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Research Article

# PHYSICO-CHEMICAL AND MICROSCOPIC STUDY OF *ARJUNA* (*TERMINALIA ARJUNA*) BARK

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**ABSTRACT**

In present study plant of *Arjuna* has been taken for physical and chemical analysis in terms of microtome of bark, powder study, loss on drying, ash values, extractive values, bulk density, Acid insoluble ash, Water-soluble Ash, Water-soluble extractive value, Alcohol-soluble extractive, pH range, TLC, Tapped density, Compressibility index, Hauser ratio, Angle of repose, Ultra violet fluorescence analysis of drug, etc. Physical and chemical analysis an important place in standardization of Ayurvedic drugs in order to make its global acceptability. The plant of *Arjuna* botanically named as *Terminalia arjuna* linn.*;* family Combretaceae, has traditionally been used to treat many diseases especially heart disease for centuries, that's why it is called as "Guardian of the heart". Transverse sections of Arjuna bark shows the calcium oxalate crystal, starch grains and lignified cells respectively shows that Xylem Vessels, Sclerenchymatous Fibers, Cork Cells, Tracheids, Sclereids, LOD value of the sample of Arjuna is 5.63%. According to result the Arjuna has three Rf vaule0.70, 0.42, 0.28 table1.4. Angle of repose of powder sample shows the flow of powder. The extractive value of *Arjuna* have different solvent like water, ethanol, isopropanol, acetone, chloroform, benzene, toluene, petroleum ether, hexsene are respectively 50.80, 41.07,30.37, 8.95, 0.96, 0.67, 0.52, 0.51, 0.46.

**KEYWORDS:** *Arjuna* bark, Ayurveda, Standardization (macroscopic and microscopic), loss of drying, ash values, Water-soluble extractive value.

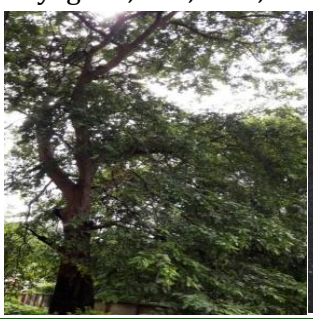
## INTRODUCTION

The name *Arjuna* for the tree occurs in the *Rig Veda* and *Atharva Veda* and means white or bright, probably denoting its creamy-white flowers or the shining quality of its bark. One of the tree's Sanskrit names is *Kakubha* which means beauty or fascination. It also means several flowers held together in a cluster. According to different Samhitas *Arjuna* is used in the treatment of different diseases like *Dant dhawan* (cleaning of teeth), *Medoroga* (obesity), *Kaphaja Prameha, Mutraghat* (urinary disorder), *Mukharog* (mouth disease), *Pittaja Prameha, Vataja* and *Kaphaja prameha*. Samhitas says that *Arjuna* different Ayurvedic formulations like *Lepa, Churna, Ghrita, Tail, Kwath, Asava, Aristha, Dhupa, Phanta* and *Modaka* etc are found.[1] In the corporative era there is an exponential growth in the field of herbal and Ayurvedic medicine in the last few decades. It is getting popularized in developing as well as in developed countries owing to its low cost natural origin and no side effects. In olden times, *Vaidyas* used to treat patients on individual basis, and prepare medicines according to the need of the patient. But the situation has changed now, Ayurvedic and herbal medicines are being manufactured on the large scale in Pharmaceutical units, where manufacturers come across many problems such as availability of good quality raw material, authentication of raw material, good standardization methodology of single drugs and formulations, quality control parameters.[2] In present time merchants for their personal benefit makes the adulteration. So the standardization of Ayurvedic drugs are very important.

## METHOD & MATERIAL

**1. Collection:** Sample *Arjuna* bark was collected from local area (Barkacha, Mirzapur).

**2. Identification** The collected sample was identified by Prof. A.K.Singh, Head of department of Dravyaguna, IMS, BHU, Varanasi.




***Terminalia arjuna*: A-tree, B-bark, C-Fruit, D-powder**

### 3. Microscopic Study

Transverse sections of *Arjuna* bark taken by using a microtome. Permanent mount of bark was prepared using saffranin fast green stain by double staining technique.[3] The morphological characters were reconfirmed by using various Floras of Gamble.[4,5,6] Images were obtained with a digital camera attached on a light microscope. For the study of crystals, starch grains and lignified cells, polarized light was employed. Magnifications of the figures were indicated by the scale-bars.

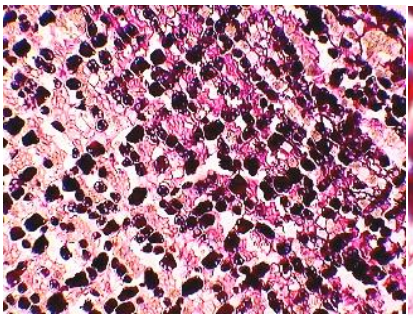


**Figure 1**

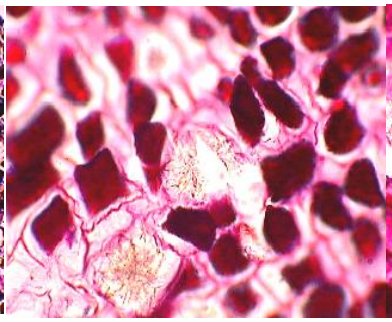


**Figure 2**

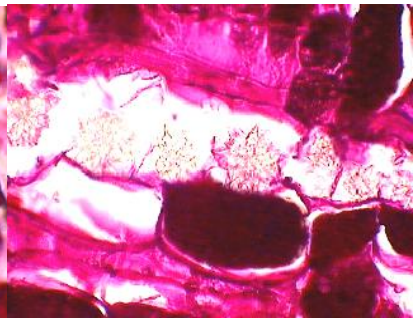


**Figure 3**

### 4. Powder Microscopy of *Arjuna* Bark

The bark powder of *Arjuna* was boiled separately with small volume of chloral hydrate solution. Cleaved powder was removed into three separate watch glasses, respectively and stained with one drop each of Reagent.

Phloroglucinol + Conc. HCl Pink colour Lignified cells are present.
Power + Ruthenium red pink colour mucilaginous cells are present in epidermis.
Powder + Sudan red III Pink colour Cuticle.
Powder +Dilute iodine solution +Conc. Sulphuric acid Blue colour Hemicellulose present.
Powder + Dil. iodine solution Blue colour Starch in endodermis is presents.

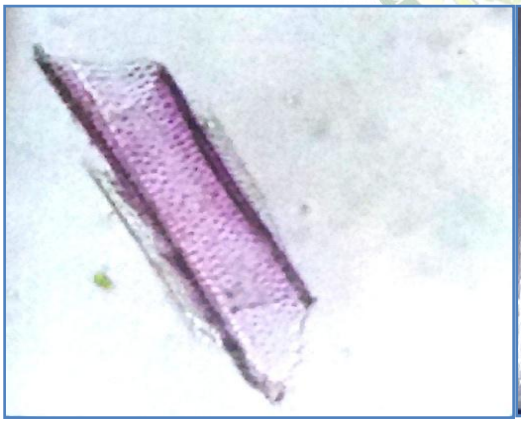


**Fig.3 Xylem Vessels**

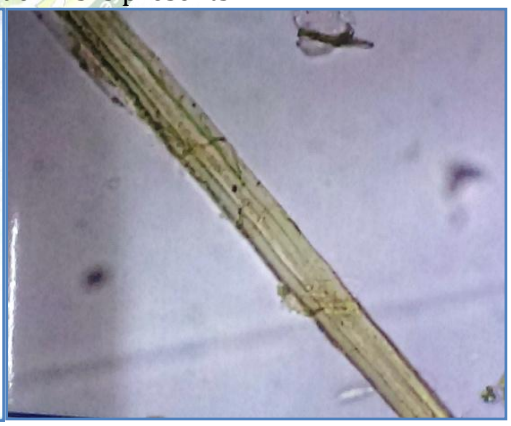


**Fig.4 Sclerenchymatous Fibers**

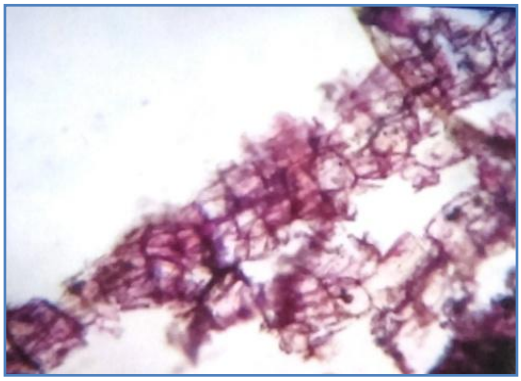


**Fig.5 Cork**

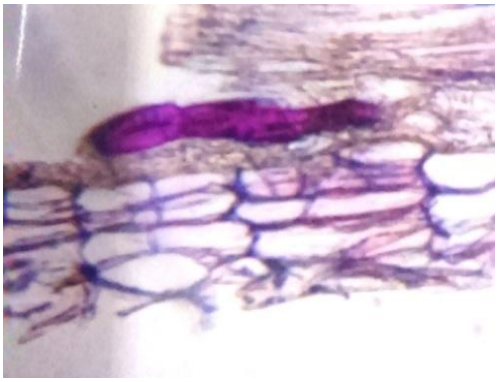


**Fig.6 Cork**

**Fig.7 Tracheids**

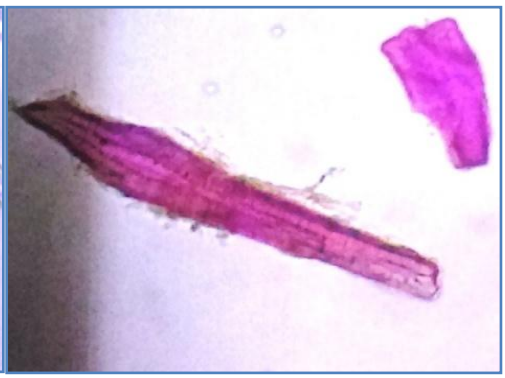


**Fig.8 Sclereids**

## 5. Physico-Chemical Analysis

### Determination of loss and drying

10gm of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish. It was dried at 105$^0$C for 5 hours and at 1 hour interval until difference between two successive weightings corresponded to a value not more than 0.25%.[7]

Calculation of LOD = $[W_2 – W_3 / W_2\text{-}W_1] \times 100$

$W_1$-wt of empty tray,$W_2$-wt of empty tray +sample,$W_3$–after drying wt of sample and tray

### Determination of Total ash

Unless otherwise stated in the individual monograph, weight accurately 2 to 3 g of the air-dried drug in a tarred platinum or silica dish and incinerate at a temperature not exceeding 450º until free from carbon, cool and weight. If a carbon-free ash is not obtained, wash the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper until the ash is white or nearly white, add the filtrate to the dish, evaporate to dryness and ignite at a temperature not exceeding 450º. Calculate the percentage of ash on the dried drug basis.[7]

Calculation of total ash = $[w_3\text{-}w_1/w_2\text{-}w_1] \times 100$

$W_1$-wt of empty crucible,$W_2$ – wt of sample + crucible, $W_3$ – weight after dry sample and crucible wt

### Determination of Acid insoluble ash

Boil the ash with 25 ml of 2M hydrochloric acid for 5 minutes, collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, ignite, cool in a desiccator and weigh. Calculate the percentage of acid-insoluble ash on the dried drug basis.[7]

Acid insoluble value = [residue wt/sample wt] x100

### Water-soluble Ash

Boil the ash for 5 minutes with 25 ml of water, collect the insoluble matter in a Gooch crucible or an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450$^0$. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash on the dried basis.[7]

### Determination of Water-soluble extractive

5gm of test sample was weighed and macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. 25ml of the filtrate was taken and evaporated to dry in a tarred flat bottomed shallow dish at 105 $^0$C, and weighed. The percentage of water soluble extractive value was calculated with reference to the air dried sample.[7]

### Determination of Alcohol-soluble extractive

Procedure for water soluble extractive was followed for the determination of alcohol soluble extractive value but 90% ethanol was used instead of chloroform water.

### Determination of Isopropenol-soluble extractive

Procedure for Isopropanol - soluble extractive was followed for the determination of

isopropanol soluble extractive value in 100% Isopropanol.

**Determination of Acetone-soluble extractive**

Procedure for Acetone - soluble extractive was followed for the determination of Acetone soluble extractive value in 100% acetone.

**Determination of chloroform-soluble extractive**

Procedure for chloroform - soluble extractive was followed for the determination of chloroform soluble extractive value in 100% chloroform.

**Determination of Benzene-soluble extractive**

Procedure for Benzene - soluble extractive was followed for the determination of benzene soluble extractive value in 100% Benzene.

**Determination of Toluene-soluble extractive**

Procedure for Toluene - soluble extractive was followed for the determination of toluene soluble extractive value in 100% toluene.

**Determination of hexane-soluble extractive**

Procedure for Hexane - soluble extractive was followed for the determination of hexane soluble extractive value in 100% hexane.

**Determination of pH range**

5gm of the powder sample of *Arjuna churna* was weighed and immersed in 100ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated pH meter.[7]

**6. Ultra Violet Flourescence Analysis of Drug**

Fluorescence analysis of the whole plant powder drugs was carried out according to the methods followed by Chase and Pratt. The fluorescence property of the powder is observed both in visible and ultra- violet for their fluorescence characters (short wave length 254 nm and long wave length 365 nm) after treatment with various chemical reagents.[8]

**7. Qualitative Phytochemical Screening [9]**

**8. TLC**

Identification of chemical can be detected by observation of spots of identical $R_f$ value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographer on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

**Preparation of plates**

Suspension of the coating substance was prepared in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, a uniform layer of the suspension, 0.25 to 0.30mm thick; on a flat glass plate 20cm long was spread. The coated plates were allowed to dry in air, heated at 100ºC to 105ºC for at least 1 hour and allowed cooling, protected from moisture. The plates were protected from moisture and used within 3 days of preparation. At the time of use, the plates were again dried, if necessary, the distance of each spot from the point of its application was measured & recorded the $R_f$ value were calculated by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.[9]

**9. Determination of Physical Characteristics of *Churna***

**Bulk density**

It is the ratio of given mass of powder and its bulk volume. It is determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of weight of the volume it occupied was calculated.

Bulk density $=W/V_0$ g/ml

Where, W = mass of the powder, $V_0$ = untapped volume

**Tapped density**

It is measured by transferring a known quantity (25gm) of powder into a graduated cylinder and tapping it for a specific number of times. The initial volume was noted. The graduated cylinder was tapped continuously for a period of 10-15 min. The density was determined as the ratio of mass of the powder to the tapped volume.

Tapped volume= $W/V_f$ g/ml

Where, W = mass of the powder, $V_f$ = tapped volume.

**Compressibility index**

It is the propensity of the powder to be compressed. Based on the apparent bulk density and tapped density the percentage compressibility of the powder was determined using the following formula.

Compressibility index = $[(V_0-V_f)/V_0] \times 100$,

% compressibility = [(tapped density – bulk density)] / tapped density] x 100

**Hausner ratio**

It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner ratio. It was calculated as following-

Hausner ratio= Tapped density/bulk density

**Angle of repose**

The internal angle between the surface of the pile of powder and the horizontal surface is known as the angle of repose. The powder is passed through funnel and distance between the funnel and table are fixed. The height and the radius of the pile were measured. Angle of repose of the powder was calculated using the formula.

Angle of repose= $\tan^{-1}(h/r)$

Where, H=height of the pile, r = radius of the pile

**RESULTS**

**Table 1: Scale of Flow Ability**

| S.No. | Flow Properties | Angle of Repose | Compressibility Index (%) | Hausner Ratio |
|---|---|---|---|---|
| 1. | Excellent | 25-30 | <10 | 1.00-1.11 |
| 2. | Good | 31-35 | 11-15 | 1.12-1.18 |
| 3. | Fair | 36-40 | 16-20 | 1.19-1.25 |
| 4. | Possible | 41-45 | 21-25 | 1.26-1.34 |
| 5. | Poor | 45-46 | 26-31 | 1.35-1.45 |
| 6. | Very Poor | 55-56 | 32-37 | 1.46-1.59 |
| 7. | Very very poor | >66 | >38 | >1.6 |

**Table 2: Physico-Chemical Analysis**

| S.No. | Parameter | Result |
|---|---|---|
| 1. | % Loss on drying | 5.63 % |
| 2. | Total Ash value | 20.35 % |
| 3. | Acid Insoluble Ash value | 2.94% |
| 4. | Water soluble Ash value | 14.70% |
| 7. | pH | 5.48 |

**Table 3: Extraction**

| S.No. | Solvent (%w/w) | Sample | % of extract | Hour |
|---|---|---|---|---|
| 1. | Water | *Arjuna* | 50.80 | 40 hrs |
| 2. | Ethanol | *Arjuna* | 41.07 | 40 hrs |
| 3. | Isopropanol | *Arjuna* | 30.37 | 40 hrs |
| 4. | Aceton | *Arjuna* | 8.95 | 40 hrs |
| 5. | Chloroform | *Arjuna* | 0.96 | 40 hrs |
| 6. | Benzene | *Arjuna* | 0.67 | 40 hrs |
| 7. | Toluene | *Arjuna* | 0.52 | 40 hrs |
| 8. | Petroleum ether | *Arjuna* | 0.51 | 40 hrs |
| 9. | Hexsen | *Arjuna* | 0.46 | 40 hrs |

**Table 4: Qualitative Phytochemical Screening**

| S.No. | Test Sample | Result |
|---|---|---|
| 1. | Test for Carbohydrates | + |
| 2. | Protine | + |
| 3. | Steroid | - |
| 4. | Flavonoids | - |
| 5. | Tannins & Phenolic compounds | + |
| 6. | Alkaloids | + |
| 7. | Glycosides | + |
| 8. | Cardiac glycosides | + |
| 9. | Saponin glycosides | + |

**Table 5: TLC**

| S.No. | Parameter | *Arjuna* Sample |
|---|---|---|
| 1. | Rf value | 0.70, 0.42, 0.28 |

**Table 6: Evaluation of *Churna***

| S.No. | Parameter | Result |
|---|---|---|
| **1.** | Bulk density | 0.38 |
| **2.** | Tap density | 0.46 |
| **3.** | Angle of repose | 46 |
| **4.** | Compressibility | 24 |
| **5.** | Hausner | 1.45 |

**Table 7: Ultraviolet Fluorescence Analysis of Drug**

| S.No | Reagent | Visible light | Long wave length | Short wave length |
|---|---|---|---|---|
| 1. | Dry powder | Reddish brown | Brown (+) | Brown (++) |
| 2. | Distilled water | Reddish brown | Dark green (++) | Light green (++) |
| 3. | Ammonia | Light brown | Dark green (+++) | Brownish green (+++) |
| 4. | Glacial acetic acid | Light brown | Light green (+) | Brownish green (+++) |
| 5. | Ethyl alcohol | Light brown | Light green (+) | Light green (++) |
| 6. | Diethyl ether | Light brown | Light green (+) | Dark green (++) |
| 7. | Acetone | light brown | Light green (++) | Brown (+++) |
| 8. | Sulphuric acid | Dark brown | Brownish green (+++) | Brown (+) |
| 9. | Hydrochloric acid | Light brown | Light green (+) | Dark green (++) |
| 10. | Ethyl acetate | Brown | Dark green (+++) | Brownish green(++) |
| 11. | Methanol | Light brown | Light green (+) | Light green (++) |
| 12. | Petroleum ether | Light brown | Light green (+) | Brown (++) |
| 13. | Petroleum spirit | Light brown | Light green (+) | Dark green (+) |
| 14 | Sodium hydroxide sol | Reddish brown | Yellowish green (++) | Dark green (++) |

+++ = High intensity
++ = Moderate intensity
+ = Low intensity

**DISCUSSIONS**

From the above method we found that fig.1, 2 and 3 shows the calcium oxalate crystal, starch grains and lignified cells, fig. 3,4,5,6,7,8 respectively shows that Xylem Vessels, Sclerenchymatous Fibers, Cork Cells, Tracheids, Sclereids, LOD value of the sample of *Arjuna* is 5.63% table1.1. This value show the moisture contained in the sample. The total ash value of the sample of *Arjuna* is found 20.35% table1.1. The total ash value represents the inorganic salt, naturally occurring in drug and deliberately added to it as a form of adulteration. Acid insoluble ash value is 2.94% found to be more for purified sample because it contains any siliceous material like sand, clay etc more than unpurified sample, due to addition of some siliceous materials and water soluble Ash value is 14.70% table1.1. Alcohol soluble extractive value indicates the active constituent of drug. According to results the *Arjuna* sample has pH 5.48 table 1.1. The Rf value of sample indicates the purity of the sample. When the Rf value is higher than compounds are pure. And when the Rf value is less then compound are impure. According to result the *Arjuna* has three Rf value 0.70, 0.42, 0.28 table 1.4. Angle of repose of powder sample shows the flow of powder. So according to results the flowing property of *Arjuna* sample was good (35). Compressibility and Hausner value of powder indicates the compactness of powder. So according to results *Arjuna* sample powder has the 24 possible flows table1.5. In the ultra violet flourescence analysis of *Arjuna* bark powder have different colure in different solvents table 1.6. The extractive value of *Arjuna* have different solvent like water, ethanol, isopropanol, acetone, chloroform, benzene, toluene, petroleum ether, hexsene are respectively 50.80, 41.07, 30.37, 8.95, 0.96, 0.67, 0.52, 0.51, 0.46 after 40 hrs in table 1.2. In the qualitative phytochemical screening water extractive sample shows the presence of carbohydrates, protein, tannins and phenolic compounds and glycosides are present table1.3.

## CONCLUSION

On the basis of the result the *Arjuna* bark powder have all the parameter within the limit and satisfactory.

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