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Prof. Madya Dr. Farid Che Ghazali

Pusat Pengajian Sains Kesihatan

# **IFM surface profiler, $\mu$ CT 3D SCAN, and electron microscopical investigation of '*Sanggul Fatimah*' (*Anastatica Hierochuntica L.*)**

Farid Che Ghazali.

School of Health Sciences Universiti Sains Malaysia, Health Campus,  
Kubang Kerian, Kelantan, Malaysia 16150.

Corresponding author: farid@kb.usm.my

Keywords: '*Sanggul Fatimah*', *Anastatica Hierochuntica L.*, VPSEM and EFTEM

## **Introduction**

Malaysia is blessed with natural products that represent a valuable source of bioactive agents with potent and unique medicinal properties. However, many of these natural products (marine or herbal) are not strictly pharmaceutical products (real medicines) but represent a novel class of dietary supplements or nutraceuticals or exploited in traditional medicines. Traditional medicines has not only been used for primary health care of the poor in developing countries but has also been used in countries where conventional medicines are predominant. This have causes commercial exploitation that has endangered species of therapeutic natural products. In order to clarify and ascertain the identities and properties of the species, effort by utilizing high-resolution electron microscopy is now being pursued as a prelude and to harness the taxonomical identity and chemical characterization of therapeutic potentials of these natural products.

'*Sanggul Fatimah*' or *Anastatica Hierochuntica L.* (The True Rose Of Jericho), a resurrection plant, is a very well-known traditional herbal remedy closely associated with and consumed minutes prior to labor. This is a rhetoric practice among the Malay Kelantanese women where its tea is believed to help ease childbirth. In the course of our research interest in identifying the most sort herbal during pregnancy and post-partum in Malaysia, we have include a surface morphological characterization of *Anastatica Hierochuntica L.* that was observed under the variable pressure scanning electron microscope (VPSEM). Scanning electron microscopical characterization of this plant is virtually a research lacuna. Information on this herb of the Negev and Sahara Desert is important, as it will affect for the women's attitudes and practices.

## **Results and discussion**

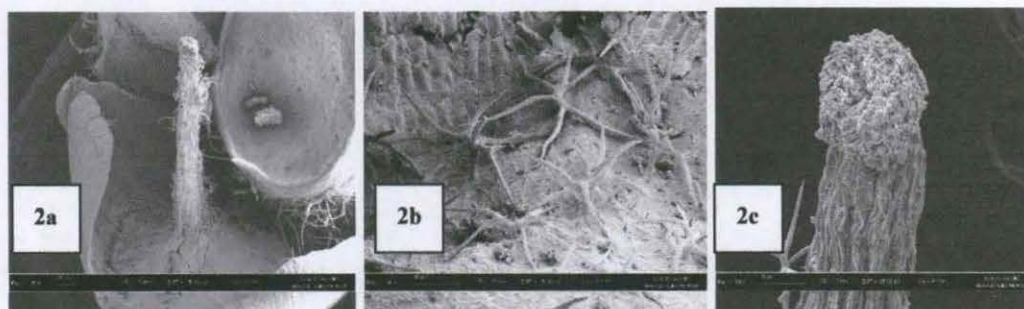
The supra variable pressure scanning electron microscope study of these plants that dies and dries up, curling its stems into a tight ball revealed a surface characterization built-up of pebbles-like structures, asinus rings with thick tunica, an overwhelming soft lamellated surface and spiky coral-like or torn-like structures closely associated with the raised pedunculated putative shoots of cactus-like feature highly prominent in the stem. Microdiffraction analysis (EDX) of the herb revealed inert significant presence of carbon, oxygen, silica, calcium, magnesium, aluminum, potassium, zinc and iron. Although silica is quite significant in major probe areas, the topographical distributions of these periodic elements are non-homogenous. The combination silica and calcium may be associated with bones repairs and help form collagen. This suggests that the folkloric use of *Anastatica Hierochuntica L.* in labor and post labor will enhance good tissue and organ repair. The microdiffraction analysis also suggest therapeutic potential role as a adjuvant supplement in the improvement in bone density and in the prevention of osteoporosis.

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**Figure 1a.** Stereo-photomicrograph of the fresh *Anastatica Hierochuntica* with white flowers. **1b.** The dried *Anastatica Hierochuntica*: indurated curled up, dormant and brown in color. **1c.** IFM true colour surface profiler of the flower and stigma.



**Figure 2a.** The central core of *Anastatica Hierochuntica L* petals revealed a raised stigma. **Figure 2b.** Numerous stellates raised as singular inoculums or out-budding from the flower petal epidermal surface. Each end point is sharp horn-like prominences. **Figure 2c.** The elongated sigma with its putative pollen.



**Figure 3.** The cross sectional image of the central core and cortical thickness of *Anastatica Hierochuntica L*, flower with a raised stigma. Scanco  $\mu$ CT-35 Scan. Energy 45 kVp, image matrix 2048 x 2048 x 925 pixel,



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Fig 3: The central core of *Anastatica Hierochuntica* L petals revealed a raised stigma. VPSEM photomicrograph



Fig 4: VPSEM photomicrograph revealing numerous globules or pebbles-like structures mess with cylindrical tread-like hairs that closely indent the stem wall of *Anastatica Hierochuntica* L. The hair-like structures are mostly bipolar although multi-polar processes (stellate formation) were



Fig 5: VPSEM photomicrograph revealing numerous stellate structures raised on a singular or inoculum or out-budding from the epidermal surface. The end points of most of these stellate arms are horn-like sharp points.



Fig 6: Another VPSEM photomicrograph revealing the stellate structures forming a rosette of stellate.



Elements	Weight%	Atomic%
C	16.98	24.81
O	52.42	57.50
Mg	1.25	0.90
Al	5.09	3.31
Si	18.51	11.57
K	1.33	0.59
Ca	0.78	0.34
Zn	3.64	0.98

Elements	Weight%	Atomic%
C	35.19	46.77
O	42.73	42.63
Mg	0.85	0.56
Al	3.61	2.13
Si	7.19	4.09
K	1.19	0.49
Ca	6.11	2.43
Fe	3.14	0.90

Table 1: VPSEM EDX weight and atomic percentage of the various elements detected.



Fig 7:  $\mu$ CT 3D Scan



Fig 8: The cross sectional image of the central core and cortical thickness of *Anastatica Hierochuntica* L, flower with a raised stigma. Scanco  $\mu$ CT-35 Scan, Energy 45 kVp, image matrix 2048 x 2048 x 925 pixel

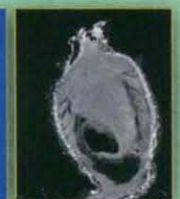


Fig 9: IFM surface profiler



Fig 10: 2D Image Flower2 with Stigma



Fig 11: Branch, 3D image in Real Color



Fig 12: Flower2, 3D image in Real Color

### References

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## **Results and discussion**

The histological and electron microscopical inspection of its thin glossy translucent tongue revealed important distinctive characteristic that may relate to the *Collocalia* nest-building prowess. There seems to be inconclusive evidence for the presence of parotid and submandibular gland per se in these species. The dominant acini were located at its buccal mucosa and the sub-epithelial lingual glands probably a specific characterization feature of these species. No taste buds and papilla was observed on its anterior dorsal surface. EFTEM revealed a significant distribution of secretory granules (serous, mucous) and the mineralized chondroid presence. There is also presence of tonsils.

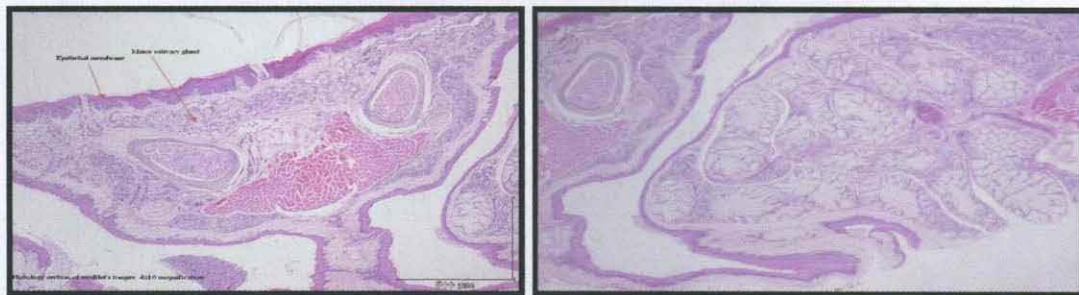
The avian tongue can be regard as the powerhouse organ that contributes to enormous salivary secretion pertaining to nest building of the *Collocalia* species. The evolutionary changes in the *Collocalia* tongue are thought to be the foundation for the abundant muciginous salivary in the nest and high thiocynate present. Histo-morphological features of the *Collocalia* tongues may be a reflection of differences among the insectivorous avian. This may suggest an important adaptation role for the tongue structure to nest building

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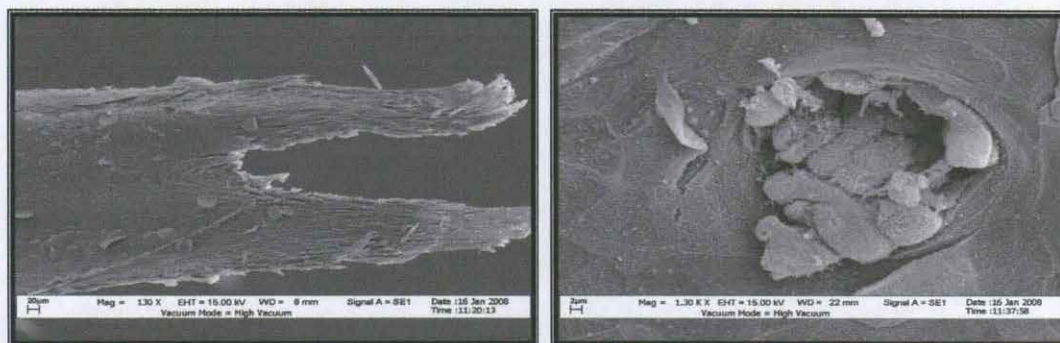
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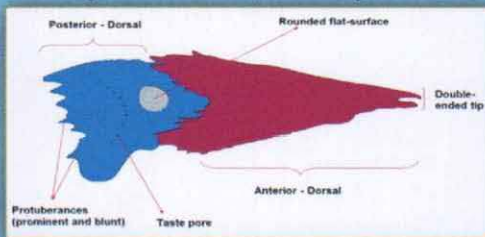
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**Fig. 4:** Histology section of swiftlet's (*Collocalia* sp.) tongue (H&E stain), 4x10 magnifications. The section revealed. Lingual epithelial that is composed of non-keratinised stratified squamous epithelium underlying its outermost layer of the tongue. There is a well-defined oval ring shapes structures suggestive of chondroid or cartilaginous like structure at both lateral borders of this tongue section. There is a significant presence of many minor mucous like acinus's of minor salivary gland structures in close proximity or below the lingual epithelial lining. The histology of the tongue also suggests the presence of only a singular transverse orientation of the intrinsic muscle. No taste buds were observed present.



**Fig 5:** Cartilagenous structure of *Collocalia*'s tongue, 400 magnification



**Fig 6:** Muscle of *Collocalia*'s tongue, 400 magnification



**Fig 7:** Light microscope of asinus, 400 magnification.



**Fig 1:** Posterior aperture **Fig 2:** Roof of the mouth



**Fig 3:** Dorsal surface of the lower jaw



**Fig 8:** EFTEM photomicrograph representation of the chondroid-like structure revealing a chondrocyte or osteocyte like structure. Embedded in a mineralised like hons structure. The cell is presented with multi satoplasmic



**Fig 9:** The is no clear demarcation called median sulcus or central grooving observed present as shown presented in some mammal and rat species. However, the intermolar eminence is observed elevated.



**Fig 7:** VPSEM photomicrograph of the anterior dorsal tip of the tongue. The tip is separated as two prominent horns. A central V-Shape invagination separate the two laterally place tips' end. These two ends dorsal surface lacks the epithelial cells covering as observed in most mammalian dorsal surface. The tips reveal a morphologicc characterization of anteriorly thick parallel running fibers forming as its anatomical body. Epithelial cells are only observed presented starting from the posterior end of the tongues' tip. And it is of almost polygonal of in shape. These epithelial cells do not show cell margin thickening between adjacent cells as observed in goats. Peeling off of it epithelial cells is also observed. Orifice of a putative taste bud isolated at the posterior dorsal surface. No such structures were observed on its anterior dorsal surface.

## Conclusion

These multivariate study have revealed unique histological and ultrastructure of the *Collocalia* species.

The findings in this study have thus provide data and novel information helping to further understand the *Collocalia* and its nest building prowess.



Maria Anna Pabst  
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*Editors*

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**Prof. Dr. Maria Anna Pabst**  
**Institut für Zellbiologie, Histologie**  
**und Embryologie**  
**Medizinische Universität Graz**  
**Harrachgasse 21**  
**8010 Graz**  
**Austria**  
**anna.pabst@meduni-graz.at**

**Prof. Dr. Günther Zellnig**  
**Institut für Pflanzenwissenschaften**  
  
**Karl-Franzens-Universität Graz**  
**Schubertstrasse 51**  
**8010 Graz**  
**Austria**  
**guenther.zellnig@uni-graz.at**

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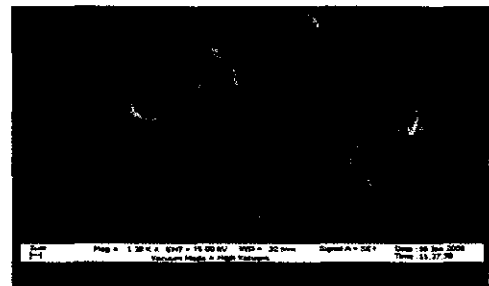
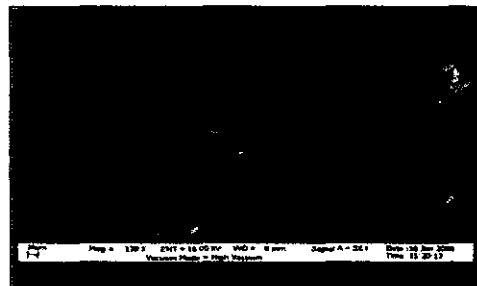
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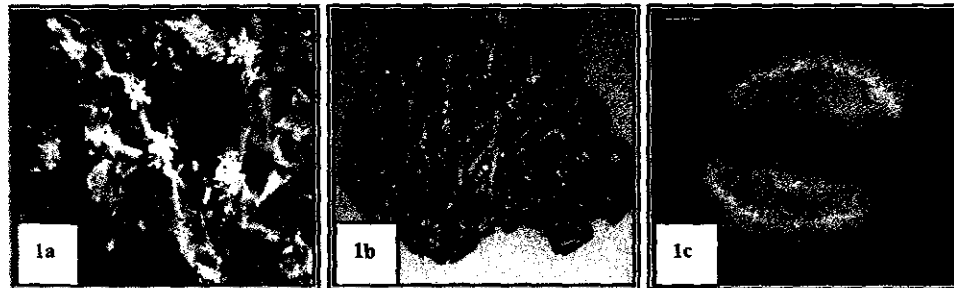


Figure 1. a Stereo-photomicrograph of the fresh *Anastatica Hierochuntica* with white flowers. b. The dried *Anastatica Hierochuntica*: indurated curled up, dormant and brown in color. c. IFM true colour surface profiler of the flower and stigma.

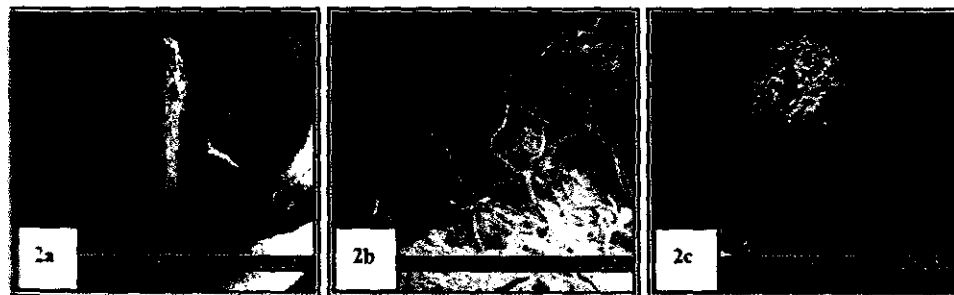


Figure 2. a. The central core of *Anastatica Hierochuntica L.* petals revealed a raised stigma. b. Numerous stellates raised as singular inoculum or out-budding from the flower petal epidermal surface. Each end point is sharp horn-like prominences. c. The elongated stigma with its putative pollen.



Figure 3. The cross sectional image of the central core and cortical thickness of *Anastatica Hierochuntica L.* flower with a raised stigma. Scanco  $\mu$ CT-35 Scan. Energy 45 kVp, image resolution 2048 x 2048 x 925 pixel