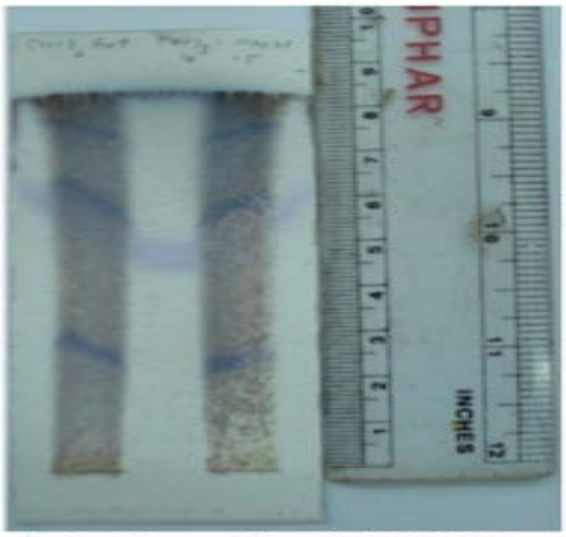
Benzene Extract – Solvent Chl:Ben::7:3
Visualization::Short Wave (254 nm) UV



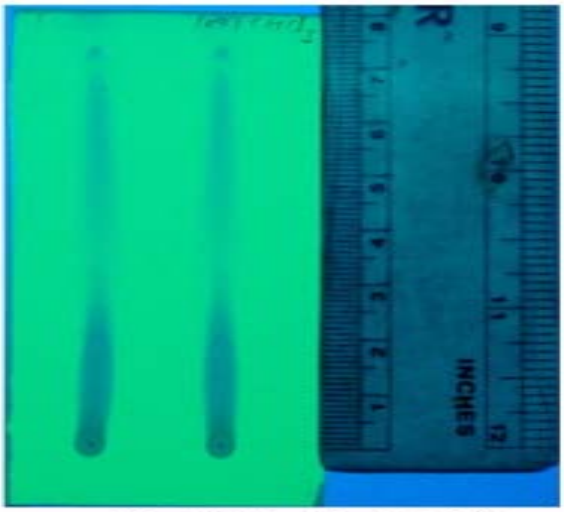
Benzene Extract = Solvent Chl:MeOH::10:2 Drops
Visualization::Vanilline + sulphuric Acid



Chloroform Extract = Solvent Chl:MeOH::10:1 Drop
Visualization::Short Wave (254 nm) UV



Chloroform Extract = Solvent Chl:MeOH::10:2 Drops
Visualization::Vanilline + Sulphuric Acid



Toluene Extract = Solvent Chl::100%
Visualization :: Short Wave (254 nm) UV

(7) Density

Density = Mass / Volume

- ✓ Density 1% resin = 1.00989 gm/cm^3
- ✓ Density 1% bark = 1.00942 gm/cm^3
- ✓ Density of 1% root = 1.0100 gm/cm^3
- ✓ Density of 1% leaf = 1.0104 gm/cm^3

(8) Determination of pH of different parts of *Shorea robusta*

- ◈ Resin = 2.84 (acidic)
- ◈ Bark = 7.40 (alkaline)
- ◈ Root = 5.36 (acidic)
- ◈ Leaf = 5.25 (acidic)

Conclusions

My study **"Phytochemical Analysis of *Shorea robusta*".** The following conclusion was drawn from this research work:

- ◈ After analysis of different Ayurvedic text about Shala, the word Shala, Sarja, Aswakarna and Ajkarna may be considered as different plants.
- ◈ As the drug having Kashaya rasa, Ruksha guna, Sheeta virya, Katu vipaka and it pacifies Pitta and Kapha, so prevent the formation and growth of Krimis.
- ◈ Phytochemical study of Shala concludes the following facts -

Inorganic matters like

- Iron is present in Resin.
- Calcium, Iron, **Phosphorus, Potassium, Sulphur** and Manganese are present in Leaf of Shala.
- Calcium, Iron and Manganese are present in Bark of Shala.
- Iron, **Phosphorus, Sulphur** and Manganese are present in Root of Shala.

Heavy metals like

- Silver is present in Leaf and Root.

Organic matter like

- Carbohydrates, Starch, Tannin, Protein, Saponin, Phenol, Glycoside and Alkaloids are present in Leaf of Shala.
- Carbohydrates, Starch, Tannin, Protein, Saponin, Phenol and Glycoside are present in Root of Shala
- Carbohydrates, Phenol, Glycoside and Alkaloids are present in Bark of Shala

Quantitative Analysis of Shala

- **Resin reveals that** Loss on Drying is 8.29 %, Total Ash value is 0.6108%., the average percentage of acid insoluble ash is 0.9820 % the average percentage of water soluble ash is 0.0580 % Density1% is 1.00989 gm/cm^3 and it has acidic pH.
- **Bark reveals that** Loss on Drying is 9.86 %. , Total Ash value is 12.2486%., the average percentage of acid insoluble ash is 2.22% the average percentage of water soluble ash is 3.886% Density1% is 1.00942 gm/cm^3 and it has alkaline pH.
- **Root reveals that** Loss on Drying is 6.72 %. , Total Ash value is 2.2460%., the average percentage of acid insoluble ash is 1.2720%
  The average percentage of water soluble ash is 0.5870% Density1% is 1.0100 gm/cm^3 and it has acidic pH.
- **Leaf reveals that** Loss on Drying is 7.40 %. , Total Ash value is 6.3337%., the average percentage of acid insoluble ash is 1.6650% the average percentage of water soluble ash is 2.4660% Density1% is 1.0104 gm/cm^3 and it has acidic pH

Extractive value of Shala in different solvents of different parts are as follows-

- In resin-Methanol 75.95% Ethanol 75.5%, Toluene 66.48%.
- In Leaf
  Hexane – 1.40 %, Chloroform – 1.19%, Ethyl acetate – 0.75%, Acetone – 1.50%, Methanol – 7.11%, Water – 7.79%,
- In Root
  Hexane – 0.68 %, Chloroform – 0.94%, Ethyl acetate – 3.04%, Acetone – 1.80%, Methanol – 5.42%, Water – 4.25%.
- In Bark
  Hexane – 0.422 %, Chloroform – 0.801%, Ethyl acetate – 0.767%, Acetone – 1.076%, Methanol – 1.047%, Water – 1.156%.

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