Ingredient Identification in 'Namak Ajeeb': A Quality Assurance Approach

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Abstract

Quality of herbal drugs is a burning issue now days. Quality is not something which can be achieved by a magic wand. It has to be built in from the concept till the end of manufacturing process by systematic and comprehensive studies. Ingredient identification in a herbal compound formulation is a pre-emptive attempt towards quality assurance that not only ensures the reproducible therapeutic efficacy but also ensures the safety. Present paper reports ingredient identification in Namak Ajeeb which is considered as Kasir-e-Riyah (carminative) and Hazim (digestive) in Unani System of Medicine. It is recommended in case of Waj-ul-meda (gastralgia), Qulanj (colic) and Waj-ul-Kulya (nephralgia). All the ingredients that are required in the preparation were examined separately (both macroscopically as well as microscopically) followed by the microscopic examination of the formulation as a whole. The study has provided key diagnostic histological characters which may serve as an important tool in laying down the standards for quality assurance of this important Unani drug.

Key words: Ingredient identification, Namak Ajeeb, Quality assurance

Introduction

Due to increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, there has been a renewed interest in the use of herbs and herbal drugs throughout the world. Because of this sweeping green wave a large number of herbal drugs and other plant derived herbal products are sold all over the world.

On account of consciousness for herbal products at global level, the quality of herbal drugs has become one of the issues of great concern for the scientists, professionals and drug enforcement authorities. Quality is not something which can be achieved by magic wand. It has to be built in from the concept till the end of manufacturing process by systematic and comprehensive studies. Ingredient identification in a herbal compound formulation is a pre-emptive attempt towards quality assurance, that not only ensures the reproducible therapeutic efficacy but also ensures the safety. Present paper reports ingredient identification in 'Namak Ajeeb' a Unani formulation which is considered as Kasir-e-Riyah (carminative) and Hazim (digestive) in Unani system of medicine and is recommended in case of Waj-ul-meda (gastralgia), Qulanj (colic) and Waj-ul-Kulya (nephralgia). Present

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studies are in continuation of several Unani drugs investigated earlier by the authors and published. All the ingredients that are required in the preparation were examined separately (both macroscopically as well as microscopically) followed by the microscopic examination of the formulation as a whole. This will provide a key of diagnostic histological characters which serves as an important tool in laying down the standards for quality assurance of the drug 'Namak Ajeeb' investigated in the present work.

Methodology

All the ingredients of the drug studied were procured from the local raw drug dealers, New Delhi. Each ingredient was authenticated (by examining both macroscopically and microscopically) and powdered separately. 'Namak Ajeeb' was prepared as per formulation composition given in NFUM part VI (Anonymous, 2011).

Formulation Composition:

S. No.	Ingredients	Scientific/English Name	Part used	Quantity
1.	Namak-e-Toam	Sodium Chloride	Crystal	8 kg.
2.	Naushadar	Ammonium chloride	Crystal	2.75 kg.
3.	Tukhm-e-karafs	*Apium graveolens* Linn.	Seed	500 g.
4.	Nankhwah	*Trachyspermum ammi* Linn.	Fruit	500 g.
5.	Filfil siyah	*Piper nigrum* Linn.	Berries	500 g.
6.	Zanjabeel	*Zingiber officinale* Rosc.	Rhizome	500 g.
7.	Zeera siyah	*Carum carvi* Linn.	Fruit	500 g.
8.	Taj Qalmi	*Cinnamomum cassia* Blume	Stem bark	250 g.
9.	Jaiphal	*Myristica fragrans* Houtt.	Seed	250 g.
10.	Jawitri	*Myristica fragrans* Houtt.	Aril	250 g.
11.	Sirka Naishakar	Vinegar	Liquid	6 lit.

Further, a pinch of Namak Ajeeb was taken on a slide and mounted in different reagents viz. (Safranin, Iodine, Ferric Chloride). The cells/ tissues/ cell contents etc. were examined under a microscope according to the methods laid down by Johansen (1940) and Trease and Evans (1983). The resulting photographs were taken from the microscope with computer attachment.

Observations

Ingredients:

1. Tukhm-e-Karafs (*Apium graveolens* Linn.)

   Part used : Seed

   Macroscopy : Dried Seed/Fruit, mostly separated mericarps, cremocarp brown, ovoid, laterally compressed, approx. 1.0 – 1.5 mm. in length, 1.5 mm. in thickness. Each mericarp has five straight, prominent ridges; odour and taste aromatic. (Nadkarni, 1986 ; Kirtikar and Basu, 1988).

   Microscopy : Sectional view of fruit shows:

   Epicarp - Single layered, rectangular, thin walled parenchyma cells covered by irregular cuticle. Mesocarp - Several layered, moderately thick walled parenchyma cells, polygonal-oval, sclereids ovoid – elongated, thick walled, innermost layer of mesocarp consists of large, elongated parenchyma cells. Endocarp - Single layered, square to rectangular shaped, thin walled parenchyma cells. Testa - Single layered, thin walled, elongated rectangular cells. Endosperm - Several layered, rectangular – polygonal, thick walled parenchyma cells filled with oval to round aleurone grains and microspheroidal crystals of calcium oxalate.

2. Nankhwah (*Trachyspermum ammi* Linn.)

   Part used : Fruit

   Macroscopy : Dried fruit consists of two mericarps, grayish brown, ovoid, compressed, length – approx. 2mm. width – 1mm. with pale coloured protuberances, each mericarp consists of 5 ridges and 6 vittae, odour : characteristic, thymolic, taste : pungent.

   Microscopy : T. S. of fruit shows :

   Epicarp - Single layered, tangentially elongated tubular cells covered by thick cuticle, unicellular trichomes present. Mesocarp - Several layered, consists of moderately thick walled, rectangular – polygonal tangentiallyl elongated cells having vascular bundles and vittae. Testa - Single layered, thin walled, tangentially elongated cells. Endosperm - Thin walled parenchyma cells filled with aleurone grains and oil globules.

3. Filfil siyah (*Piper nigrum* Linn.)

Part used: Berries

Macroscopy: Fruits globular, hard, dark brown to black, 3-5 mm. in diameter with a characteristic coat of deep set wrinkles; odour aromatic, taste pungent. (Nadkarni, 1986; Kirtikar and Basu, 1988).

Microscopy: T.S. of fruit shows:

Epicarp-Single layered epidermis covered by cuticle; epidermal cells polygonal (tabular) containing dark brown- blackish content followed by 2- 3 layers of thin walled parenchyma cells intermingled with thick walled isodiametric to radially elongated lignified stone cells. Mesocarp - Broad zone of tangentially elongated parenchyma cells having larger secretion sacs with suberised walls and oil or resin contents. Cells in the inner mesocarpic region are compressed having few fibro vascular bundles. Endocarp - Single row of beaker shaped stone cells (cells whose radial and inner walls of cells were more strongly lignified than the outer ones). Testa - single layer of yellow coloured cells. Perisperm - Broad zone of thin walled, radially elongated parenchyma cells filled with abundant starch grains, aleurone grains, oleoresin cells containing oil globules and masses of resin.

4. Zanjabeel (*Zingiber officinale* Rosc.)

Part used: Rhizome

Macroscopy: Rhizome irregularly branched (sympodial), laterally compressed, different sizes, externally pale yellowish-buff, longitudinally striate, ends of branches with depressed stem scars, fracture short, mealy, uneven with projecting fibres, odour agreeably aromatic with characteristic pungent taste.

Microscopy : A cross section of rhizome shows:

Phellem or outer cork : Few layered, dark brown, irregular parenchyma cells. Phellogen or inner cork: Few layered, colourless parenchyma cells, radiallly arranged in regular rows. Phelloderm or cortex : Several layered, thin walled, round- polygonal, parenchyma cells with intercellular spaces containing abundant starch grains which are mostly simple, fairly large, flattened, oblong or sub-rectangular to oval or sac shaped with terminal beak like projection in which eccentric hilum is situated. Numerous oleo-resin cells and vascular bundles present. Endodermis : Single layered

with radial walls thickened, starch grains absent. (Stele broad central zone, thin walled, round to polygonal, parenchyma cells with intercellular spaces same as cortex) just inside the endodermis i.e. to the periphery of the ground tissue a ring or narrow zone of vascular bundle present. Larger, closed, collateral, fibrovascular bundles were observed irregularly scattered throughout the remainder of the stele.

5. Zeera siyah (*Carum carvi* Linn.)

Part used : Fruit

Macroscopy : Dried fruit, greenish brown, slightly curved, elongated mericarp, odour and taste aromatic and characteristic.

Microscopy : The fruit has typical structure with six vittae and five primary ribs in each mericarp, small schizogenous secretion canal present in each rib just above the vascular bundle.

T. S. of fruit shows:

Pericarp : Single layered epidermis, tabular cells covered by cuticle. Mesocarp – Several layered parenchyma cells without reticulate thickenings.

Endocarp : Elongated sub rectangular cells arranged parallel to each other.

Endosperm : Thick walled celllulosic parenchyma cells containing fixed oil and aleurone grains upto 10µ in diameter; small rosette crystals of calcium oxalate present.

6. Taj Qalmi (*Cinnamomum cassia* Blume)

Part used : Stem bark

Macroscopy : Pieces of bark, length – approx. 5-40 cm., width – approx. 1-2 cm. channeled, colour – dark earthy brown, smooth, inner surface light brown, fracture short, granular in outer part and fibrous in inner part; odour – delicate, fragrant, aromatic and mucilaginous; taste – astringent. (Nadkarni, 1986; Kirtikar and Basu, 1988).

Microscopy : T. S. of bark shows phellem consisting of few layers of cork cells, polygonal – tubular cells arranged in alternating layers of thick and thin walled cells with reddish-brown contents; phellogen and phelloderm not distinguished; cortex several layered, parenchymatous with abundant

oval – round, simple, starch grains. 20µ in diameter, scattered sclereids with more lignified and pitted tangential and lateral walls present in this region, pericycle fibre embedded among stone cells. Secondary phloem consists of parenchymatous cells with starch grains and acicular crystals; medullary rays 1 -3 celled, narrow on inner side and wider towards periphery.

7. Jaiphal (*Myristica fragrans* Houtt)

Part used : Seed

Macroscopy : Seed ellipsoid, length – 20-30 mm, width – approx. 20mm., greenish-brown, marked with small irregular dark brown patches and lines reticulately furrowed, a groove running along the perisperm with infoldings appearing as dark ruminations in the endosperm; odour – strong and aromatic; taste – pungent and aromatic.

Microscopy : T. S. of endosperm shows several layers of peripheral perisperm, flattened polyhedral cells containing prismatic crystals, inner layers of perisperm consists of thin walled parenchyma cells infolding into the tissue of endosperm to form ruminations containing a vascular strand and numerous large oil cells; endosperm parenchypmatous with occasional tannin, idioblasts and abundant starch grains that are simple or compound, round, approx 20µ in diameter. Aleurone grains small and irregular but each cell contains one large grain with a well developed crystalloid.

8. Jawitri (*Myristica fragrans* Houtt)

Part used : Aril

Macroscopy : Reddish pieces , approx. 2-4 cm. in size, flat, smooth, irregularly slit, slightly flexible or brittle, rich in oil, when pressed exudes reddish or orange coloured oily substance; odour strong with agreeable taste.

Microscopy : The cross section of aril shows single layered epidermis on either side; simple thick walled cells without intercellular space in between, oil cavities present in abundance.

Test Sample (Formulation)

Microscopic examination of ‘Namak Ajeeb’ shows following components of diagnostic characteristics:-

Epicarp : Fragment of epicarp in surface view showing striated cuticle.

Parenchyma cells : Parenchyma cells of different size and shape, some in the form of groups of parenchyma cells densely packed with polyhedral masses of numerous starch grains, parenchyma cells of the endosperm slightly thick walled, tightly packed and filled with aleurone grains and oil globules, few parenchyma cells thin walled, either single or in groups having scattered starch grains.

Starch grains: Abundant starch grains, present either scattered or within the parenchyma cells, mostly simple, fairly large, flattened, oblong to oval shaped with a pointed hilum situated at the narrower end.

Sclereids: Abundant, various size and shape, either single or in groups, few sclereids irregularly shaped, moderately thick walled with numerous well marked pits, few were small oval – rectangular shaped, thick walled showing striations and wide lumen.

Fibre: Pieces of fibres of various sizes few thick walled lignified with uneven lumen.

Vittae: Fragment vittae showing polygonal thin walled cells having slight thickness at the corners.

Endocarp cells: Elongated cells of the endocarp with their long axis parallel to one another.

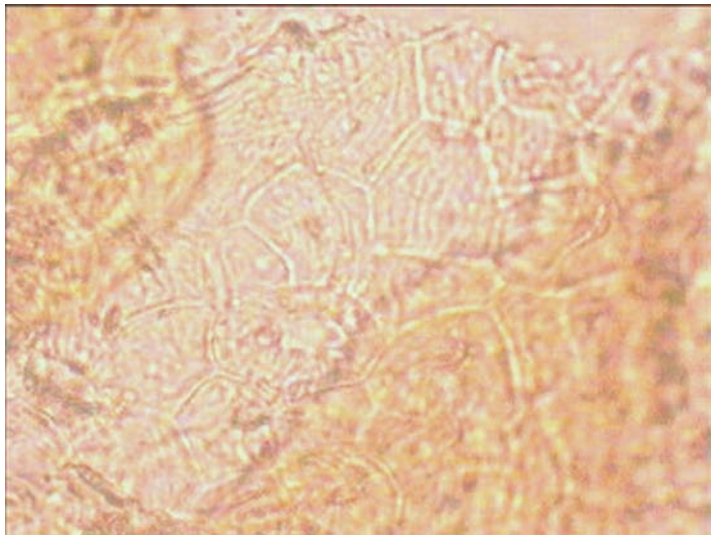

Fig 1. X100 Fragment of epicarp of 'Tukhm-e-Karafs'

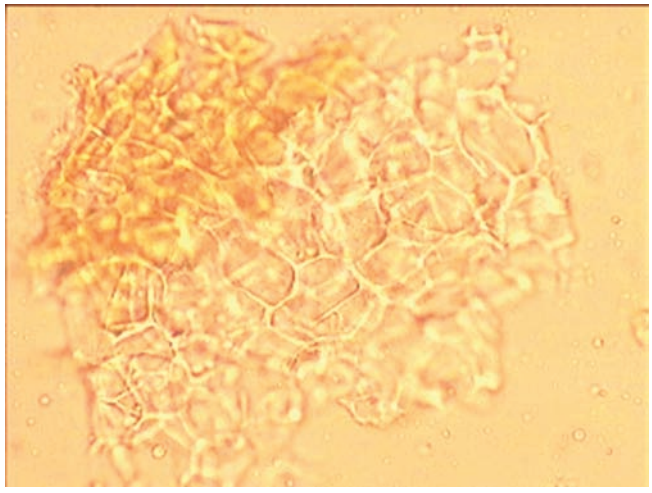

Fig 2. X40 Fragment of vittae of ‘Tukhm-e- Karafs’

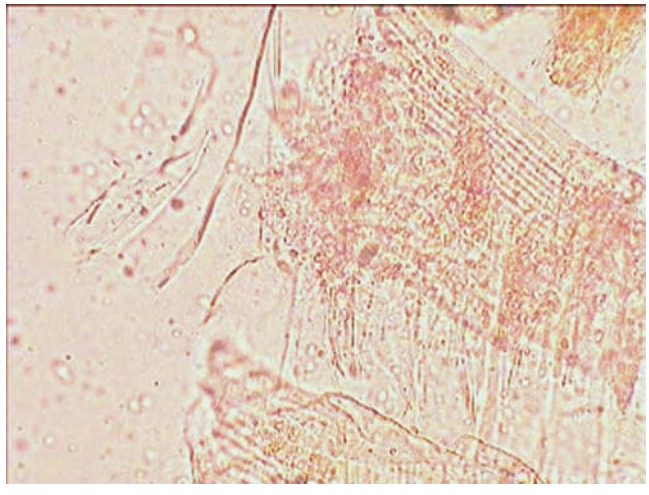

Fig 3. X40 Fragment of endocarp of ‘Tukhm-e-Karafs’

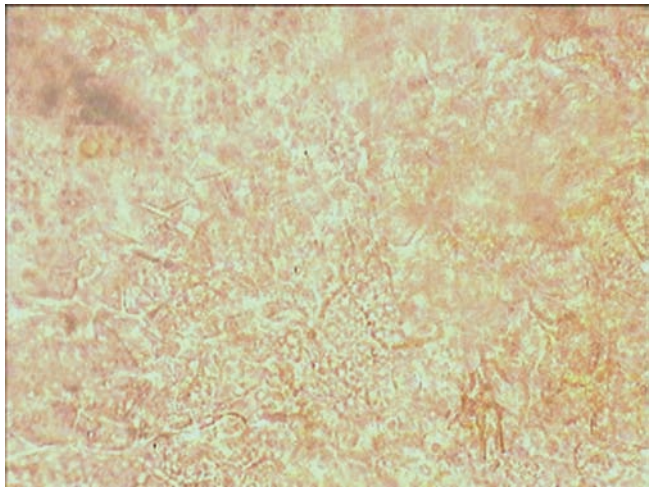

Fig 4. X40 Endosperm cells showing Aleurone grains & oil globules of ‘Nankhwah’

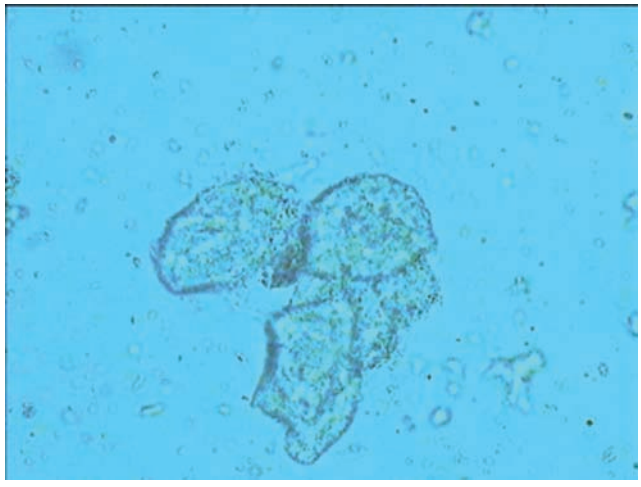

Fig 5. X40 Parenchyma cells filled with starch

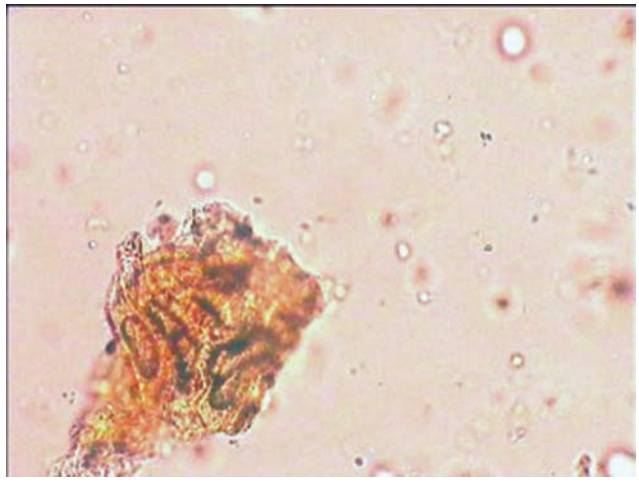

Fig 6. X40 Stone cells of ‘Filfil Siyah’ grains in ‘Filfil Siyah’

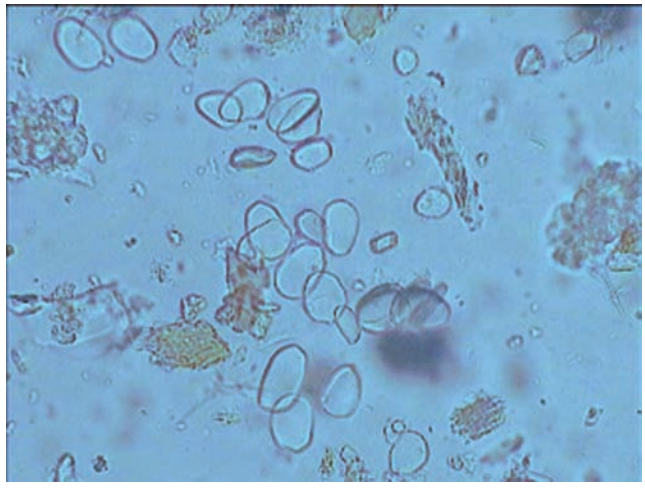

Fig 7. X40 Starch grains of ‘Zanjabeel’

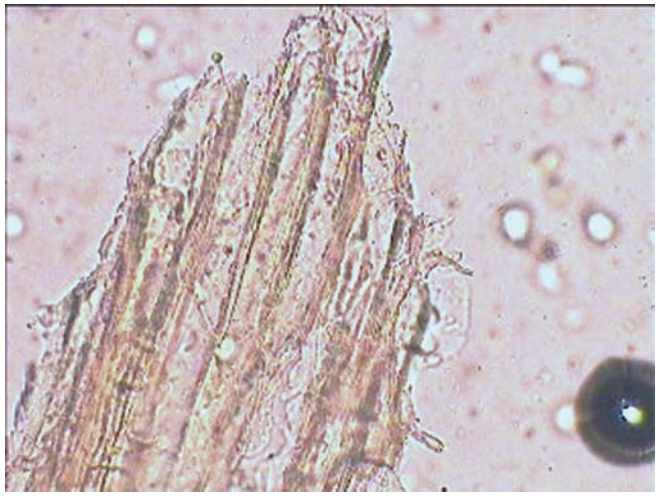

Fig 8. X40 Fibers in groups of 'Zanjabeel'

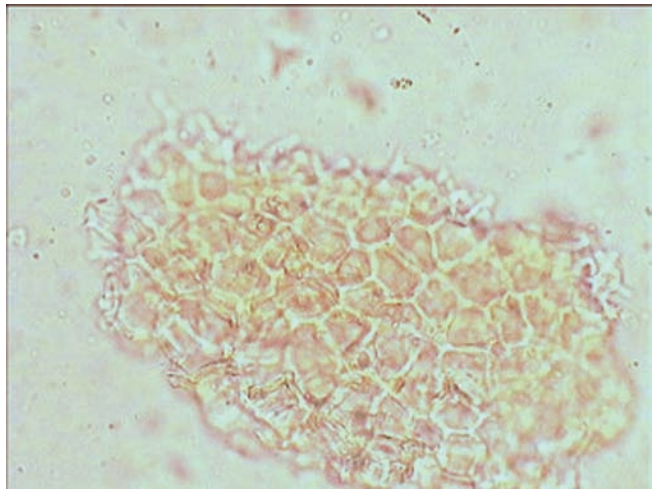

Fig 9. X40 Fragment of Vittae of 'Zeera Siyah'

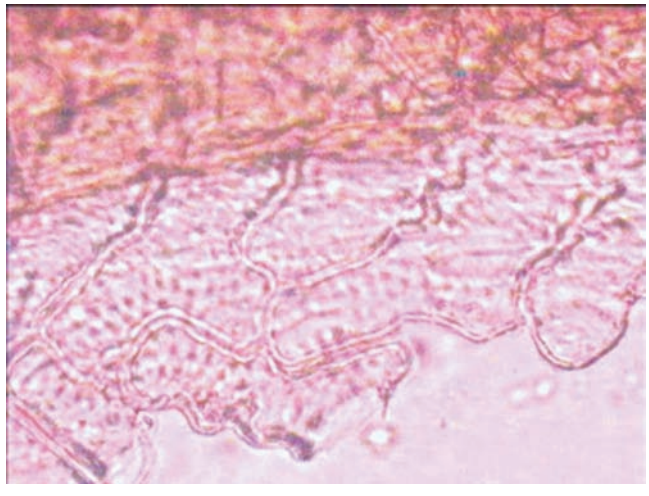

Fig 10. X100 Sclereids from mesocarp of 'Zeera Siyah'

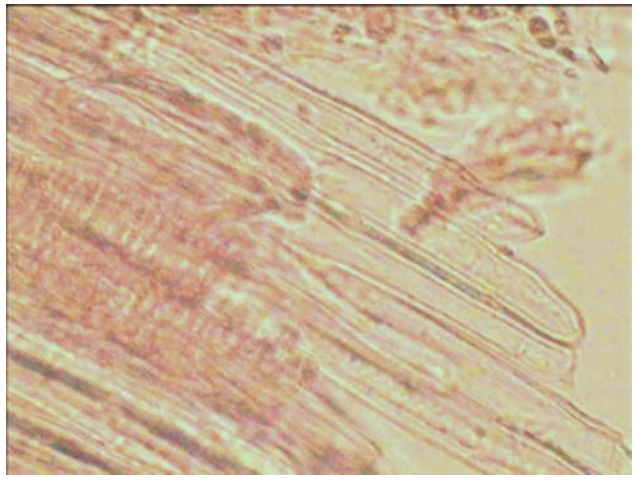

Fig 11. X100 Cells from endocarp of 'Zeera Siyah'

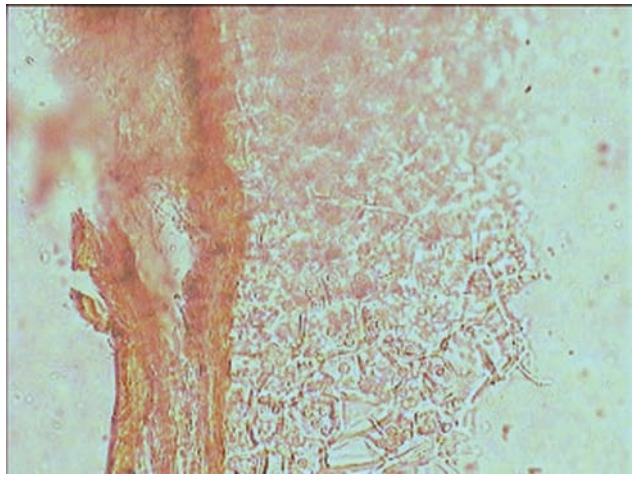

Fig 12. X40 Parenchyma cells filled with aleurone grains & oil globulaes of 'Zeera Siyah'

Fig 13. X40 A piece of fibre of 'Taj Qalmi'

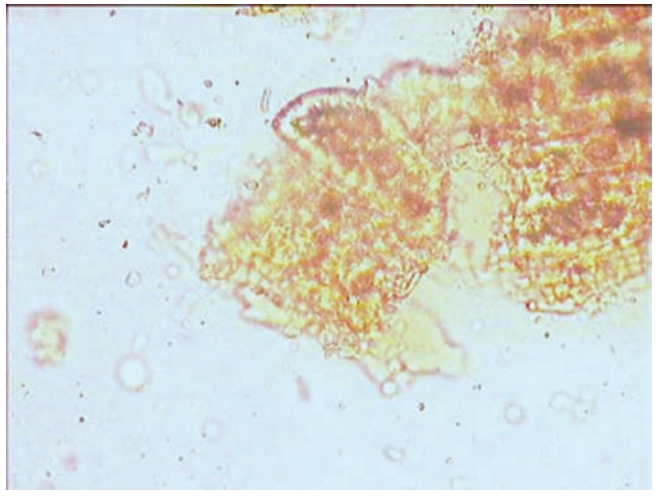

Fig 14. X40 Sclereids of ‘Taj Qalmi’

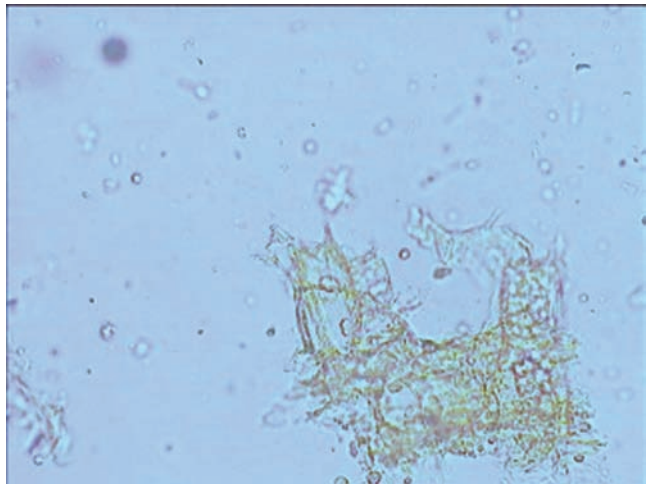

Fig 15. X40 Parenchyma cells filled with starch grains of ‘Taj Qalmi’

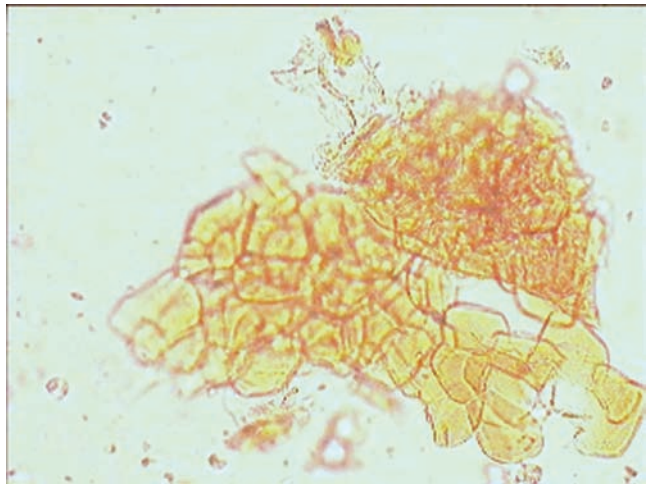

Fig 16. X40 Fragment of Perisperm of ‘Jaiphal’

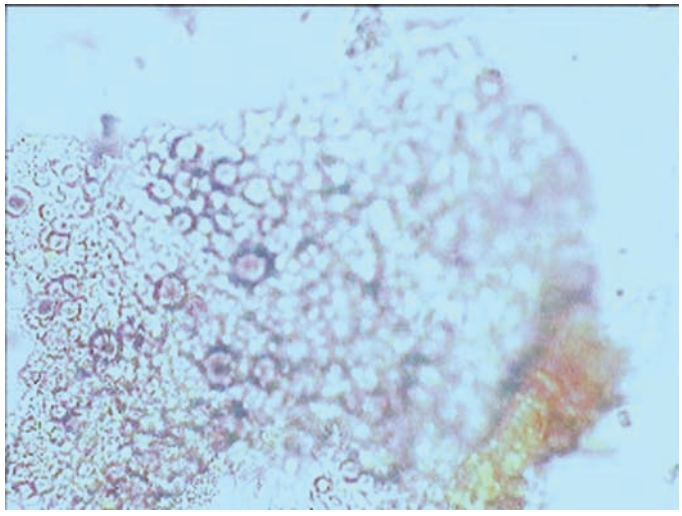

Fig 17. X40 Parenchyma cells from endosperm filled with starch granules of 'Jaiphal'

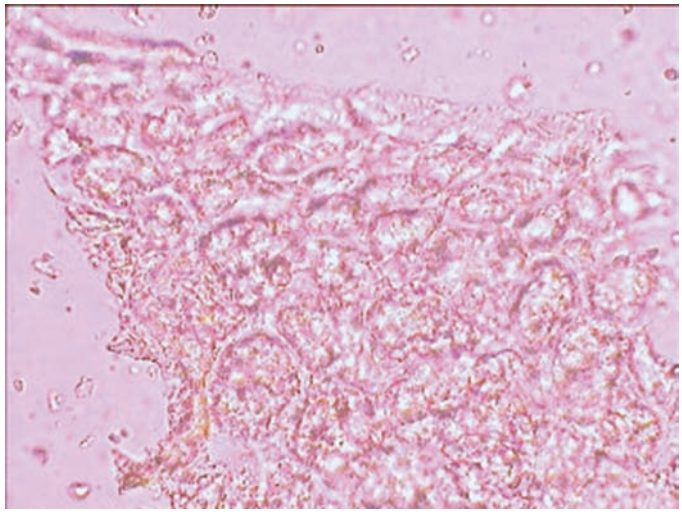

Fig 18. X40 Parenchyma cells filled with oil globules of 'Jawitri'

Results and Conclusion

Namak Ajeeb is yellowish-brown powder with salty taste and pungent odour. On the basis of histological characters studied, presence of following ingredients was established in 'Namak Ajeeb':-

Fragment of epicarp in surface view showing striated cuticle, fragment of vittae composed of polygonal, thin walled cells showing slight thickness at the corners, elongated cells of the endocarp in surface view (Tukhm-e-Karafs) Fig. 1-3.

Parenchyma cells of the endosperm filled with aleurone grains and oil globules (Nankhwah) Fig. 4.

Group of parenchyma cells densely packed with polyhedral masses of numerous starch grains, group of stone cells (Filfil siyah) Fig. 5, 6.

Abundant starch granules which are mostly simple, fairly large, flattened, oblong to oval in shape with a small pointed hilum situated at the narrower end, pieces of non lignified , thin walled fibers present in groups (Zanjabeel) Fig. 7, 8.

Fragments of vittae, sclereids of the mesocarp which are irregularly shaped, moderately thick walled with numerous well marked pits, elongated cells of the endocarp with their long axes parallel to one another, parenchyma cells of the endosperm filled with aleurone grains and oil globules (Zeera siyah) Fig. 9 – 12.

Pieces of fibre which are thick walled, lignified with uneven lumen, group of sclereids, starch granules scattered in parenchyma cells (Taj Qalmi) Fig. 13 – 15.

Fragment of perisperm, parenchyma cells of the endosperm packed with starch granules (Jaiphal) Fig. 16, 17.

Parenchyma cells filled with oil globules (Javitri) Fig. 18.

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