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PHARMACOGNISTIC AND PHYTOCHEMICAL STUDY OF THE STEM BARK OF A FOLK DRUG *VISHAGNA- Alstonia venenata* R. Br.

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ABSTRACT

Back ground and objective: With enormously diversified ethnic groups and rich biological resources, India represents one of the great emporia of ethno botanical wealth. These plants are under threat due to deforestation, overgrazing and their reckless utilization. It indicates the urgent need of their conservation. *Alstonia venenata* R. Br is a small tree belonging to Apocynaceae family. Its stem bark is used by tribes in fever, epilepsy and as anti-venom in snake bites. This study is to describe the pharmacognistic and phytochemical characters of the stem bark of Alstonia venenata R. Br. **Materials and methods**: The test drug was collected from the natural habitat. The pharmacognistic and phytochemical analysis of the stem of *Alstonia venenata* R. Br. Was done using the standard procedures. **Result:** Microscopic study of bark shows brownish coloured cork with sclerae's, and thickly packed cells. Cortex is present with starch grains and prismatic crystals. Secondary cortex shows presence of tannin. Phloem is seen next to a layer of pericycle. Physicochemical analysis shows loss on drying: 10.73 %, Values of total ash, acid insoluble ash and water soluble ash are 5.83%, 0.34% w/w, 30% w/w respectively. The value of water soluble extract and alcohol soluble extract of the drug is 31.6%w/v and 30%w/v respectively. Qualitative analysis shows presence of Alkaloids, Carbohydrate, Steroids, Tannin, Flavonoids, Phenols, Glycosides and Proteins.

KEYWORDS: ethno botanical, pharmacognistic, phytochemical, *Alstonia venenata* R. Br.

INTRODUCTION

Folklore and exotic plants yet not been stated in *Samhitas* (classics) or *Nighantus* (lexicons) are termed as *Anukta Dravyas*. Inclusion of new drug has been a tradition of Ayurveda. Recently it has been noticed that the manufacturers of Indian medicine and pharmaceutical companies are finding it extremely difficult to obtain raw materials, mainly due to destruction of the forest.

Vishagna- Alstonia venenata R. Br. is a large shrub or a small tree, with grayish brown bark grows in low to mid elevation deciduous forests of peninsular India[1]. People believe that the presence of this plant in the premises keeps snake away. It is in practice among Kurichyars tribes of Wayanad as anti-venomous, analgesic, anticonvulsant and anti-pyretic drug[2]. It is an ethno medicinal drug used among tribes extensively in the treatment of epilepsy, fever, poisoning etc. earliest reference of this drug is available in books[3]. As it is an *Anukta Dravya* we cannot find reference of drug in any of the *Samhita*s (classics) and *Nighantus (*lexicons). According to the book "Indian Medicinal Plants an Illustrated Dictionary" by Khare. C. P, Stem bark contains: alkaloids like alstovenine, venoxidine, 3- dehydroalstovenine, androalstonatine, reserpine, veneserpine, kopsinine, 5, 22- dioxokopsane[4].

If the ethno botanical information's are not processed in the laboratory clinically, any investigation on folklore medicine leading to new drugs remains incomplete. This study aims to provide the analytical data helpful for the standardization of the test drug by finding the physicochemical and preliminary phytochemical constituents and thereby helps to identify, authenticate and standardize the drug.

IDENTIFICATION AND COLLECTION OF DRUG

Vishagna- Vishagna- *Alstonia venenata* R. Br. during its flowering season was identified at the natural habitat from forest of Kollam district of Kerala authenticated referring features in local flora. Photograph from natural habitat was taken. Fresh stem bark of the plant was collected and the identity authenticated by Prof. Radhakrishna Rao, visiting professor of botany MIAMS. Fresh material was studied for pharmacognostic characters, as microscopic features are not expected in the dried samples. The collected stem bark was dried under shade and preserved in air tight container.

Detailed macroscopic and microscopic study was conducted as per standardized procedure. The study has been carried out in Muniyal Institute of Ayurveda Medical Sciences.

MACROSCOPIC STUDY

The plant parts were observed for macroscopic features by placing on a white paper surface. The organoleptic features such as size, shape, color, odour, of leaves flowers fruits were evaluated. The macroscopic features were compared to local flora for authentication.

MICROSCOPIC STUDY

Sample was preserved in fixative solution for 48 hours. The fixative used was FAA (Formalin- 5ml+ Acetic acid- 5ml+ 70% ethyl alcohol-90ml). Then thin transverse sections of the preserved bark were taken using sharp blade. These sections were stained using safranin. The slides were stained with iodine in potassium iodide for detection of starch. These sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axicam Camera under bright light field light. Magnification of the figures is indicated by the scale- bars.

Bark is the outermost layer of stem and root of woody plants. Bark refers to all the tissues outside the vascular cambium. It overlays the wood and consists of outer bark and inner bark. The inner bark on old plants is the living tissue, includes the innermost area of periderm. The outer bark includes the dead tissue, innermost part of periderm, tissue on the outer side of periderm. The outer bark is called rhytidome.

ORGANOLEPTIC STUDY

Material required- Petri dish, raw sample, powder of the sample.

Procedure

The organoleptic features of the stem bark of Vishagna- *Alstonia venenata* R. Br., like colour, odour, taste, appearance and sound of its fracture were recorded using sense organs.

PHYTOCHEMICAL STUDY

The authorization over effectiveness and safety of traditional medicinal plants often relies on long term medicinal use. However, the pharmacovigilance data of medicinal plants reveal need for continuous re-evaluation. Safety issues might be caused by minor compounds in drug, which are exerting side effects after long term, sometimes decades, of application. Phytochemical analysis of the drugs helps in solving this issue. The crude powder or drugs

extracted in different solvents are tested for various phytochemicals present in them by standard procedures. They are generally tested for the presence of alkaloids, flavonoids, tannins, phenols, glycosides

PREPARATION OF DRUG POWDER

The dried bark is coarsely powdered and sieved through sieve no. 180 as per WHO standards for medicinal plant material.

PHYSICO-CHEMICAL STUDY[5]

Tests such as Loss on drying, total ash, acid and water insoluble ash, aqueous and alcohol extractive values were conducted following the standard pharmacological procedures.

PRELIMINARY PHYTOCHEMICAL ANALYSIS[6]

1. Tests for Alkaloids

Alkaloids are class of naturally occurring organic compounds that contain basic nitrogen atoms. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke bitter taste. Most alkaloids are precipitated from neutral or slightly acidic solutions by following tests:

Dragendorff's test

Few drops of alcoholic extract of the drug taken in a test tube added with few drops of acetic acid and Dragendorff's reagent and shaken well to observe an orange red precipitate.

Wagner's test

To the drug extract dissolved in acetic acid few drops of Wagner's reagent is added to notice a reddish brown precipitate.

Mayer's test

To the drug extract dissolved in acetic acid few drops of Mayer's reagent is added to form a dull white precipitate.

Hager's test

To the drug extract dissolved in acetic acid 3ml of Hager's reagent is added and the formation of a yellow precipitate.

2. Test for Carbohydrate

Carbohydrates are the condensation products of polyhydroxy aldehydes or polyhydroxy ketones and their derivatives. They are soluble in water so from plant tissue, aqueous extract provides most of the sugars available in the specimen.

Molisch test

A few drop of sample solution is taken in a test tube to which few drops of ∝- naphthol solution is added and shaken. When H2SO4 is slowly poured a violet or purple ring is formed at the junction of the two liquids.

Fehling's test

In a test tube 1ml of Fehling's solution A and 1ml of Fehling's solution B are taken and mixed well and boiled for a minute. Then equal amount of sample solution is added and boiled for 5-10 minutes. Observed for formation of yellow color first then brick red.

Benedict's test

To a test tube containing about 3ml of sample solution, 2-3 ml of Benedict's reagent is added and boiled for 5 minutes. The solution turns to green, yellow and finally red depending on the amount of sugar present in the sample.

3. Test for Steroids

Steroids are biologically active organic compounds with four rings arranged in a specific molecular configuration. Plant steroids include steroidal alkaloids, cardiac glycosides, the phytosterols and brassinosteroids. Steroids and their metabolites function as signaling molecules and steroids and phospholipids are components of cell membrane. Steroids such as cholesterol decrease membrane fluidity.

Lieberman- burchard test

When the extract of the drug dissolved in chloroform is treated with 1ml each of acetic acid and acetic anhydride and then heated on water bath to which concentrated Sulfuric acid is poured along the sides of the test tube, bluish green color ring formed.

Salkowski test

When the chloroform solution of drug extract is treated with equal quantity of Concentrated Sulfuric acid, in the presence of steroid bluish red to cherry red color in the chloroform layer and green florescence in the acidic layer is formed.

4. Test for Tannin

Tannins are class of astringent, polyphenol biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. These resulting substances are insoluble and resistant to decomposition. Tannin stored in plant bark protects it from fungi or bacterial infections. Tannin can leach out of the plants and seeps in to ground water giving water a brown color.

Test

Few drops of diluted Ferric chloride solution added to the drug extract, if showed formation of dark blue color elicit the presence of tannin.

5. Test for Flavonoids

Flavonoids are plant compounds that are found in almost all fruits and vegetables. Flavonoids are a diverse group of phytonutrients which are powerful antioxidants with anti-inflammatory and immune system benefits. They play variety of functions in plant like regulating plant development, pigmentation and UV protection, to an array role in defense and signaling between plants and microorganisms.

Shinoda test

Pieces of magnesium ribbon and few drops of concentrated HCL were mixed with aqueous extract of the plant. Presence of red to pink color ensures presence of flavonoids.

6. Test for Phenol

Phenols are aromatic benzene ring compounds with one more hydroxyl groups produced by plants mainly for protection against stress. Phenols play important roles in plant development, particular in lignin and pigment biosynthesis. They also provide structural integrity and scaffolding support to plants.

Test

Mix several drops of drug extract in alcohol with alcoholic ferric chloride, formation of blue to bluish black color elicit presence of phenol.

7. Test for Glycosides

Glycoside is a molecule in which sugar is bound to another functional group via glycoside bond. Glycosides play numerous important functions in living things.

Keller-Kiliani test

A solution of 1ml glacial acetic acid with 1 drop of 2% $FeCl_3$ mixture was mixed with the 10ml of aqueous extract of the drug and 1 ml concentrated H_2SO_4. The bluish green ring formation between the layers showed entity of cardiac steroidal glycosides.

8. Test for Amino Acids

Amino acids are organic compound that contain amine and carboxyl functional group along with a side chain specific to each. They help with chlorophyll synthesis in plants and the increased chlorophyll leads to higher degree of photosynthesis.

Ninhydrin test

In the presence of amino acid, 0.1% w/v solution of ninhydrin in- butanol when added with extract of the drug gives a violet or purple appearance.

9. TEST FOR PROTEINS

Plant stores proteins in embryo and vegetative cells to provide carbon, nitrogen and sulfur resources for subsequent growth and development.

Biuret test

The residue in water is mixed with 1 ml of 4% sodium hydroxide solution, followed by a drop of 1% copper sulfate solution the presence of protein in it gives pink-purple color.

Xanthoproteic test

Residue is taken in 2ml of water. To which 0.5ml of concentrated nitric acid was added, development of yellow color indicates presence of protein.

10. Test for Saponins

5ml of distilled water was mixed with aqueous extract of drug in a test tube.

Add olive oil and shake. The frothing appearance showed presence of saponins.

11. Thin Layer Chromatography (TLC) [7]

TLC is a widely accepted tool for quantitative and qualitative evaluation of drug.

Procedure was conducted in R and D lab of MIAMS, Manipal.

Procedure

Silica gel G was used as adsorbent for preparing TLC plates. Plates of size were air dried and heated at 110ºc for 30 minutes. Extract of the drug was spotted using a TLC capillary tube and placed in a closed chamber and allowed to run until the solvent reached the top. Two different mobile phases used were Toluene: Ethyl acetate: Diethyl amine (7:2:1) and Chloroform: Methanol (7:1).

The solvent system developed where first observed under natural light and then under UV light and yellow spots were noted.

Later plates were sprayed with dragendorff's reagent for alkaloids. The R*f* value was calculated using the formula.

Rf = distance travelled by solute/distance travelled by solvent.

RESULTS OF PHARMACOGNISTIC STUDY

1. Macroscopic study

- Studying the morphology on comparison with the details of the drug given in the flora of presidency of Madras, following observations where made
- A small tree of 2.5 m height, bark is thin, greyish brown with white grains.
- Leaves are whorled, simple, 10-20 cm x1.2-3.8 cm, lanceolate, finely acuminate, petiole is short and slender
- Flowers are in cymes, sub-umbellate; petals white twisted 2.5 cm long, slender, top barrel-shaped, sepals- 1mm long and ovate,
- Fruits are fusiform follicles and beaked. Seeds are smooth, flattened, comose at both ends.

2. Microscopic study

Microscopic study of stem of Vishagna- *Alstonia venenata* R. Br. shows following parts of stem bark - Cambium, Cork, cortex, pericycle, phloem, pith, stone cells, starch grains, rosette crystals, sclerae's, calcium oxalate crystals, starch grains, vessels, xylem rays, xylem.

Bark shows:

- Cork, cambium, pericycle, cortex and phloem

- Cork brownish in colour, shows presence of sclerae's, and thickly packed cells
- Cortex is present with starch grains and prismatic crystals. Secondary cortex shows presence of tannin
- Phloem is seen next to a layer of pericycle.

3. Organoleptic features

Table: no 5 organoleptic characters of Vishagna- *Alstonia venenata* R. Br.

Sl.no	Parameters	Stem bark
1	Colour	Greyish brown outside and dark yellow inside
2	Odour	Peculiar smell
3	Taste	Bitter
4	Appearance	Woody
5	On fracture	Fibrous

4. Foreign matter

Percentage of foreign matter present in the sample was 0.8%w/w**.**

RESULT OF PHYSICO-CHEMICAL PARAMETERS

Table no 6: Result of physico-chemical parameters analysis of stem bark of Vishagna- *Alstonia venenata* R. Br.

Parameters	Observation
Loss on drying	10.73%
Total Ash value	5.838 % w/w
Water soluble ash	12.41%
Acid insoluble ash	0.34% w/w
Water soluble extract	30 %w/v
Alcohol soluble extract	31.6 % w/v
pH	5.31

RESULT OF PRELIMINARY PHYTOCHEMICAL ANALYSIS

Table no 7: Results of phytochemical analysis of stem bark of Vishagna- *Alstonia venenata* R. Br.

Parameter	Test	Cold infusion	Methanol	Petroleum ether	Chloroform
Alkaloids	Dragendroff's test	+ve	+ve	+ve	+ve
	Wagner's test	+ve	+ve	+ve	+ve
	Mayer's test	+ve	+ve	+ve	+ve
	Hager's test	-ve	-ve	-ve	-ve
Carbohydrate	Molisch's test	+ve	+ve	+ve	+ve
	Fehling's test	+ve	+ve	+ve	+ve
	Benedict's test	+ve	+ve	+ve	+ve
Steroids	Libermann-Burchard test	+ve	+ve	+ve	+ve
	Salkowski test	+ve	+ve	+ve	+ve
Tannin	Ferric chloride test	+ve	+ve	+ve	+ve
Flavonoids	Shinoda's test	-ve	-ve	-ve	-ve
Phenols	Ferric chloride	-ve	+ve	+ve	+ve
Glycosides	Keller-Kiliani test	+ve	+ve	+ve	+ve
Amino acids	Ninhydrin test	-ve	-ve	-ve	-ve
Proteins	Biuret test	+ve	+ve	+ve	+ve
	Xanthoproteic test	+ve	+ve	+ve	+ve
Saponins	Foam test	-ve	-ve	-ve	-ve

+ve -Positive, -ve –Negative

RESULT OF TLC

TLC was done to generate chromatographic profile of methanol extract of stem bark of Alstonia *venenata* R. Br.

Table no 8: Result of TLC report of methanol extract of stem bark of Vishagna- *Alstonia venenata* R. Br.

Rf value of sample in Toluene: Ethyl acetate: Diethyl amine (7: 2:1)		Rf of sample in Chloroform: Methanol (7:1)	
Short UV	**Long UV**	**Short UV**	**Long UV**
6.31 (Red)	6.31 (Red)	7.11(Red)	7.11(Red)
5.70 (purple)	5.70 (purple)	6.34 (purple)	-
1.42 (F.green)	-	5.72 (F. green)	5.72 (F. green)
0.54 (Blue)	-	3.64 (D. yellow)	3.64 (D. yellow)
-		2.27 (Blue)	2.27 (Blue)
-		1.81 (Blue)	1.81 (Blue)
-		1.42 (D. green)	1.42 (D. green)
-		0.55 (D. blue)	-

D- Dark, F- Fluorescence.

ANNEXURE

MORPHOLOGY OF Vishagna- *Alstonia venenata* R. Br.

Fig: 1 & 2 Alstonia venenata R. Br.

	
Fig:3 Stem	**Fig:4 Stem cutting**
	
Fig:5 Raw drug	**Fig:6 Bark**
	
Fig:7 Storage of course and fine powder	**Fig:8 Kashaya preparation**

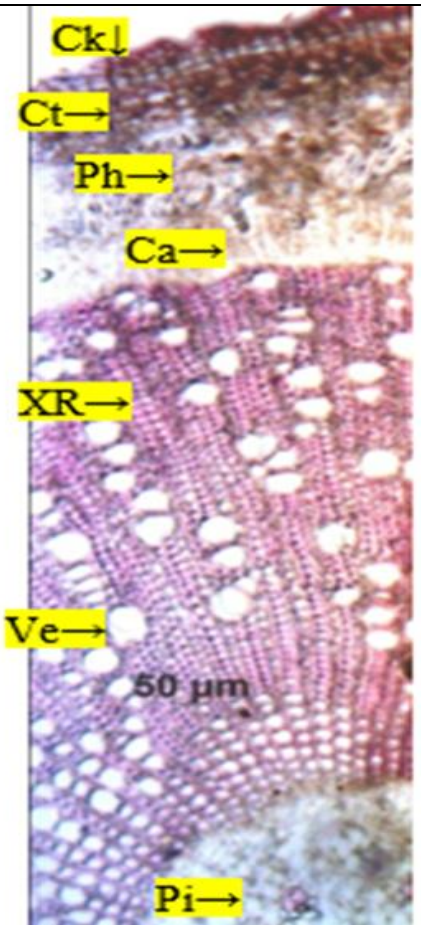


Fig 9 T.S of stem

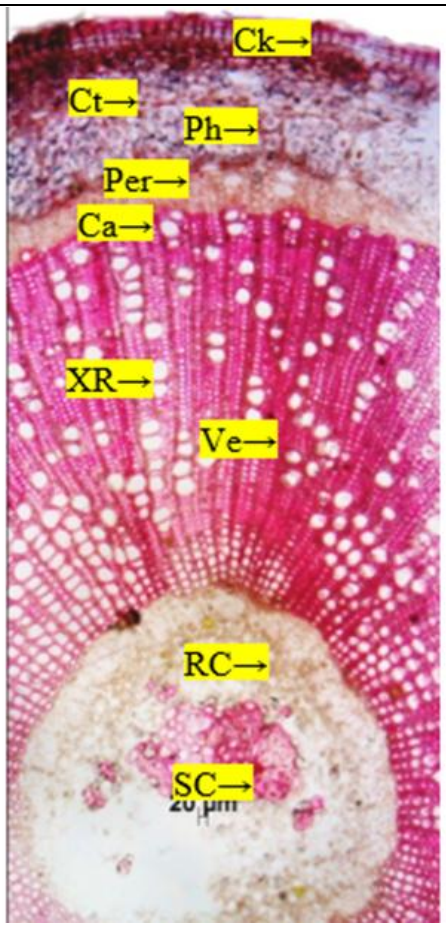


Fig: 10 T.S of stem (detailed

MICROSCOPIC VIEW OF STEM OF *Alstonia venenata*

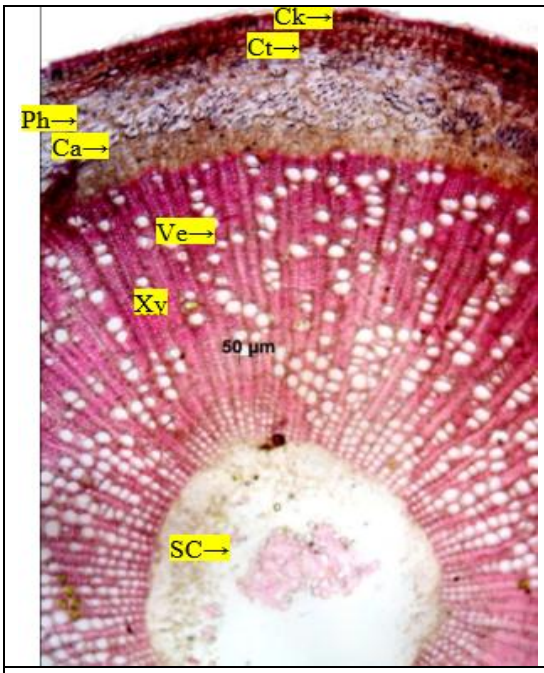


Fig 11 Cork, cortex and xylem

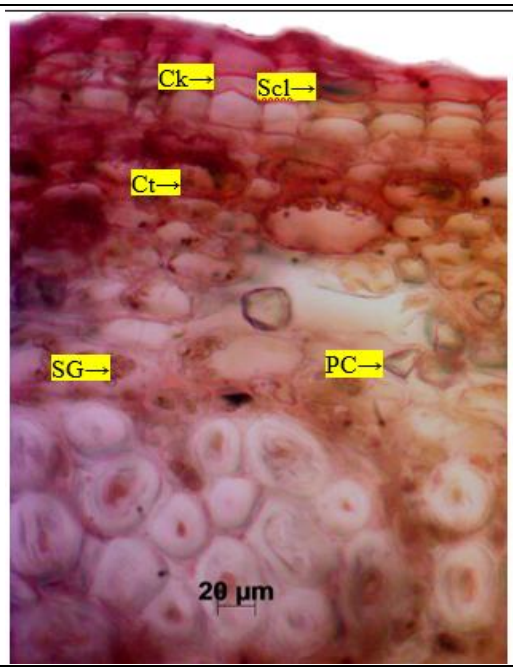
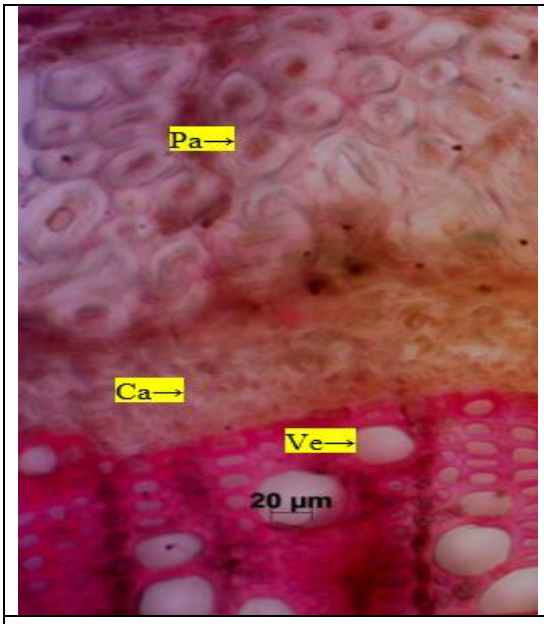
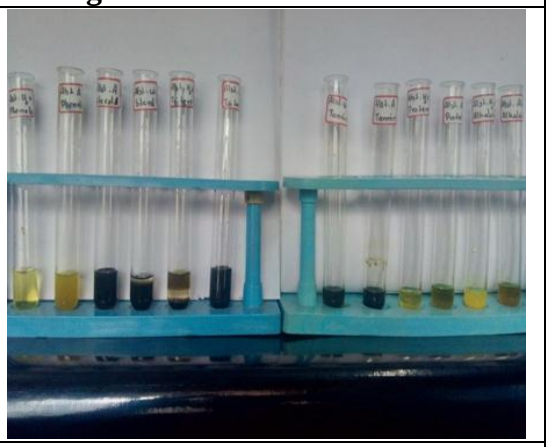


Fig: 12 Cork and cortex

	Ck – cork; Ct – cortex; Per – pericycle; Ph – phloem; Pi – pith; SC–stone cells; SG – starch grains; Ve – vessel; XR – xylem rays rays; XY – xylem,
FIG: 13 Pitted Cortical parenchyma, Cambium, Xylem	

TLC plates in Toluene: Ethyl acetate: Diethyl amine (7:2:1)

	
Fig: 14 Muffle Furnace –Ash value	**Fig: 15 water bath- extractives**
	
Fig: 16 Water and alcohol extractives	**Fig: 17 Qualitative analysis**

DISCUSSION

Macroscopic and Microscopic study

Samples of stem bark of Vishagna- *Alstonia venenata* R. Br. used were botanically authenticated by botanist. By performing macroscopic and microscopic study of the samples of stem bark of Vishagna- *Alstonia venenata* R. Br. were found to be genuine.

PHYTOCHEMICAL STUDY

- Physico-chemical analysis of Vishagna- *Alstonia venenata* R. Br. shows following result

Loss on drying: shows 10.73 %, it is designed to measure the amount of water and volatile matter in the sample.

- Ash value: indicate presence of inorganic materials in the sample. Values of total ash, acid insoluble ash and water soluble ash are 5.83%, 0.34% w/w, 30% w/w respectively.
- Extractive values: it plays an important role in determining the amount of active constituents present in the sample when extracted with solvent. The value of water soluble extract and alcohol soluble extract of the drug is 31.6%w/v and 30%w/v respectively.
- Qualitative analysis of Vishagna- *Alstonia venenata* R. Br. shows the presence of following phytoconstituents in different solvents like aqueous , alcohol, ether and petroleum: Alkaloids, Carbohydrate, Steroids, Tannin, Flavonoids, Phenols, Glycosides and Proteins.

Whereas saponins and amino acids were absent.

TLC

TLC was done to generate chromatographic profile of methanol extract of stem bark of *Alstonia venenata* R. Br.

- Many spots appeared on the plate of sample in Toluene: Ethyl acetate: Diethyl amine (7: 2:1). The spots were at Rf-6.31 (Red), Rf-5.70 (purple), Rf-1.42 (F. green), and Rf-0.54 (Blue).
- Spots present on the plate with sample in chloroform: methanol (7:1) at different Rf of 7.11(Red), 6.34 (purple), 5.72 (F. green), 3.64 (D. yellow), 2.27 (F. Blue), 1.81 (Blue), 1.42 (D. green), 0.55 (D. blue)

From the above data the spots may be representing different phytochemicals .Blue, blue green colours may shows presence of alkaloids, presence of green colour may shows

presence of tannins', formation of red colour may indicates glycosides, and Dark yellow, fluorescent blue or green colour may show presence of flavonoids.

CONCLUSION

Macroscopic and microscopic study, gives data regarding identification of the stem bark of Vishagna- *Alstonia venenata* R. Br.

Quantitative analysis shows percentage of foreign matter as 0.8%w/w, loss on drying: 10.73 %, Values of total ash, acid insoluble ash and water soluble ash are 5.83%, 0.34% w/w, 30% w/w respectively. The value of water soluble extract and alcohol soluble extract of the drug is 31.6%w/v and 30%w/v respectively.

Qualitative analysis shows presence of Alkaloids, Carbohydrate, Steroids, Tannin, Flavonoids, Phenols, Glycosides and Proteins.

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