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Research Article

# COMPARATIVE PHARMACOGNOSTIC PROFILES OF KERALA MARKET SAMPLES OF *TERMINALIA ARJUNA* ROXB.EX.DC (WIGHT & ARN) [ARJUNA], AN AYURVEDIC CARDIOPROTECTIVE DRUG

Pooja Ravindran *[1], P. Jayasree [2]
[1]PG Scholar, Department of Dravyaguna vijnana. Government Ayurveda College, Thiruvananthapuram, Kerala, India
[2]Professor (Retd.), Department of Dravyaguna vijnana, Government Ayurveda College, Thiruvananthapuram, Kerala, India
*Corresponding Author Email: drpoojaravindran@gmail.com

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**ABSTRACT**

The drug Arjuna in Ayurveda, identified as *Terminalia arjuna* Roxb.ex.DC (Wight &Arn) of Combretaceae family has gained much importance in the recent times as a potent cardio protective drug. The drug is given the near threatened status by the Foundation for Revitalisation of Local Health Traditions, Bengaluru, being included in the list of 178 prioritized species in high trade with annual trade of over 100 metric tonnes. The doubt of adulteration led to the present study to screen the drug *Terminalia arjuna* Roxb.ex.DC (Wight &Arn) by collecting the samples from the raw drug markets of Kerala using the tools of pharmacognosy.

**Key words**: Arjuna, *Terminalia arjuna* Roxb. ex. DC (Wight &Arn), standardization, Pharmacognosy, cardioprotective drug, Kerala market

## INTRODUCTION

The main source of raw materials to the Ayurveda drug industry is the forest resources which accounts for 90% and only 10% is cultivated. These are often harvested in a destructive and unsustainable manner[1]. Adulteration in market samples is one of the greatest drawbacks in the promotion of herbal drugs. It is invariably found that majority of adverse events related to the use of herbal products are usually not due to the intended herb but due to the presence of an unintended herb. Various adverse events may arise from the use of products contaminated with potentially hazardous substances, such as toxic metals, pathogenic microorganisms and agrochemical residues[2]. The bark is proved to be a potent anti-ischemic, cardio protective agent in hypertension and ischemic heart disease and possesses diuretic, prostaglandin enhancing and coronary risk factor modulating properties. The bark is also credited with hypocholestrolemic property[3]. It is one of the major ingredients of several Ayurveda formulations like Arjunarishta, Arjunaghrita, Lakshaguggulu, Kakubhadi Choorna[4]. Several active compounds are being isolated in recent times from this drug like Arjunahomo sesquiterpenol, Stigmasteryldigalactoside and Termiarjunosidel[5]. Due to over exploitation of the tree for both medicinal and economic purposes, it has been included in the priority list of 178 medicinal plant species in high trade i.e. species with annual trade of >100MT each, to focus attention on its management. It is also included in the list of 70 medicinal plant species in high trade from tropical forests. This drug is also included in the list of 85 medicinal plant species assigned with RL status of near threatened species of Kerala[6].

## MATERIALS AND METHODS

### Sample Collection

**Collection of Genuine Samples:** The genuine samples of Arjuna [*Terminalia arjuna* (Roxb.ex DC) Wight & Arn] bark were collected from the Botanic Garden of Government Ayurveda College, Poojappura, Thiruvananthapuram and was authenticated by botanist at Pharmacognosy Unit, Poojappura, Thiruvananthapuram. They were shade dried and packed in zip lock polythene bag and labeled as genuine sample.

**Collection of Market Samples:** For the purpose of the study, Kerala Market was divided into 3 zones viz. North Zone, Central Zone and South Zone.2 Market samples were collected from each of the 3 zones, randomly. Thus, total 6 market samples were collected for the study. They were separately packed in zip lock polythene bags and labeled as sample A and sample B for each zone.

### Sample Evaluation

**Pharmacognostical Evaluation:** As the second phase of the study, samples of Arjuna were evaluated for their pharmacognostical characters including powder microscopy. These characteristics were evaluated for both the genuine samples and the market samples and were compared to the standards of the genuine drug mentioned in Ayurvedic Pharmacopoeia of India (API).

### Macroscopic Evaluation

**Materials:** Magnifying lens, dissection microscope, and measuring scale were used for the purpose.
**Methods:** The samples were subjected to macroscopic evaluation by observation with naked eyes and by tactile and other sensory inspection. A magnifying lens with a dissecting

microscope was used for a better evaluation of surface characters. The characters of the genuine samples and the market samples were done separately and then these characters of the market samples were compared with those of the genuine one.

**Microscopic Evaluation**

Microscopic evaluation was done under two phases:

- Histological evaluation
- Powder microscopy

**A) Histological Evaluation**

**Materials:** Razor, Safranin stain, Glass slides, cover slips, Water, Glycerin and Compound microscope.

**Methods:** The genuine bark sample of Arjuna were cut into pieces of 10 – 15 cm in size and put in water taken in a petri dish. The fine transverse, of almost 100 sections, were taken and the best 5 sections were taken for the study of anatomical characteristics. These fine uniform sections were mounted in glycerin on glass slides, covered with cover slip and viewed under compound microscope. The specimens were viewed under both low power and high power magnification. Photographs of the sections were taken with the digital camera attached with the Digital Microscope (Olympus digital-CS41, Japan, with CCD camera with analysis software digital image-Pro) at Pharmacognosy lab, Drug Standardization Unit, Government Ayurveda College, Thiruvananthapuram. Ocular 2x, 4x, 10x and 40x objectives were used for all observations. The same procedure was repeated for all the market samples.

**B) Powder Microscopy**

**Materials:** Powdered drug, Glass slides, Cover slips, Glycerin, Digital microscope.

**Methods:** The genuine bark sample of Arjuna [*Terminalia arjuna* (Roxb. ex.DC) Wight &Arn] and the market samples were shade dried for one week, stored in separate air tight containers and labeled accordingly. They were powdered in a mini pulverizer, labeled and stored in separate zip lock packets. For examining characters of the powder, sufficient amount of powder of the samples were mounted on glass slide after mixing with glycerin. The slide was then examined under a compound microscope and the diagnostic features were recorded.

## RESULTS AND DISCUSSION

**Macroscopic Evaluation:** The macroscopic characters of market samples of Arjuna were evaluated. Then the results were compared with the characters of the bark of *Terminalia arjuna* Roxb. ex. DC (Wight &Arn) mentioned in Ayurvedic Pharmacopoeia of India (API) and also with the stem bark of *Terminalia arjuna* collected from the genuine source plant. Characters observed were as follows:

**Macroscopic Evaluation of Genuine Sample In Comparison with the Characters Mentioned in API**

**Table 1: Organoleptic Characters of Genuine Sample**

| Macroscopic features | API | Genuine Sample |
|---|---|---|
| Shape | Flat, curved, recurved, channeled to half - quilled | Flat,recurved pieces were obtained |
| Size | 0.2 – 1.5cm thick<br>10cm in length<br>7cm in width | 1.3-1.5cm thick<br>7-10cm in length<br>6-7cm in width |
| Texture | Outer surface: somewhat smooth<br>Inner surface: somewhat fibrous | Outer surface: smooth<br>Inner surface: fibrous |
| Color | Outer surface: grey<br>Inner surface: pinkish | Outer surface: whitish grey<br>Inner surface: pinkish; on drying turns to dark brown |
| Fracture | Inner: short<br>Outer: laminated | Inner: short<br>Outer: laminated |
| Taste | Bitter and astringent | Bitter and astringent |
| Odour | Odorless | Odorless |

Organoleptic characters of the genuine sample are comparable to that mentioned in API.

**Macroscopic Evaluation Of Market Samples:** Characters were assessed from the randomly picked pieces of samples from each zone. 10-15 replicates from each sample were assessed. The results are charted below:

**i) Organoleptic Evaluation Of Market Samples Of SOUTH ZONE**

**Table 2: Organoleptic Evaluation of Market Samples of South Zone**

| Macroscopic Features | Sample A | Sample B |
|---|---|---|
| Shape | Medium – sized rectangular to square curved pieces. | Large flat irregular pieces |
| Size | 1.5 – 1.7cm thick<br>6-8cm in length<br>6-7cm in width | 2-2.3cm thick |
| Texture | Outer surface: somewhat smooth<br>Inner surface: somewhat fibrous | Outer surface: smooth<br>Inner surface: longitudinally striated fibrous |
| Fracture | Outer: laminated<br>Inner: fibrous | Short fracture |
| Color | Outer surface: muddy grey<br>Inner surface: reddish brown | Outer surface: dusty light brown<br>Inner surface: dusty light brown |
| Taste | Bitter | Bitter |
| Odor | Odorless | Odorless |

**ii)Organoleptic Evaluation of Market Samples of central zone**

**Table 3: Organoleptic Evaluation of Market Samples of Central Zone**

| Macroscopic features | Sample A | Sample B |
|---|---|---|
| Size | 4 -8 cm long<br>2 – 5 cm width<br>0.3 – 0.5 cm thick | 4 -8 cm long<br>2 – 5 cm width<br>0.3 – 0.5 cm thick |
| Shape | Long recurved pieces | Long recurved pieces |
| Texture | Outer: rough with longitudinal striations<br>Inner: Smooth | Outer: rough with longitudinal striations<br>Inner: Smooth |
| Fracture | Outer and inner: laminated | Outer and inner: laminated |
| Color | Outer: creamish – white with blackish coatings<br>Inner: Blackish | Outer: creamish – white with blackish coatings<br>Inner: Blackish |
| Odor | Odorless | Odorless |
| Taste | Bitter | Bitter |

**iii) Organoleptic Evaluation of Market Samples of north zone**

**Table 4: Organoleptic Evaluation of Market Samples of North Zone**

| Macroscopic features | Urban sample | Rural sample |
|---|---|---|
| Size | 5 – 10 cm long<br>3 – 5 cm width<br>0.3 - .6 cm thick | 5 – 8 cm long<br>3 – 5 cm width<br>0.3 - .6 cm thick |
| Shape | Flat and curved rectangular long pieces | Flat rectangular long pieces |
| Texture | Outer: undulated<br>Inner: Smooth with longitudinal striations | Outer: Slightly rough with longitudinal ridges.<br>Inner: Smooth with longitudinal ridges. |
| Fracture | Outer and inner: laminated | Outer: short<br>Inner: Laminated |
| Color | Outer: creamish white with dark green to brown patches.<br>Inner: light brown to dark brown. | Outer: creamish – white<br>Inner: dark brown |
| Odor | Odorless | Odorless |
| Taste | Astringent | Astringent |

**Microscopic Evaluation:** The microscopic evaluation of the genuine bark sample of Arjuna[*Terminaliaarjuna* (Roxb. ex.DC) Wight &Arn] and all the market samples were carried out. The microscopic evaluation also included powder microscopy of the genuine and market samples. The results are charted below:

**Histological Evaluation of Genuine Sample**

The transverse section of the genuine bark sample of Arjuna[*Terminaliaarjuna* (Roxb. ex.DC) Wight &Arn] was taken and evaluated.

**Table 5: Histological Evaluation (TS) of Genuine Sample in Comparison with the Features Mentioned in API**

| Microscopic characters | API | Genuine stem bark |
|---|---|---|
| 1) Periderm (Cork) | | |
| i) Phellem | 8-10 layered thin-walled rectangular cells; consists of tannin | 6-10 layered tangentially elongated cells arranged in tiers.<br>Few crystals seen. |
| ii) Phellogen | Single layered with rectangular colorless cells | Single layered rectangular colorless cells |
| iii) Phelloderm | One to two seriate with rectangular colorless cells | 1-2 layered rectangular colorless cells |
| 2) Cortex: Consists of parenchymatous cells | | |
| i) Tannin containing cells | Present | Present |
| ii) Crystals | Rosette shaped Calcium oxalate crystals present | Rosette shaped Calcium oxalate crystals present |
| 3) Secondary phloem | | |
| i) Phloem fibres | Present as bands alternating with crystal containing cells | Present as bands alternating with crystal containing cells |
| ii) Crystals | Rosette shaped and clustered calcium oxalate crystals seen | Rosette shaped and clustered calcium oxalate crystals seen |
| iii) Medullary rays | 1-2 seriate traversing up to the cortical layer | 1-2 seriate present traversing up to the cortical layer |
| iv) Tanniferous cells | Present | Present |

**Histological Evaluation Of Market Samples**

The transverse sections of the market samples from the 3 zones of Kerala were evaluated. The results are as follows:

**i) Histological Evaluation Of South Zone Samples**

**Table 6: Histological Evaluation Of South Zone Samples**

| Microscopic characters | Sample A | Sample B |
|---|---|---|
| 1) Periderm | | |
| i) Phellem | A disrupted cork layer is seen. The three layers cannot be distinguished. 8-10 layers identified. A few rosette shaped calcium oxalate crystals seen. | Cork layer was not visible. |
| ii) Phellogen | | |
| iii) Phelloderm | | |
| 2) Cortex | Highly shrunken | Cortex region was not visible. |
| i) Tannin containing cells | Present | |
| ii) Crystals | Rosette shaped calcium oxalate crystals seen. | |
| 3) Secondary phloem | | |
| i) Phloem fibres | Seen as bands alternating cells containing calcium oxalate crystals. | Fibres were not seen as the whole region looks shrunken in 10x. Some phloem fibres seen in 40x. |
| ii) Crystals | Bands of cells containing rosette shaped calcium oxalate seen. Clustered crystals also seen. | Bands of rosette shaped calcium oxalate crystals were seen. Clustered crystals also seen. |
| iii) Medullary rays | Uniseriate medullary rays seen. | Were not seen. |
| iv) Tanniferous cells | Present. | Bands of tanniferous cells seen. |

- Several vacant spaces seen throughout the specimen of Sample B.

**ii) Histological Evaluation Of Central Zone Samples**

**Table 7: Histological Evaluation Of Central Zone Samples**

| Microscopic characters | Sample A | Sample A |
|---|---|---|
| 1) Periderm | | |
| i) Phellem | Disrupted cork layer seen. | Cork layer not visualized. |
| ii) Phellogen | | |
| iii) Phelloderm | | |
| 2) Cortex | | |
| i) Tannin containing cells | Absent | Cortical layer not visualized. |
| ii) Crystals | Absent | |
| 3) Secondary phloem | | |
| i) Phloem fibres | Not identified. | Bands of phloem fibres present. |
| ii) Crystals | Absent | Absent. |
| iii) Medullary rays | Multiseriate medullary rays seen. | Absent. |
| iv) Tanniferous cells | Absent. | Absent. |

**iii) Histological Evaluation Of North Zone Samples**

**Table 8: Histological Evaluation Of North Zone Samples**

| Microscopic characters | Sample A | Sample B |
|---|---|---|
| 1) Periderm | | |
| i) Phellem | 4-5 layers of longitudinally elongated cells with unknown inclusions present. | Thin cork layer identified. |
| ii) Phellogen | | |
| iii) Phelloderm | | |
| 2) Cortex | A separate cortical and secondary phloem region was not identified | A highly shrunken cortical region |
| i) Tannin containing cells | | Present |
| ii) Crystals | | Absent |
| 3) Secondary phloem | | |
| i) Phloem fibres | A separate cortical and secondary phloem region was not identified | Layers of phloem fibres present. |
| ii) Crystals | | Prismatic crystals present. |
| iii) Medullary rays | | Uniseriate medullary rays present. |
| iv) Tanniferous cells | | Present. |

**Powder Microscopy Of Stem Bark Of Genuine Arjuna [*Terminalia Arjuna* Roxb. Ex.DC (Wight &Arn)]:** The powder of stem bark of genuine Arjuna [*Terminalia arjuna* Roxb. ex. DC (Wight &Arn)] as well as the market samples were screened for the presence of rosette crystals, phloem fibres, cork fragment, tannins etc. Based on the similarity shown by the market samples, the features of representative samples are tabulated.

**Powder Microscopy Of Stem Bark Of Genuine Arjuna**

**Table 9: Powder Characters Of Stem Bark Of Genuine Arjuna**

| Powder characters | API | Genuine stem bark |
|---|---|---|
| i) Colour of powder | Reddish brown | Reddish brown |
| ii) Cork fragment | Present | Present |
| iii) Parenchyma with rosette crystals | Present | Present |
| iv) Phloem fibre | Present | Present |
| v) Longitudinally cut fragment of phloem fibre | Present | Present |
| vi) Rosette crystals | Present | Present |
| vii) Tannin | Present | Present |
| viii) Starch grain | Present | Present |

**Powder Microscopy Of Market Samples Of Arjuna**

Powder characters of all market samples were screened. Observations from the market samples are compiled and grouped into two categories as representative samples. Market sample I which matches with genuine sample and Market sample II, which differs from the genuine sample. The results are tabulated below:

**Table 10: Powder Characters Of Market Sample I And Market Sample II**

| Powder characters | Market Sample I | Market Sample II |
|---|---|---|
| i) Colour of powder | Reddish brown | Greyish |
| ii) Cork fragment | Present | Absent |
| iii) Parenchyma with rosette crystals | Present | Absent |
| iv) Phloem fibre | Present | Present |
| v) Longitudinally cut fragment of phloem fibre | Present | Present |
| vi)Calcium oxalate crystal | Rosette shaped | Prismatic |
| vii) Tannin | Present | Absent |
| viii) Starch grain | Present - few | Present - numerous |

**Fig 1: Genuine Stem Bark**

Outer surface Inner surface



**Fig 2: Market Samples from South Zone**



**Fig3: Market Samples from Central Zone**



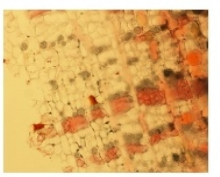
**Fig 4: Market Samples from North Zone**



**Fig 5: TS of Genuine stem bark**

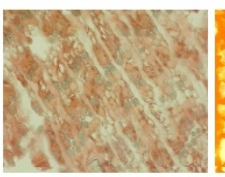


**TS of cork at 4x**



**TS of phloem region10x**

**Fig 6: TS of market samples from South zone**

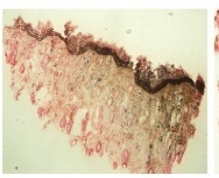


**Sample A 10x**



**Sample B 10x**

**Fig 7: TS of samples from Central zone**

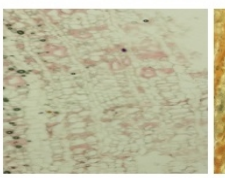


**Sample A 4x**

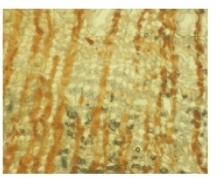


**Sample B 4x**

**Fig 8: TS of samples from North Zone**



**Sample A 10x**



**Sample B 10x**

## DISCUSSION

From the pharmacognostical analysis of the genuine and market samples collected, it is very evident that pharmacognostical profiles of the market samples differ from that of the genuine sample. But the market samples from the South zone somewhat matched with the genuine sample, but they were also infected with fungal infestations, which also amounts to adulteration. From microscopic evaluation, the genuine sample had the crystal containing cells and the phloem cells arranged in an alternate fashion. Moreover, they were rosette shaped calcium oxalate crystals. The market samples collected from the South zone also showed the same features. The market samples collected from the Central zone and Northern zone did not show this pattern. Also the oxalate crystals present in the North zone samples were prismatic in nature. No crystals were found in the Central zone samples and the medullary rays were multiseriated compared to the uniseriate to biseriate nature of the collected genuine sample. Further enquiry in the raw drug market, revealed that the raw drug sellers in the southern region of Kerala got their drugs from some markets of Central and Northern India. But that of Central and Northern markets were supplied from the local forest dwellers.

## CONCLUSION

There were variations in pharmacognostic profile of market samples of Arjuna from the genuine standard drug *Terminalia arjuna* Roxb.ex.DC (Wight &Arn), suggesting the adulteration of Arjuna stem bark in raw drug markets of Kerala with stem bark of some closely related and other species. Moreover such adulterated and low quality drugs available in commercial markets cannot be thought to bring about the desired pharmacological action.

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