# MICROSOPICAL CHARACTERIZATION OF MALAYSIAN *Trigona spp* HONEY AND PROPOLIS

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*Honey is a carbohydrate-rich syrup produced by bees from nectars, while propolis is the resinous compound. Together, honey and propolis are well purported with potentials in antioxidant, antimicrobial and anti-inflamatory related to wound healing. Currently most honey and propolis are derived from highly domesticated Apis Mellifera. Trigona spp is a genus of stingless bees which can only be found in tropics and subtropics regions (South America, Australia, Asia and Africa). The present research was conducted as a prelude to more detailed characterization studies. In this research Trigona's honey and propolis were examined under light microscope, FESEM and VPSEM to visualize the morphological features. Micro diffraction analysis or EDX was also pursued to identify periodic table compound in both samples. The various presentations observed includes thin biofilm topography, crater-like surface, fiber-like and seed like structure .*

**Keywords:** Honey, Propolis, Fiber like, Trigona spp

## INTRODUCTION

Bee-hive products have always been popular choice in health management since ancient time [1]. Honey and propolis are among bee-hive product known for their therapeutic values. Honey is nectar based, carbohydrate-rich syrup produced by honey bee [2]. Propolis is mixture of beeswax with plant resin used as building material and defence mechanism of the hive [3]. Previous researches showed that both honey and propolis posses antioxidant [4],[5], antibacterial [6],[7], anti-inflammatory [8],[9] and anti-proliferative [10],[11] properties. Most of the research conducted focus on the effect of substances in disease state. Little is known on actual morphology of both compounds. Microscopic techniques are usually used in botanical origin research. Microscopical observation allows surface morphology identification. Data on surface morphology is essential in understanding the actual compound present. In this research the morphological characteristic will be covered to give detail information which will assist in therapeutic-based research.

## MATERIALS & METHODS

The study utilize light microscope, field emission electron microscope (FESEM) and variable pressure electron microscope (VPSEM) integrated with EDX analysis. Honey sample was prepared through freeze-drying in order to obtain solid type of honey. Raw propolis, propolis in 70% ethanol and the sediment of propolis taken from extraction were used in light microscope study. Only raw propolis were examined under scanning electron microscope. Both samples were sputter coated with a thin layer of gold for 10 minutes and analyzed using FESEM and EDX.

## RESULTS & DISCUSSION

Light microscope observation of Trigona honey illustrated presence of shell-like component with peripheral rim of auto fluorescence. There were putatitive spore-like structure embedded within.

Honey showed thin-biofilm form arranged in muscovite-like structure. Bubble-shaped structures also found embedded within the thin-layer of honey. As compared to previous reserched by Farid 2009 [12], Gelam honey was also presented as thin biofilm form. It differed by presentation of Gelam honey in more intact form. Backscatterd EDX showed no presence of trace elements in Trigona's honey.

The difference of morphology of honey are hypothesized due to different method used to solidify the honey.Light microscope observation of propolis illustrated presence of sphere-like structure and fiber-like structure embedded within.

Propolis revealed homogenized flat surface with presence of crater-like structure. Further observation through higher magnification show presence of tulip-like shape compound embedded within the structure. VPSEM examination show abundant compound embedded within the surface of propolis.

EDX analysis shows no trace element present in both the sample as shown in Fig.14 and 15. Previous research had recorded that stingless bee nest is build from mixture of resin collected from plant with wax which is term as propolis [13] and further mixed it with other compound such as pollen, seed, plant gums, mud, faeces and wood to stabilize the nest [14]. Such

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phenomena can explain presence of compound in Fig.10 and Fig.12. The mixture much depend of the site of the nest where different part build up with different compound. There are little characterization and definition relative to the organisms found in stingless bee nest especially in propolis. One of organism that associated with bee is mites. Honeybees generally have about 100 known species of mite associates. There are a lot of possibilities regarding the actual interaction of mites with bees from commensalism to mutualism. Table 1 by Walter et al 2002 summarize the interaction. Most association begin in the nest and end in the nest [15]. Fig.8 and Fig.9 revealed presence of structure similar to dorsal shield of Hypoapis (Mesostigmata) in propolis surface. Hypoaspis had been reported present in Malaysian stingless bee nest by Robert et al 1996 [16]. Fig.16 shows the image of the mites by Robert et al 1996. The presence of Laelapidae mite is still lacunae pertaining to honey bee.

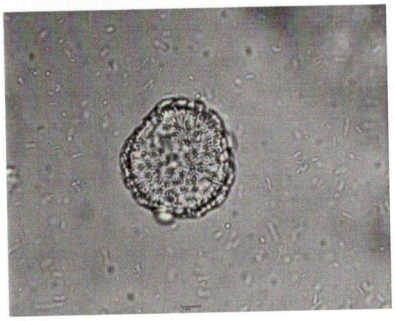

**Fig.1a Light microscope image of honey, 100x magnification. Shell-like structure**

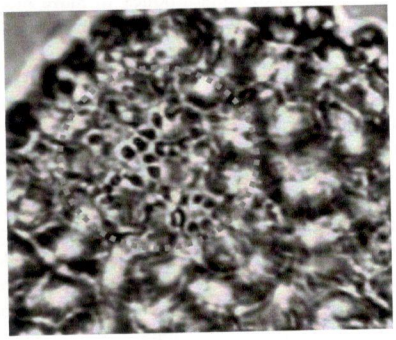

**Fig.1b Light microscope image of honey, 100x magnification. Close up image showed putative spore-like structure embedded within**

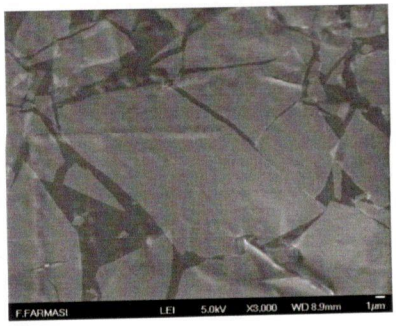


**Fig.2a. FESEM of freeze-dried Trigona honey, 3000x magnification at 9.0mm W.D. Muscovite topography of thin biofilm.**


**Fig.2b FESEM of freeze-dried Trigona honey, 3500x magnification at 9.0mm W.D. Globular compound embedded within layer of thin biofilm.**

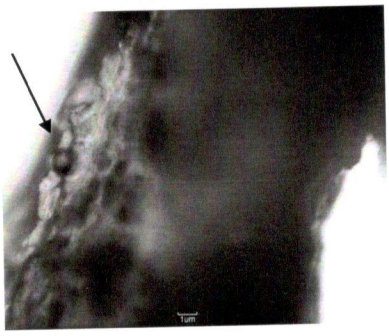

**Fig.3 Light microscope image of raw propolis, 40x magnification. Sphere-like object embedded (marked with arrow) within resinous surface of propolis.**

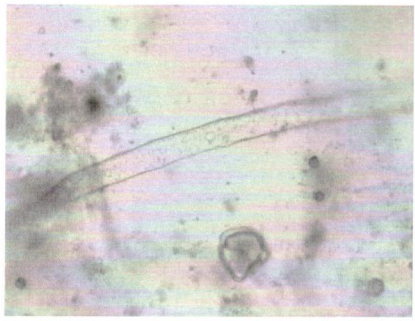

**Fig.4 Light microscope image of propolis tincture, 40x magnification. Fiber-like structure**

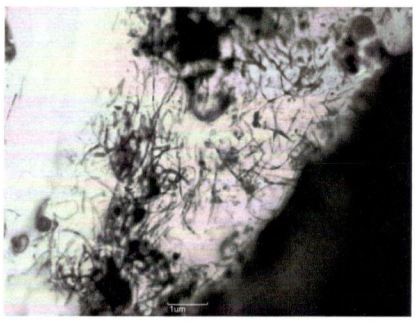

**Fig.5 Light microscope image of propolis sediment, 40x magnification. Fiber-like structures.**

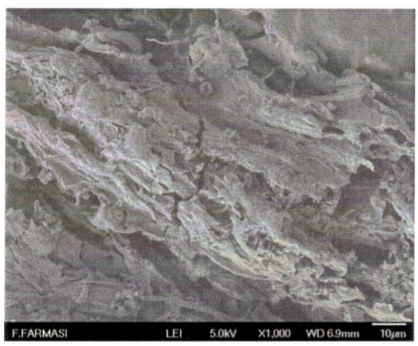


**Fig.6 FESEM of raw propolis, 1000x magnification at 6.9 mm W.D. A crater composed of flaking-out top structure**


**Fig.7 FESEM of raw propolis, 16000x magnification at 6.9mm W.D. A tulip like structure embedded within crater surface of propolis.**

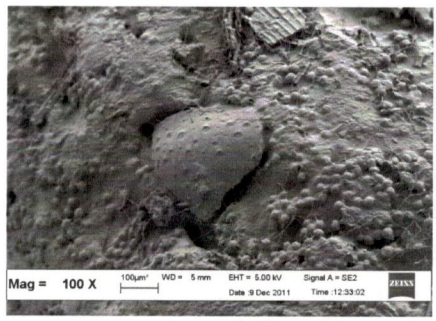


**Fig.8 VPSEM of raw propolis, 100x magnification at 5.0mm W.D. Dorsal shield of Hypoaspis embedded in propolis surface.**

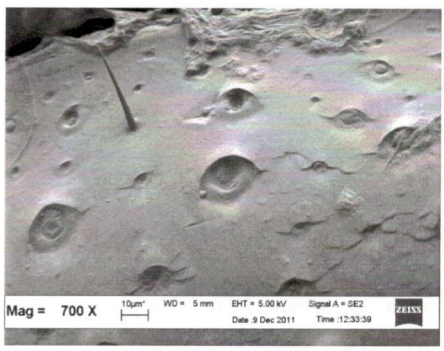


**Fig.9 VPSEM of raw propolis, 700x magnification at 5.0 mm W.D. Close up image of dorsal shield.**

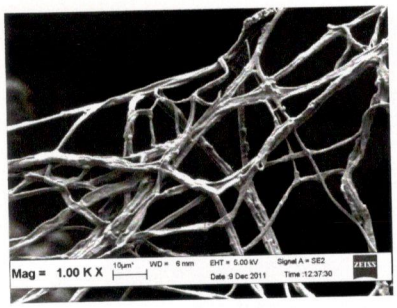


**Fig.10 VPSEM of raw propolis, 1000x magnification at 5.0mm W.D. Flatten fiber network.**

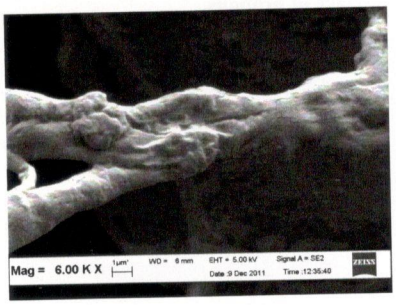


**Fig.11 VPSEM of raw propolis, 6000x magnification at 5.0mm W.D. Close up of fragile mesh-like structure**

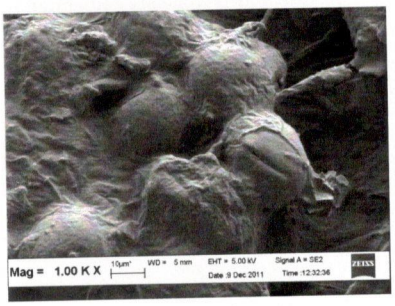


**Fig.12 VPSEM of raw propolis, 1000x magnification at 5.0mm W.D. Globular nut-like structure with blanketing thin biofilm coat observe within propolis surface**


**Fig.13 VPSEM of raw propolis, 2090x magnification at 5.0 WD. Outer skin of nut-like globular structure.**

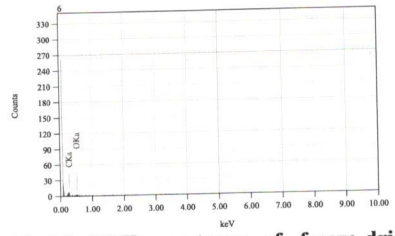


**Fig.14 EDX spectrum of freeze-dried Trigona's honey in backscattered mode.**

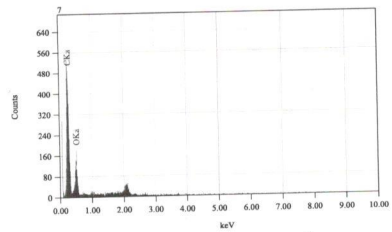


**Fig.15 EDX spectrum of raw propolis in backscattered mode.**

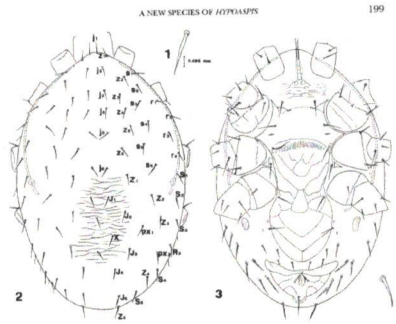


**Fig.16 Image of Hypoaspis [16]**

**Table 1 Genera of mites phoretic or potentially phoretic on Australian bees [15]**

| Family/ Genus | Host Relationship | Status |
|---|---|---|
| Podapolipidae | | |
| *Locustacarus* | Tracheae of *Bombus*/parasite of adult | Not reported |
| Scutacaridae | | |
| *Imparipes* | Nest of soil-nesting bees/unknown | Known from soil (Mahunka 1967) |
| Trochometridiidae | | |
| *Trochometridiidae* | *Lasioglossum eremaean* Walker/ parasite larva? | **New record** |
| Tarsonemidae | | |
| *Acarapis woodi* | *Apis* tracheae/ parasite of adult | Quarantine intercepts |
| *Acarapis* spp. | *Apis* thorax/ parasite of adult | Halliday *et al.* (1997) |
| *Tarsonemus* | Body of acarinaria of Xylocopini/scavenger | B. OConnor, pers. comm. 2001 |
| Erythraeidae | Various bees/ ectoparasite of adult | See Southcott (1989) |
| Cheyletidae | | |
| *Cheyletidae* | *Xylopoca*/ predator of mites in nest | **New record** |
| Wintershmidtiidae | | |
| Undetermined | *Hylaeus, Halictus*/ commensals? | Rayment (1954) |
| Chaetodactylidae | | |
| *Sennertia* | Megachile, Xylocopa/ predator | Rayment (1954), UQIC |
| *Chaetodactylus* | Native bee (Torres Strait)/brood parasites | UQIC |
| Histiostomatidae | *Anoetus*/scavengers, mutualists? | Rayment (1954), UQIC |
| Acaridae | | |
| *Ctenocolletacarus* | *Ctenocolletes*/scavenger | Fain & Houston (1986) |
| *Horstia* | *Megachile*/scavenger | Rayment (1954) |
| *Kuzinia* | *Bombus*/scavenger | O. Seeman, pers. comm. 2000 |
| *Cerophagopsis* | *Trigona*/scavenger | Fain & Heard (1987) |
| Undetermined | Various Colletidae, *Coelioxys*, *Xylocopa* | Rayment (1954) |
| Ameroseiidae | | |
| *Afrocypholaelaps* | *Apis mellifera*/flower mite | Seeman & Walter (1995) |
| *Neocypholaelaps* | *Lipotriches tomentifera*/flower mite | **New record** |
| *Hattena* | *Apis mellifera*/flower mite | Halliday (1997) |
| Varroidae | | |
| *Varroa* | *Apis mellifera*/brood parasite | Not reported |
| Laepidae | | |
| *Melittiphis* | *Apis mellifera*/pollen thief | Knihinicki & Halliday (1995) |
| *Tropilaelaps* | *Apis* spp./brood parasite | Not reported |
| *Raymentia* | *Lasioglossum*/unknown | Walter et al 2002 |
| *Eumellitiphis* | *Trigona*/unknown | Not reported |
| *Stigmatolaelaps* | Xylocopini/unknown | Not reported |
| *Dinogamasus* | Xylocopini/unknown | Not reported |
| Undetermined | *Lasioglossum* | Rayment (1954) |

## CONCLUSIONS

Microscopical examination through light microscope and scanning electron microscope has shown potential in characterizing and exploring morphological properties and identifying unknown compound embedded within samples. Trigona honey is presented as thin biofilm layer with bubble-shape embedded within it while propolis is presented with flat surfaced homogenous layer with abundant compound embedded within. Microscopical examination allows characterization of actual morpholgy and compound of honey and propolis which will help in further characterizing therapeutic properties of both substances.

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