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Research Article

Scanning Microscope-Energy Dispersive X-Ray (Sem-Edx) Studies of *Quercus infectoria* Gall

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ABSTRACT
Quercus infectoria gall extract are popular among Malaysian. Therefore there is vast availability of the galls supply in Malaysian local market. However, these galls found in Malaysian market were brought out from another country such as Iran and India. In this regard, maintaining the quality of the source is very important to ensure the expected health benefit. In this study we used scanning microscope-energy dispersive X-ray (SEM-EDX) to analyse the microscopical image and basic elements present in crude and aqueous extract of *Quercus infectoria* gall in comparison with macroscopical view. Galls of *Q. infectoria* were divided into external surface and cross section inner parts were performed using hard cutter. Macroscopical view showed that the gall looked alike ball-shaped with numerous protruding blunt horn-like lumps on external rugae like surface (1.4-2.3 cm in diameter). The cross section revealed three major parts; outer, middle and inner layer containing whitish core separated by barrier. The parenchyma cells could be seen in most part of the layer and elongated to formations of tracheid cells. Elemental analysis showed that the galls contain various useful minerals of carbon, calcium, oxygen, magnesium, silicon, potassium, sodium and chlorine. The elements distributions were different in each part. Most of the elements found in the external surface. The cross section part showed the highest number of elements on the outermost layer and it was the only part where the sodium and chlorine were found. These present study serve as a valuable information about macroscopic and microscopic features of the galls besides trace elements composition that will be useful for the establishment of quality standards and future reference for galls authentication.

Keywords: *Quercus infectoria* gall, Energy dispersive X-ray (EDX), Scanning electron microscope (SEM), microscopical view.

INTRODUCTION

Quercus infectoria Olivier (Fagaceae) (QI) or also known as nut galls found on a small tree in Greece, Asia Minor and Iran [1]. Studies indicated that galls of QI have a variety of pharmacological properties including being an astringent [2], antidiabetic [3], antitremorine, local anesthetic[4], antiviral potential[5], antibacterial[6], antifungal[7], larvicidal[8] and anti-inflammation[9]. The main constituents found in the galls of QI are tannin (50-70%) and small amounts of free gallic acid and ellagic acid [1]. Tannin is a phenolic compound, which is soluble in water, alcohol and acetone. It gives precipitates with protein[10].

The wide range of pharmacological activities of this plant might support the efficacy of extract preparation of *Q. infectoria* that are widely used in Malaysia for treating many kinds of health problems since many decades ago. In Malaysia, QI galls are known as “manjakani”. The extract of manjakani was claimed to be highly beneficial for the Malay Kelantanese postpartum women. Hazardous effects of the extract were not reported so far[11]. In addition, the Arabs, Persians, Indians, Malays and Chinese have traditionally used the galls after childbirth to treat vaginal discharge and related postpartum infections[12].

The popularity of manjakani used among Malaysian might contribute to the vast availability of the supply in local market. However, these nuts galls found in Malaysian market were brought out from another country such as Iran and India. For research purposes, maintaining the standard of parameters such as physicals constants, macroscopical, microscopical and phytochemicals[13] analysis are very important for authenticity of the sources. Several studies have been carried out on those mentioned parameters but limited study reported the scanning microscope electron-EDX image and mineral content of the galls as the standard procedure to maintain the quality. Thus, in this study, we reported the macroscopical and microscopical analysis of outer and inner part of the galls in order to be used as referral in the future study for validation of the source.

MATERIALS AND METHOD

Cross section of *Quercus infectoria* oliv: *Q. infectoria* used in this study were obtained from the local market. The cross section of QI was prepared by using a hard cutter equipped with diamond band saw and was observed under the scanning electron microscope Leica EM SCD005. For elemental analysis of QI cross section, the scanning electron microscope with EDX (Energy Dispersive X-ray)

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Fig.1: Scanning electron micrograph of (A) protruding blunt horn-like lumps of the Q. infectoria surface, (B) pole of Q. infectoria gall, (C) rugae like surface of Q. infectoria gall

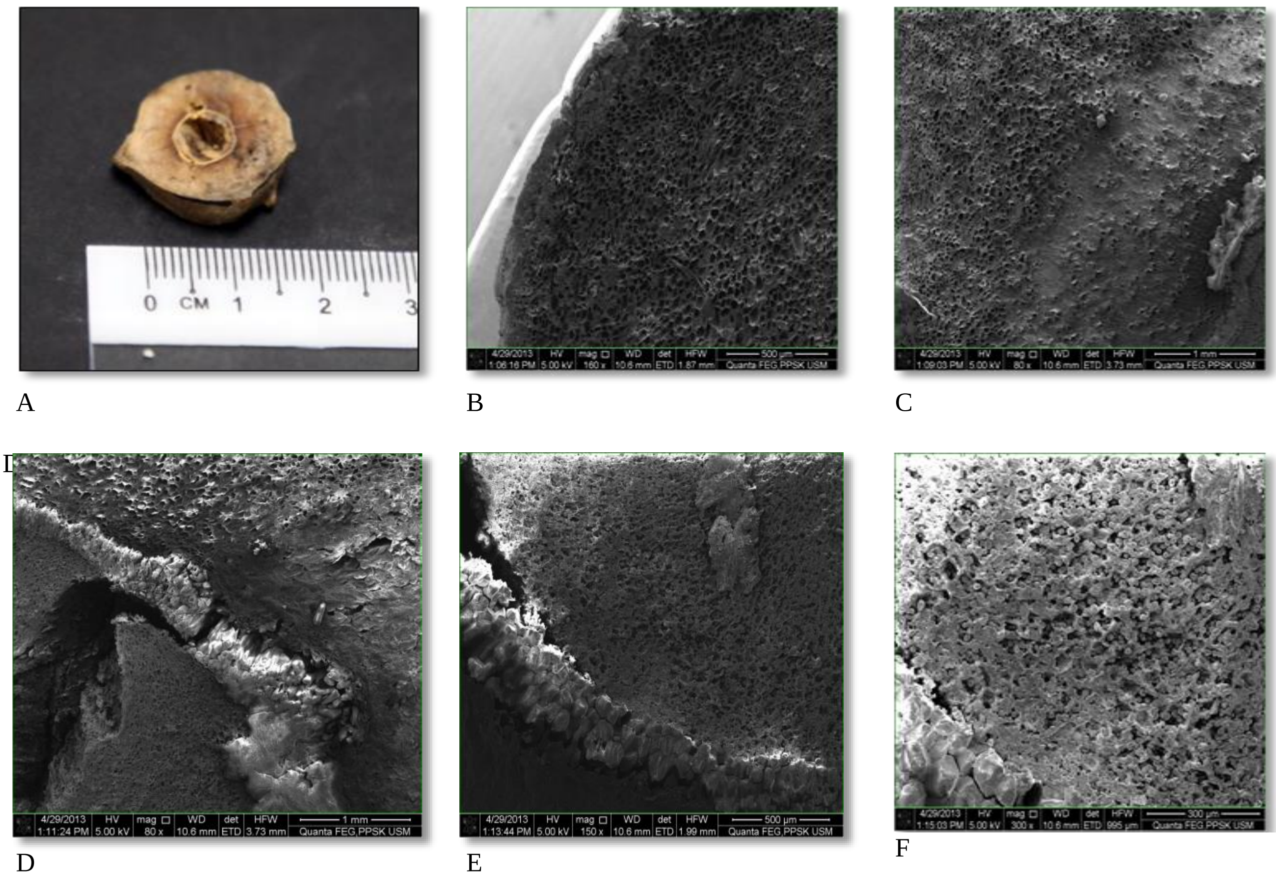


Fig. 2: (A) Cross section of Quercus infectoria gall, Scanning electron micrograf showed the cross section image of the Quercus infectoria gall (B) outer layer, (C) middle layer, (D) barrier between middle and inner layer, (E) inner layer and (F) whitish core of inner layer.

Oxford Instrument INCA 5.02 was used.

Sample preparation**:** Methods for preparation of QI galls extract was modified based on[14]. The crushed galls of QI

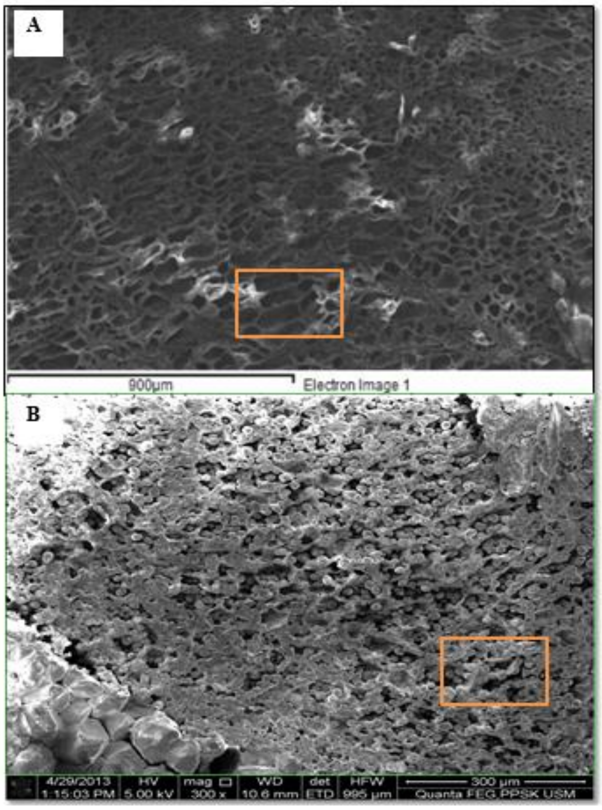


Fig. 3: (A) Elongated parenchyma cells between upper and lower epidermis were found. (B) Representative of vascular strands that present at all places and radially elongated sclerides touched the lower epidermis.

were obtained from the local market and were extracted with distilled water and refluxing at 50˚C for 24 hours. The extract was then being filtered through Whatman filter paper IV to remove other residual from the extract. The extract was concentrated using a rotary evaporator. Concentrated extract was taken out from rotavapor, and further dried using freeze drier. For the elemental analysis of QI cross section, the scanning electron microscope with EDX (Energy Dispersive X-ray) Oxford Instrument INCA 5.02 was used.

RESULT AND DISCUSSION

Macroscopical analysis: Macroscopically, the nut (Figure 1A) is globose with protruding horny appearances on external surface with a whole at pole site. The gall looked alike ball-shaped with numerous protruding blunt horn-like lumps on external rugae like surface (1.4-2.3 cm in diameter). The external appearance of the gall looks greyish-brown to brownish-black in colour. Surface is smooth with numerous horny protuberances giving rough touch, and unpleasant odour.

Figure 1: Scanning electron micrograph of (A) protruding blunt horn-like lumps of the *Q. infectoria* surface, (B) pole of *Q. infectoria* gall, (C) rugae like surface of *Q. infectoria* gall

The protruding horny like surfaces are lumpy when viewed under scanning electron microscope (Figure 1C). The pole site indicated the traced of insect gate. The outer surface

Fig. 4: A) Powder of QI and A) Aqueous extarct of QI and B) elemental distribution by SEM-EDX

Table 1 :Elemental distribution by SEM-EDX

Element	Weight %		Atomic %	
	A	B	A	B
C	55.98	52.62	63.06	59.87
O	43.44	46.71	36.74	39.90
K	0.58	0.67	0.20	0.23

(exsocarps) seems rugae texture (Figure 1A-C) as observed with the naked eyes. Previously,[11] had reported the observation on the outer layer and inner layer of the nut (Figure 2A-F). We have found the same observations as reported. Cross sectional cut of the nut indicated three layer differentiated by thickness of the textures. The outer layer comprises the exsocarps part. The middle layer is more visible between barrier and outer layer. The inner layer is situated in the whitish core.

Figure 2: (A) Cross section of *Quercus infectoria* gall, Scanning electron micrograf showed the cross section image of the *Quercus infectoria* gall (B) outer layer, (C)

middle layer, (D) barrier between middle and inner layer, (E) inner layer and (F) whitish core of inner layer.

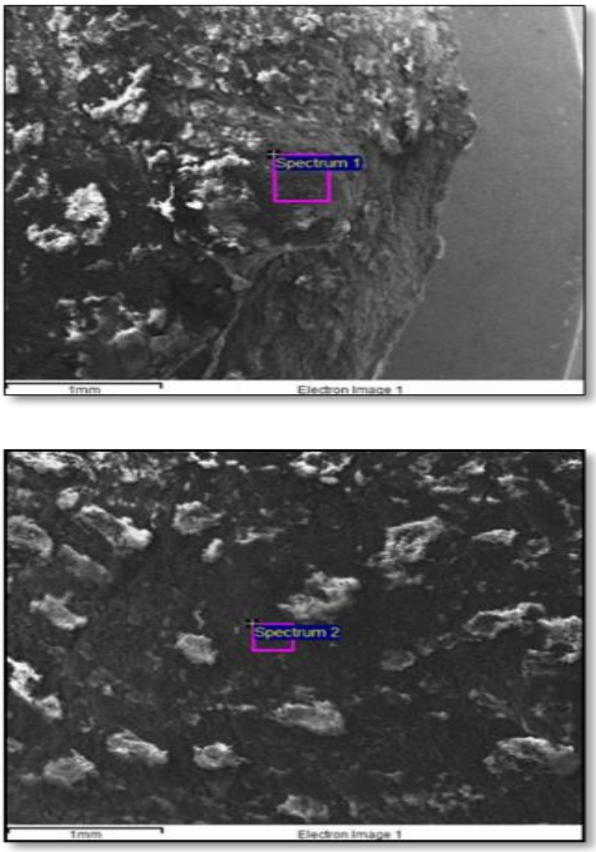


Fig. 5: A) Outer surface, B) outer rugae like surface of Q. infectoria elemental

Table 2: Elemental distribution by SEM-EDX

Element	Weight %		Atomic %	
	A	B	A	B
C	52.23	60.95	54.84	65.72
O	44.21	38.00	32.86	29.58
K	0.47	0.16	n.d	n.d
Ca	1.13	0.39	10.68	3.84
Mg	0.35	0.20	n.d	n.d
Si	0.61	0.30	1.62	0.83

On the other hands, the horn-like protuberance, hard and blunt structure distributed all over the surface is the characteristic specific to galls of Q. *infectoria*. The similarity of the structure confirmed that our sample procured from local markets were galls of Q. *infectoria*. With this confirmation, we have conducted the study to visualise the shape of parenchyma cells of the plant after cross sectional cut.

SEM EDX microscopical analysis: The cross sectional cut were performed to view the microscopical features of inner layer of nut galls. Microscopically, a wide zone of radially elongated parenchyma cells between upper and lower epidermis were found. The vascular strands were present at all places and radially elongated sclerides touched the lower epidermis [15]. The cells were made up by rounded to polygonal parenchyma (Figure 3A). The thickened and pitted walls distinct the intercellular spaces with variations of sclereids shape from less ovoid to rectangular (Figure 3B). Previously,[16] had reported the distribution of parenchyma and sclereids cells in powder nutgalls observed under microscope. Our SEM EDX image showed clear shape of both cells and supported the findings by[16].

The packed parenchyma cells might explain the rounded hard body of the galls[11].

People consumed nutgalls extract to maintain health stability and cure some related health problems. The richness in polyphenols and phenolics compound in the galls might contribute the health benefit to the consumers[14]. Besides phytochemicals, the galls also rich in trace minerals content such as calcium, potassium and many mores. Mineral elements are acquired from the soil

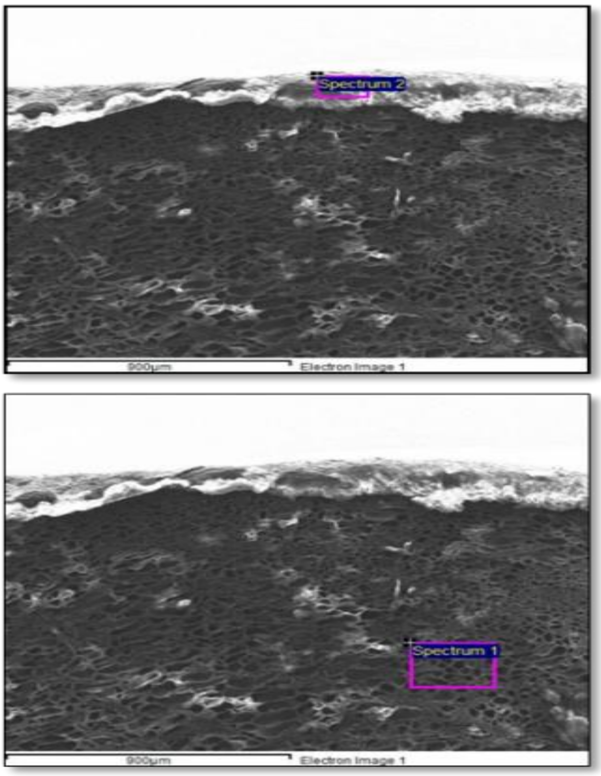
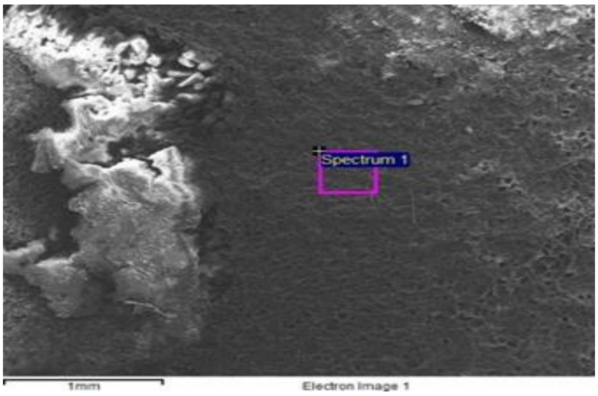


Fig. 6: A) Outermost layer of QI (pericarp) and B) outer layer of QI cross section

Table 3: *Elemental distribution by SEM-EDX*

Element	Weight %		Atomic %	
	A	B	A	B
C	61.02	68.51	50.31	57.80
O	35.78	30.1	48.40	41.75
K	0.26	0.09	0.71	0.25
Ca	1.35	0.45	0.58	0.20
Na	0.46	0.27	n.d	n.d
Mg	0.26	0.15	n.d	n.d
Si	0.48	0.23	n.d	n.d
Cl	0.37	0.14	n.d	n.d

solution by plant roots. The uptake of minerals elements by plant root and their subsequent distribution within the plant varies. Plants require at least 14 minerals elements for their nutrition except carbon and oxygen. The present of trace minerals in the galls would provide the soil environment during the growth period[17].



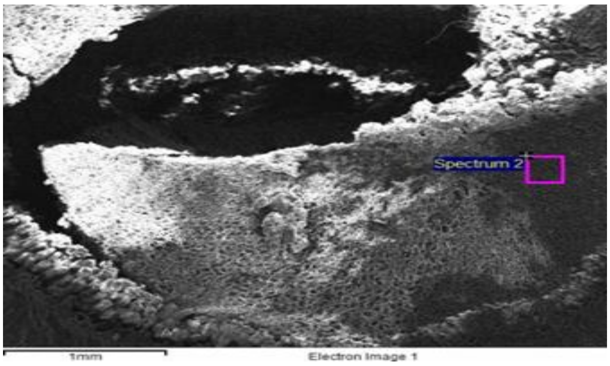


Fig. 7: A) middle layer of Q. infectoria cross section and elemental distribution by SEM-EDX B) Inner layer of Q. infectoria cross section and elemental distribution by SEM-EDX

Table 4: Elemental Disribution by SEM-EDX

Element	Weight %		Atomic %	
	A	B	A	B
C	52.56	49.42	59.72	56.83
O	47.07	49.63	40.14	42.84
K	0.38	0.43	0.13	0.17
Ca	n.d	n.d	0.47	0.16

The apoplastic (extracellular) or symplastic (intracellular) pathways bring the minerals from the root to the entire plants[17]. The minerals content of the galls are produced with the aid of enzymes and soil-based microorganisms from soil existed in the form of mineral salts[18]. Through various metabolic processes, these minerals will no longer exist as salts, and become complexed with various carbohydrates, lipids, and proteins present in the plant as part of the living organism[19]. Most mineral supplements contain minerals in the form of rocks (e.g. calcium carbonate). Mineral salts are natural food for plants, not a natural food for humans. Our current study measured the trace minerals in whole galls of *Q. infectoria* including water extract. The measurements of trace minerals in the galls could provide the standard for maintaining the quality of the galls used in preparation of any types of supplement or extract from this plant.

Analysis of powder nut revealed the starch granules (Figure 4A). The lignin bodies seen in image of water extract (Figure 4B). The elemental distribution revealed the presence of carbon, oxygen, magnesium, silica, potassium and calcium in the outer surface of the nutgalls (Figure 4C).

Cross section of outer layer of nutgalls revealed the distribution of elements carbon, oxygen, kalium, calcium, magnesium and silicon (Figure 5A). The surface with rugae like showed the distribution of fewer elements without the present of magnesium and kalium. (Figure 5B).

Figure 5: A**)** Outer surface, B**)** outer rugae like surface of *Q. infectoria* elemental and C) elemental distribution by SEM-EDX

We had found that, the pericarp layer exhibits more abundant elements with the addition of natrium, magnesium, silicon and chlorine (Figure 6A). The richness of elemental distribution in this section might support its role for nutgalls protection of minerals deficiency. This part is the hardest structure of the nutgalls. However, outer layer (Figure 6B) only consist of basic elements of carbon, oxygen. kalium and calcium (Figure 6C).

Figure 6: A) Outermost layer of QI (pericarp) and B) outer layer of QI cross section and C) elemental distribution by SEM-EDX

The middle layer only comprises of carbon,oxygen and kalium (Figure 7A-B). Therefore this part seemingly comprised a softer structure compare to the other parts of the galls. Our findings suggested that calcium is the most present elements in most of the galls part except in the middle layer.

Plants like *Q. infectoria* use these minerals as structural components in carbohydrates and proteins; organic molecules in metabolism, such as magnesium in chlorophyll and phosphorus in ATP; enzyme activators like potassium, and for maintaining osmotic balance. Calcium is highly implicated in maintenance the firmness of fruits [20] and its requirements in fruits are related to cell wall stability and membrane integrity[21]. The richness of these minerals in *Q. infectoria* nutgalls might contribute to the hard and blunt structure.

CONCLUSION

In conclusion, the used of SEM-EDX microscopy would provide the microscopical image for identification and authentication of the *Q. infectoria* galls obtained elsewhere. The data from this study supported previous reports regarding the parameters used for identification and authentication of the galls. The elemental distribution might indicate the favorability of growth environment from its origin and supported structure and function of this plant.

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