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Research Article

COMPARATIVE PHARMACOGNOSTIC STUDIES OF ROOTS OF *ASHWAGANDHA* (WILD, NAGORI AND POSHITHA VAR)

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KEYWORDS: *Nagori Ashwagandha, Poshitha Ashwagandha, Wild Ashwagandha.*

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ABSTRACT

Background: *Ashwagandha* is a very well-known herb in Ayurveda. It is widely used in pharmaceutical, cosmetic, agricultural, food industries, and is a constituent of over 200 Ayurvedic formulations. The annual demand of *Ashwagandha* is 7000 tons but its actual availability is just 1500 tons, this demand is not met by using the wildly grown *Ashwagandha*. To meet this demand, it has been brought under cultivation many centuries ago, in India. Numerous cultivated varieties of *Ashwagandha* are developed for high yield and better quality roots. Amongst them, *Nagori Ashwagandha* is a popular variety, cultivated for its demand. *Poshitha Ashwagandha* is a high yielding and improved variety released by CSIR-CIMAPS. The current study aims at comparing the roots of wildly grown *Ashwagandha, Nagori Ashwagandha* and *Poshitha Ashwagandha*, pharmacognostically.

Methodology: The study deals with macroscopic parameters, microscopic study of transverse sections physicochemical, phytochemical analysis and powder microscopy of roots of the three varieties of *Ashwagandha*.

Results: Significant macroscopic differences were noted between the wild and the two cultivated varieties of *Ashwagandha roots*, there was no much variation in the physico-chemical and phyto-chemical parameters amongst the three, also there was no much difference found between the two cultivated varieties- morphologically and phyto-chemically.

INTRODUCTION

Since ancient times, *Ashwagandha* is being used indiscriminately from wild habitats. Deforestation and destructive harvesting has led to the endangerment of many wildly growing medicinal plants, including *Ashwagandha*.

Ashwagandha is a constituent of more than 200 Ayurvedic formulations. The annual demand of *Ashwagandha* is 7000 tons but its actual availability is 1500 tons.[1] Collection of *Ashwagandha* from wild is not able to suffice this demand. Hence, cultivation of *Ashwagandha* has been started at a large scale. The increase in demand of *Ashwagandha* has led to an increase in area under cultivation for higher production and good quality. The wide applicability of this plant and the high demand for its root, provide ample scope to cultivate this plant on a commercial scale. Cultivation is one of the methods of conservation of medicinal plants.

Varieties of *Ashwagandha* are not mentioned in any of the Classical texts or Nighantus. The commentator of Bhavaprakasha Nighantu, K.C Chunekar mentions two kinds of *Ashwagandha*: Wild and *Nagori Ashwagandha.*

Nagori variety is said to be *Balya, Brimhaniya, Rasayana* and *Vrishya.* Wild variety of *Ashwagandha* is told to be *Vatagna* and *Avasadaka*, it has *Swapnajanana* and *Mutrajanana* actions.[2]

One of the major differences between cultivated and wild type plants is that the former are annual, whereas the latter are perennial.[3]

The therapeutic benefits of *Ashwagandha* as described in classics pertain to *Ashwagandha* collected from wild.[4]

The cultivated variety of *Ashwagandha* which is thin and lean is mainly brought from Nagori district of Madhya Pradesh. Hence the name Nagori variety.[5]

Poshitha was released by CIMAPS for commercial cultivation. All cultivated varieties of *Ashwagandha* were bred through repeated self-pollination of selected plants from wild or from local cultivar from farmers.[6]

Cultivation usually aims at improving the quality and quantity of raw materials by selective control and improvement of certain factors of plant growth.[7] Irrespective of the source of *Ashwagandha*, it is being used in the Ayurveda pharmaceutical industries.

The change of factors of plant growth, geo-climatic conditions, and natural habitat varies from wild to cultivated plant. This can have an effect on morphological, physicochemical and phyto-constituents of the plant. Since *Ashwagandha* is obtained from various kinds of sources, there is a possibility of encountering changes amongst them.

Therefore it is essential to study the variations between *Ashwagandha* collected from wild and the cultivated varieties.

MATERIALS AND METHODS

Botanical Identification

Botanical Name: *Withania somnifera* (L) Dunal.

Family Name: Solanaceae

Collection and Identification of Plant Material

The Wild *Ashwagandha* roots were collected from Bagalkote district. It was washed properly, to remove all the soil particles and debris. It was chopped into pieces and shade dried.

Poshitha Ashwagandha roots were procured from a farmer cultivating it at Kurnool district of Andhra Pradesh (improved and high yielding variety by CIMAPS) it was in small pieces and in dried form.

Nagori Ashwagandha roots were procured from Kannauj, Uttar Pradesh. It was in small pieces and in dried form.

All the three varieties were botanically identified and authenticated (DGMP005, DGMP006, and DGMP007) as *Ashwagandha* by Dr.Shiva Manjunath. M.P, Botanist, Dept of Dravyaguna at Sri Sri College of Ayurvedic Science and Research.

The dried material were ground in a mixer grinder and sieved. The powder was stored in air sealed polythene bags at room temperature.

Macroscopic Evaluation[8]

The morphology of the roots was studied with the help of available literature. The macroscopic characters of *Withania somnifera* roots were observed for the following features- colour, texture, length and diameter of the roots. (Fig no. 1)

Microscopic evaluation[9]

For qualitative microscopic analysis transverse section of the three roots were made. Various identifying characteristics were studied and the pictures were taken in Digital camera microscope. (Fig No.3, 4, 5)

Powder microscopy of the root powder was also conducted according to standard procedure.

Physicochemical Evaluation [10]

Physico-chemical parameters such as Total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, and concentration of withanolides (HPLC), total alkaloids were determined according to standard procedures done for medicinal plants.

Phytochemical Evaluation[11]

The qualitative chemical tests carried out for the identification of the natural phyto-constituents present in the water and alcohol extract of the three powdered crude drugs. The tests were carried out using conventional protocols. Estimation of total alkaloids and HPLC for withanolides was done using standard protocol.

RESULTS AND DISCUSSION

Morphological features of roots of three varieties of *Ashwagandha*:

The following observations were made with respect to three varieties of *Ashwagandha* roots:

Table 1: length and diameter of the roots of 3 varieties

Root	Length	Diameter
Wild	30-50cm	5-8cms
Poshitha	5-10cm	0.5-1cm
Nagori	4-10cm	0.3-0.7 cm

Table 2: Comparative macroscopic and microscopic observations of roots of 3 varieties

Macroscopical	**Wild *Ashwagandha***	***Poshitha Ashwagandha***	***Nagori Ashwagandha***
External surface	Rough with many fissures	Smooth	Smooth
Nature of cork	Dark brown	Buff to Grey-yellow	Buff to Grey-yellow
Odour	Characteristic strong odour	Characteristic	Characteristic
Microscopical			
Cork	Exfoliated, 6-20 layers, tangentially elongated cells with calcium oxalate crystals	Exfoliated, 5-8 layered, oval-spherical cells, compressed	Exfoliated, 3-6 layers oval - spherical cells. Not much compressed
Cork cambium	Cambium layer and the cells are not distinct	Cambium layer and cells are not distinct	Cambium layer and cells are not distinct
Cortex	10-15 layers of thin walled rectangular parenchymal cells	10-15 layers of parenchymal cells with abundant starch grains	10-12 layers of spherical-oval parenchymal cells with abundant starch grains
Secondary cortex	Compact with a number of vascular tissue, interrupted with medullary rays	Not so compact with vascular tissue, interrupted with medullary rays	Not so compact with vascular tissue, interrupted with medullary rays
Medullary rays	Bi-serrate	Bi-serrate	Bi-serrate
Phloem	Crushed	Crushed	Crushed and not distinguishable
Starch cells	Simple, circular, not so abundant	Simple, circular to oval, abundant	Simple, Spherical to oval, abundant
Central xylem (pith)	Arranged compactly	Tetrarch	Triarch
Vessels	reticulate	Reticulate	Reticulate
Pigment cell	Not found	Found	Found

Table 3: Comparative characters of powder of 3 varieties: (fig no.2)

Characters	***Wild Ashwagandha***	***Poshitha Ashwagandha***	***Nagori Ashwagandha***
Colour	Light Brown	Greyish white	Greyish white
Odour	Characteristic sweet odour	Characteristic sweet odour	Characteristic sweet odour
Touch	Coarse	Soft	Soft
Taste	Bitter	Bitter	Bitter

Table 4: Physico-chemical analysis of 3 varieties

S. No.	Method adopted	Wild *Ashwagandha*	*Poshitha Ashwagandha*	*Nagori Ashwagandha*
1.	Total ash	4.915%	6.695%	7%
2.	Acid insoluble ash	0.87%	1%	1%
3.	Alcohol soluble extractive	18%	14.8%	22.4%
4.	Water soluble extract	8.8%	14.4%	24.8%
5.	Concentration of withanolides (HPLC)	0.006%	0.004%	0.005%
6.	Total alkaloids	0.018%	2.08%	0.48%

The above results of *Wild, Poshitha* and *Nagori Ashwagandha* were compared with Standards of The Ayurvedic pharmacopoeia of India and were found within normal limits.

Table 5: Analysis of organic constituents of 3 varieties *Ashwagandha churna*: Aqueous extract

S.No.	Phyto-constituents	Test	Wild *Ashwagandha*	*Poshitha Ashwagandha*	*Nagori Ashwagandha*
1	Alkaloids	Wagner's	-	+	-
2	saponins	Foam test	-	-	-
3	carbohydrates	Molisch's test	+	+	+
4	Starch	Iodine test	+	+	+
5	Non reducing sugar	Benedict's test	-	-	-
6	glycosides	Molisch's test	-	-	-
7	Steroids	Salkowski's test	+	-	-
8	proteins	Biurets test	-	-	-
9	Phenols	Ferric chloride test	+	-	-
10	Reducing sugar	Benedict's test	+	+	+
11	-	Anthocyanin test	+	+	+
12	Tannins		-	-	-

Table 6: Analysis of organic constituents of 3 varieties of *Ashwagandha churna*: Ethanolic extract

S. No.	Phyto-constituents	Test	Wild *Ashwagandha*	*Poshitha Ashwagandha*	*Nagori Ashwagandha*
1	Alkaloids	Wagner's	+	+	+
2	Saponins	Honeycomb test	-	-	-
3	carbohydrates	Molisch's test	+	+	+
4	Starch	Iodine test	+	+	+
5	Non reducing sugar	Benedicts test	-	-	-
6	glycosides	Molisch's test	+	+	+
7	Steroids	Salkowski's test	-	-	-
8	Proteins	Biurets test	-	-	-
9	Phenols	Ferric chloride test	-	-	-
10	-	Anthocyanin test	-	-	-
11	Reducing sugar	Benedict's test	+	+	+
12.	Tannins		-	-	-

DISCUSSION

One of the synonyms of *Ashwagandha* is *Vanaja*, which suggests that it grows abundantly in forests and that previously the source of *Ashwagandha* was from the wild variety.

Macroscopically Wild *Ashwagandha* roots were sturdy and more in length and diameter than the other two varieties. This could be because of the more fibre and starch content in the Wild *Ashwagandha* roots than the cultivated types. Microscopically the root cross-section of the varieties showed that the starch cells were more in the cultivated varieties than in the wild variety.

These differences may be because to the change in geographic and climatic conditions. The wild variety has to grow in atmosphere of repeated stress and strain, by variations in rainfall, the sunlight, pests attack, nutrient variation etc., whereas the cultivated are regularly taken care of, in a suitable steady environment for the plant.

To survive in an environment the plant may adapts itself by showing changes in morphology. In wild, the plants generally develop conservative responses to adapt and survive in the given environment. All these factors may have an influence on the morphological difference between wild and the cultivated varieties.

The cultivated plants are miniature of the wild types. Cultivated plants are either the wild plants grown in a controlled environment or developed through repeated self-pollination of selected plants from wild or from local cultivar from farmers. The *Ashwagandha* breeding efforts mainly focus on higher root yield with desirable secondary metabolites. The fundamental morphology remains the same in both of them.

During powdering the drug, it was difficult to powder the *Wild Ashwagandha* roots, the fibre content was more. The quantity of fine powder of wild variety got was less compared to *Nagori* and *Poshitha Ashwagandha.*

Phyto-chemical analysis

Study of Wild*, Nagori* and *Poshitha Ashwagandha* aqueous extract showed the presence of organic constituents like alkaloids in *Poshitha*, steroids in wild variety, starch, carbohydrates, and reducing sugars in all three drugs.

Presence of alkaloids, carbohydrates, starch, glycosides, reducing sugar in all the three varieties of *Ashwagandha* in its alcoholic extract.

Physico-chemical analysis of the three varieties

Withanolides were more in the wild variety than the other two varieties. Quantity of total alkaloids was more in *Poshitha* variety than the other two varieties, this could be because of the fact that *Poshitha* being an improved, high yielding and cultivated variety. The geographical area, soil, climatic conditions, might have had an impact on the quantity and quality of the phyto-constituents of the drug, though a major difference was not observed in the values.

The configuration of a drug depends on the *Mahabhautika* composition of a drug. The plants under cultivation and the plants growing in wild get different soil conditions (*Prithvi*), sunlight hours (*Agni*), water *(Jala*), climatic conditions (*Vayu*) which may foster change in the *Mahabhautika* composition and consequently alteration in phyto-constituents of the plants belonging to the same species.

Since cultivated fields and wild sources are not the same with respect to geo-climatic parameters and geo-climatic conditions play a role on the plant behaviour and its phyto-chemical profile it is logical to expect variation in phyto and physico-chemical constituents of medicinal plant materials collected from, wild and cultivated fields.

CONCLUSION

Morphologically, the Wild *Ashwagandha* is more in length and girth, it contains more fibres, and microscopically the cultivated varieties have more starch content. Phyto-constituents were found to be almost similar in all the three varieties.

Withanolides content was found to be more in Wild *Ashwagandha* and total alkaloids in *Poshitha Ashwagandha*. It can concluded that there are marked morphological differences between wild and cultivated varieties, though not much difference was found in phyto-chemical and physicochemical constituents. And no much difference was found between the two cultivated varieties (*Nagori* and *Poshitha*) morphologically and phyto-chemically.

Since *Ashwagandha* is at the verge of becoming endangered in the wild, cultivation is one of the best ways of conservation.

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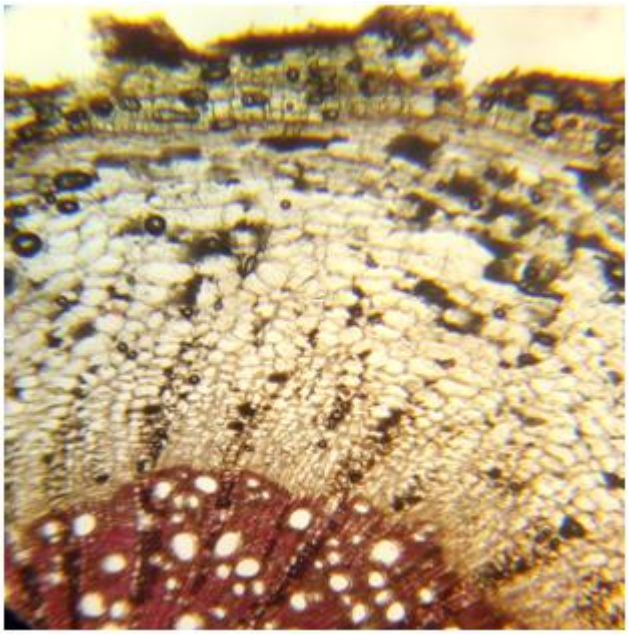
Fig no.1- *Ashwagandha* Roots- 3 Varieties

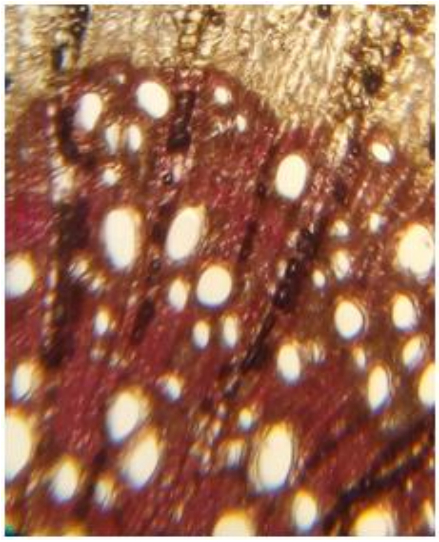
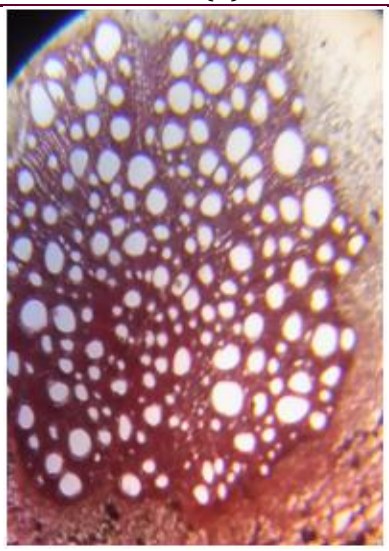
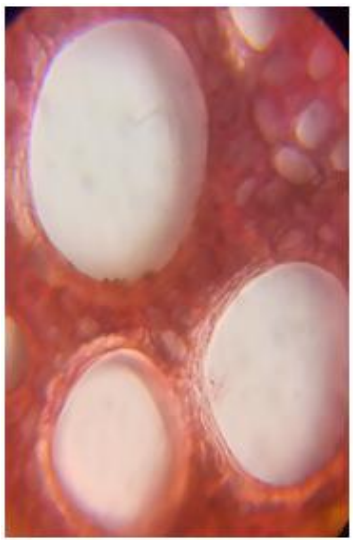
Fig no.2-*Ashwagandha Choorna* -3 Varieties

Fig no. 3- *Ashwagandha* (Wild) Root Transverse Section



Cross Section of Root

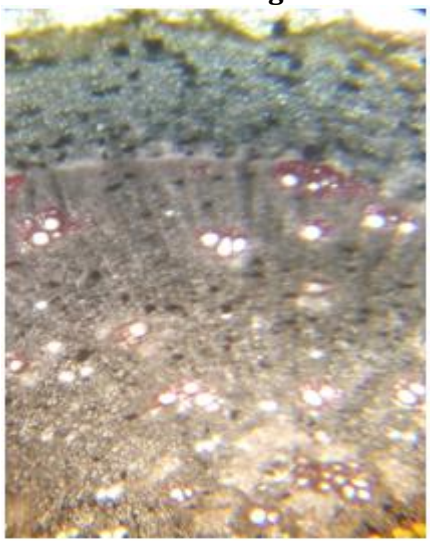
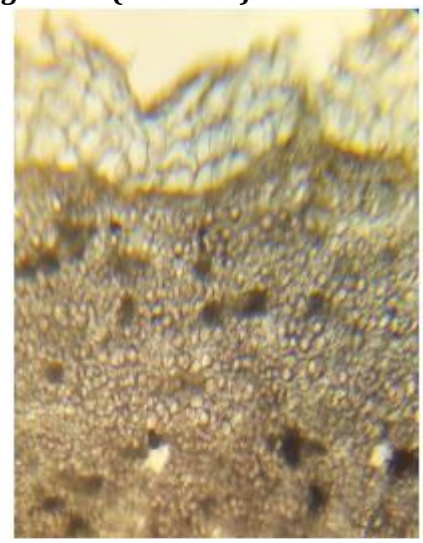
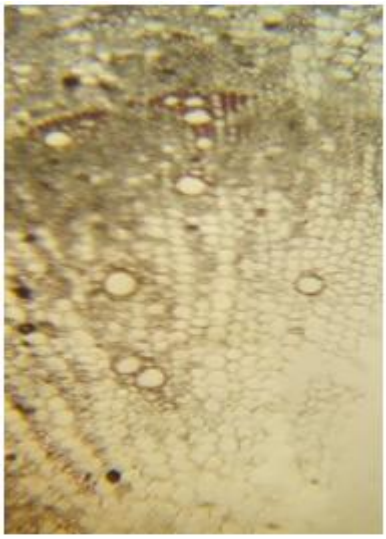
Cortex Portion Enlarged

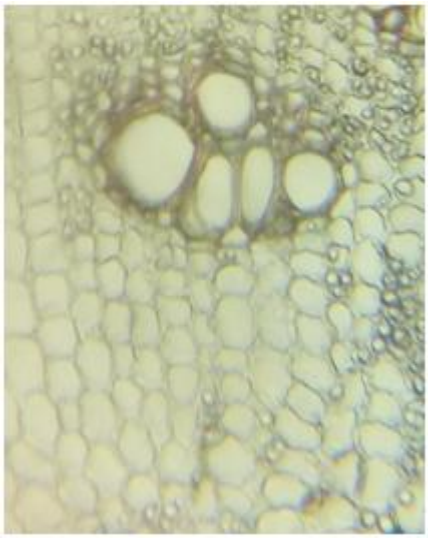
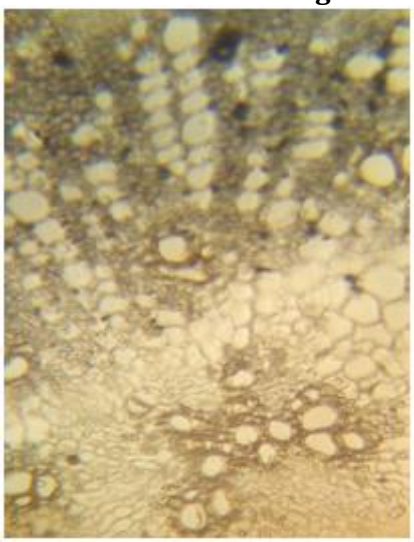
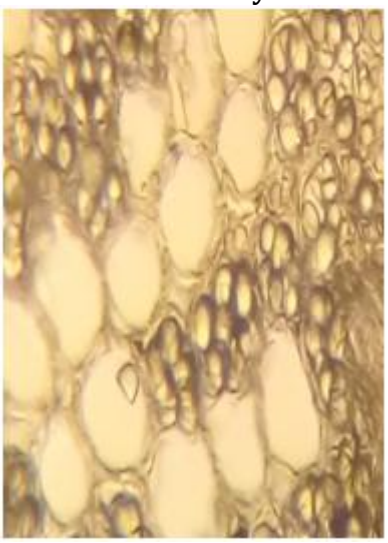
Portion of Secondary Growth **Pith** **Xylem**

a. Cork, b. Secondary cortex, c. Phloem, d. Xylem, e. Medullary rays, f. Secondary xylem, g. Metaxylem, h. Protoxylem, i. Pith, j. Secondary growth, k. Calcium oxalate crystals

Fig.no.4-*Ashwagandha* (*Poshitha*) Root Transverse Section

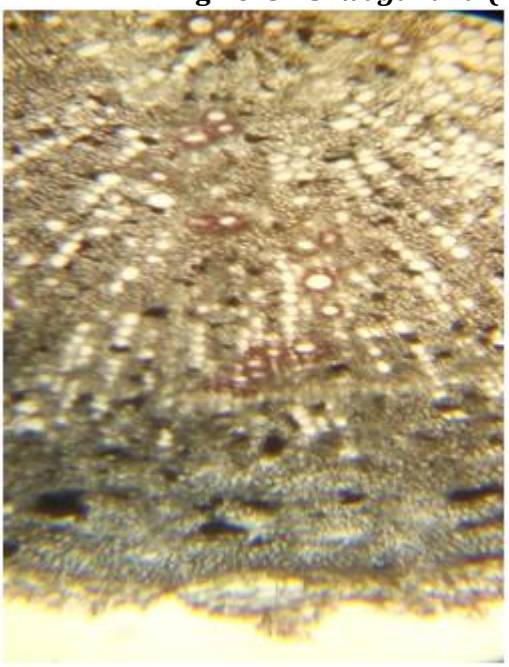
Cross Section of Root **Cortex Portion Enlarged** **Portion of Secondary Growth**

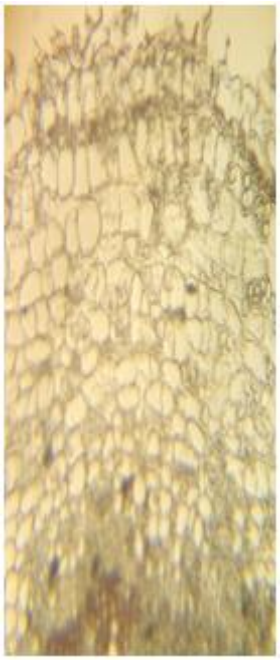
Vascular Bundle **Pith** **Starch**

a. Cork, b. Secondary cortex, c. Phloem, d. Xylem, e. Medullary rays, f. Secondary xylem, g. Metaxylem, h. Protoxylem, i. Pith, j. Secondary growth, k. Calcium oxalate crystals., l. Starch, m. Parenchymal cells

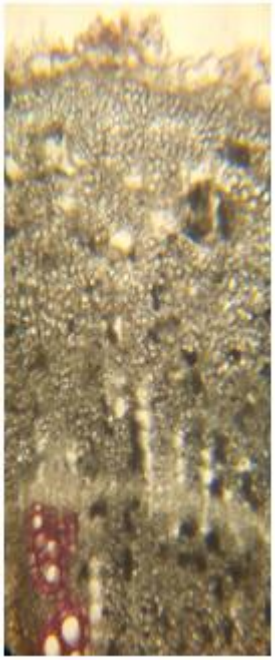
Fig no. 5-*Ashwagandha* (*Nagori*) Root Transverse Section



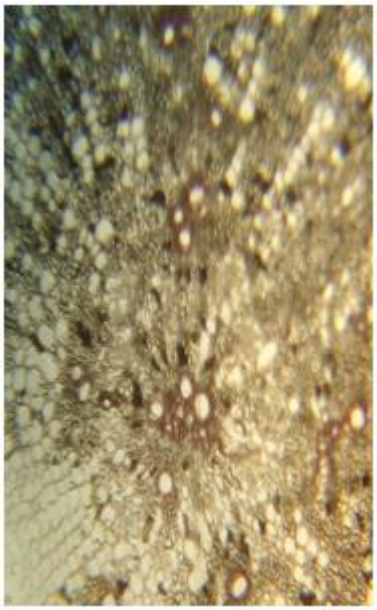
Cross Section of Root



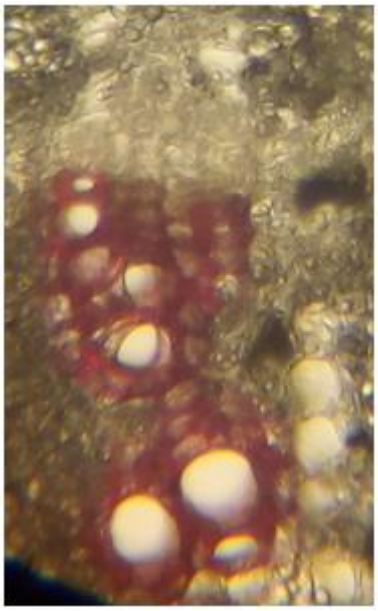
Cortex Portion



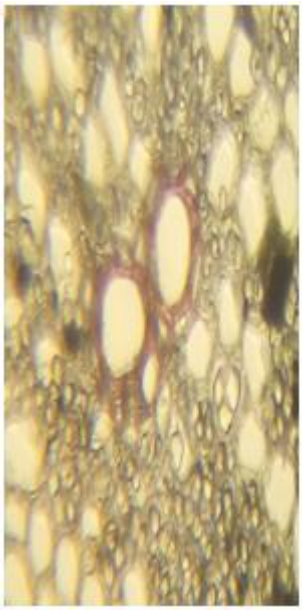
Enlarged Secondary Growth



Pith



Vascular Bundle



Starch

a. Cork, b. Secondary cortex, c. Phloem, d. Xylem, e. Medullary rays, f. Secondary xylem, g. Metaxylem, h. Protoxylem, i. Pith, j. Secondary growth, k. parenchymal cells. l. starch cells.