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Features of *Vespa affinis* Nest Based on X-ray Diffraction, Spectroscopic, and Surface Morphological Studies

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Abstract

The main focus of this research is to ascertain the X-ray diffraction (XRD) pattern, Fourier transform infrared (FTIR) spectroscopic pattern, and surface morphology of a hornet (*Vespa affinis*) nest. The optical microscopic (OM) analysis demonstrated that the nest presented an irregular formation of partition materials. This was confirmed via scanning electron microscopy (SEM), which revealed the presence of a unique form with a honeycomb-like pattern. Meanwhile, the materials largely consisted of carbon and oxygen elements, as was revealed by the energy dispersive X-ray (EDX) analysis, while the XRD analysis indicated that the nest was composed of amorphous materials of cellulose and lignin. Finally, the (FTIR) spectroscopy analysis revealed the presence of a number of peaks, which indicated that the material also consisted of a mixture of cellulose, hemicellulose, and lignin materials. It is envisaged that these results would widen the possibilities of fundamental scientific research on forest products, particularly the use of the *V. affinis* nest for further biological and chemical research.

Keywords: Vespa affinis, hornets, morphology, biominerals, nest.

Abstrak (Indonesian)

Fokus utama penelitian ini adalah untuk mengkaji pola difraksi sinar-X (XRD), pola spektroskopi *Fourier transform infrared* (FTIR), dan morfologi permukaan sarang tawon (*Vespa affinis*). Analisis mikroskopis optik (OM) menunjukkan bahwa sarang tawon tersebut memiliki partisi-partisi yang tidak teratur. Hal tersebut juga diperkuat melalui pemindaian mikroskop elektron (SEM), yang mengungkapkan keberadaan bentuk unik pada pola seperti sarang tawon tersebut. Sementara itu, bahan penyusun sarang tawon tersebut, sebagian besar terdiri dari unsur karbon dan oksigen, sebagaimana terungkap dari analisis *energi dispersive X-ray* (EDX), sedangkan analisis menggunakan XRD menunjukkan bahwa sarang tersusun dari bahan amorf selulosa dan lignin. Selanjutnya, analisis berbasis FTIR mengungkapkan adanya sejumlah puncak yang menunjukkan bahwa bahan tersebut juga terdiri dari campuran bahan selulosa, hemiselulosa, dan lignin. Temuan ini diharapkan akan dapat memperluas cakrawala penelitian dasar tentang hasil hutan, khususnya pemanfaatan sarang *V. affinis* untuk penelitian-penelitian bidang biologi dan kimia.

Kata Kunci: Vespa affinis; tawon; morfologi, biomineral, rumah tawon

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INTRODUCTION

Vespa affinis is one of the most popular vespid species in Southeast Asia, one that is highly feared by humans since their attack can be extremely dangerous. Indeed, certain reports have stated that a *V. affinis* attack can cause an acute kidney injury leading to rhabdomyolysis [1], acute pulmonary edema, and acute renal failure [2]. Meanwhile, a further investigation revealed that a *V. affinis* attack can lead to serious issues in young humans, including acute liver injury and acute renal failure [3]. In fact, phospholipase A1 is regarded as the main compound responsible for fatal vespid allergy [4-5]. However, a *V. affinis* is also regarded as an edible insect in some areas, including North East India [6]. A study using an aqueous extract of *V. affinis* revealed that the extract exhibits good antioxidant activities [7], while other studies have demonstrated that the *V. affinis* has circular molecules that are larger than those in other hornets [8]. Further studies have focused on the effects of the *V. affinis* attack and the possible uses of the *V. affinis* itself as an edible material [9-11]. Apart from these dangerous things, it turns out that this type of wasp plays an important role in the pollination process in cucumber plants (*Cucumis sativus* Linn.) [12].

However, the chemical features of the *V. affinis* nest have been rarely investigated. Therefore, the aims of this descriptive research were (i) to microscopically characterize the surface of the *V. affinis* nest, (ii) to assess the elemental composition and diffraction pattern of the *V. affinis* nest, and (iii) to assess the functional groups of the *V. affinis* nest. The findings are expected to be useful to further research; especially in relation to the use of the *V. affinis* nest for both biological and chemical applications.

MATERIALS AND METHODS

Materials

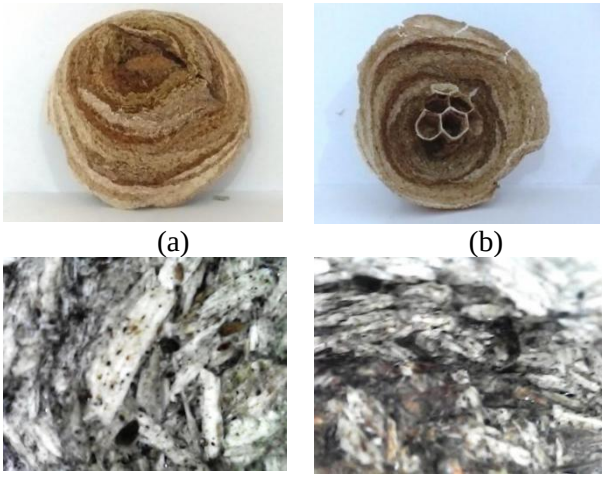
The *V. affinis* nest was obtained from the city of Bengkulu. The nest was collected by cutting it from a flower tree of *Clitoria ternatea* and was then transferred to a sample bottle and kept in a desiccator.

Methods

The morphology of the nest was investigated using an optical microscope (1600x) and a scanning electron microscope (SEM), with an EDX analyzer (JEOL JSM 6510 LA) incorporated to identify the nest's elemental composition. Meanwhile, the functional group analysis was performed using a FTIR spectrometer with a scan range of 4000–500 cm^{-1} without sample pre-treatment (Compact FTIR Spectrometer ALPHA II). Finally, XRD analysis was conducted (Bench-top powder X-ray diffractor, 600 W X-ray tube, D/teX Ultra silicon strip detector) to investigate the phase of the nest's material.

RESULT AND DISCUSSION

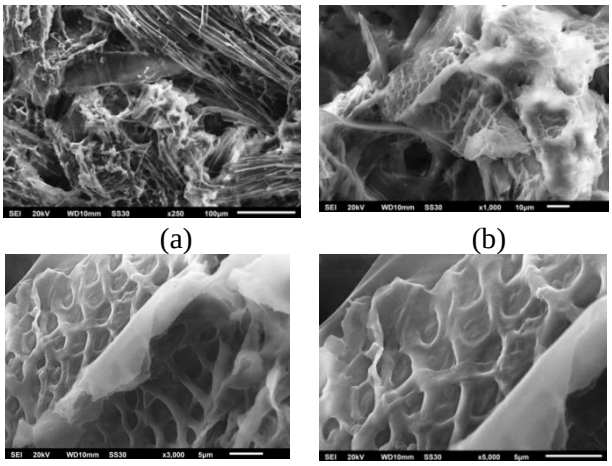
The newly built *V. affinis* nest was taken from a tree of *C. ternatea* and a photograph was taken immediately, as shown in Fig. 1. Generally, the inside of these nests takes on a honeycomb-like form, much like in common wasp nests (**Fig. 1a–b**); however, the OM analysis indicated that the surface of the nest was irregular in terms of form (**Fig. 1c–d**). It is mentioned that some wasps use a special adhesive made by their saliva glands to build their nests [13].



(a) (b) (c) (d)

Figure 1. (a) Photograph of outer of *V. affinis nest* (b) inner of *V. affinis* nest, and (c-d) optical microscope pattern of *V. affinis* nest (magnification 1600x)

The OM result was further investigated using SEM. As **Fig. 2** shows, the surface of the nest was actually arranged in an orderly manner and had an artistic texture that is very beautiful to the eye.



(a) (b) (c) (d)

Figure 2. (a) SEM pattern of *V. affinis* nest

The surface of the nest contained a number of cavities, which likely serve to help the *V. affinis* to live and breed in the nest. Further EDX analysis of the *V. affinis* nest were carried out to investigate the major elements contained in the material used by the wasp to form its home, with the results shown in **Fig. 3**. Here, the main elements were determined to be carbon (C) at 0.227 (55.4%) and oxygen (O) at 0.525 keV (44.21%) with very low contents of aluminum, sulfur, and potassium [14-15].

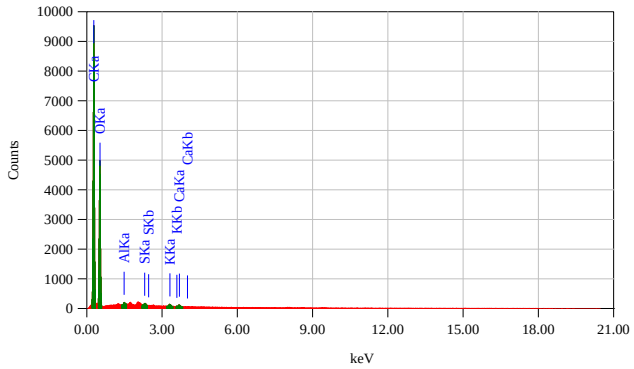


Figure 3. EDX pattern of V. *affinis nest*

Given the major components, it can be stated that the main material is organic material from the hydrocarbon groups. It is common that the elucidation of organic materials can be assisted by FTIR analysis [16], and further investigation using FTIR analysis also revealed that the *V. affinis* nest contained a number of organic functional groups, as shown in **Fig. 4**.

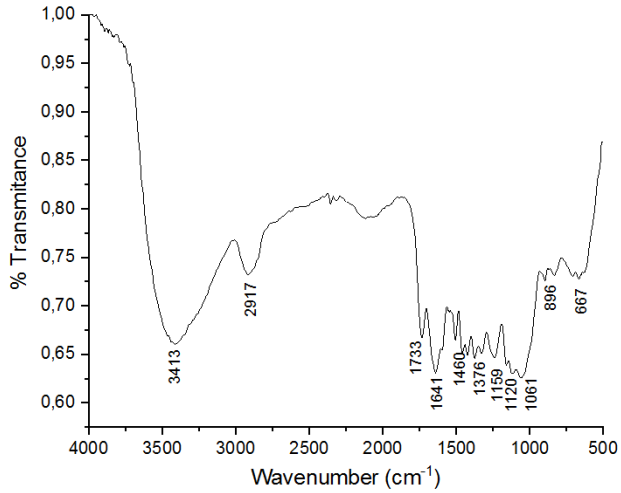


Figure 4. FTIR pattern of *Vespa affinis* nest

Based on F**ig. 4**, the *V. affinis* nest gives FTIR spectrum, which displayed characteristic absorption patterns with the explanations as follow: The peak at 897 cm^{-1} is because of C-H rock vibration of glycosidic linkages between sugar units [17]. The peak at 1061 cm^{-1} may be due to C-O-C glycosidic band stretching vibration of pyranose ring skeletal while the peak at 1120 and 1159 cm^{-1} corresponds to C-O asymmetric bridge stretching [18]. The peak at 1460 cm^{-1} may be due to methylene bending vibration [19]. The peak at 1641 cm^{-1} may be formed due to the bending mode of adsorbed water [20-22]. The peaks at 2917 and 3414 cm^{-1} correspond to C-H stretching vibration and O-H groups stretching vibration, respectively; two specific markers that common for cellulose [23]. The presence of shoulder peak at 1733 cm^{-1} indicates that there are acetyl and uronic ester groups of the hemicelluloses or the ester linkage of lignin [18]. In addition, the presence of peaks at 1376 and 1506 cm^{-1} indicates the presence of C=C of the aromatic ring of lignin [24]. So, the presence of peaks at 1376, 1506, 1733, cm^{-1} indicates the presence of hemicelluloses and lignin. Given the presence of these functional groups, it could be predicted that the *V. affinis* nest is built from a mixture of cellulose, hemicellulose, and lignin compounds. More research is needed to figure out exactly what this *V. affinis* nest is constructed of.

This prediction was also supported by the presence of an amorphous phase of natural cellulose (mixture of alpha and beta forms) and lignin, as was indicated by the appearance of a broad peak at 2θ = 22.8° and sharp peak at 2θ = 17.4°, respectively under X-ray Diffraction (XRD) analysis as shown in **Fig. 5**. The peak at 2θ = 17.4° is in line with the reported results related to the common commercial lignin [25].

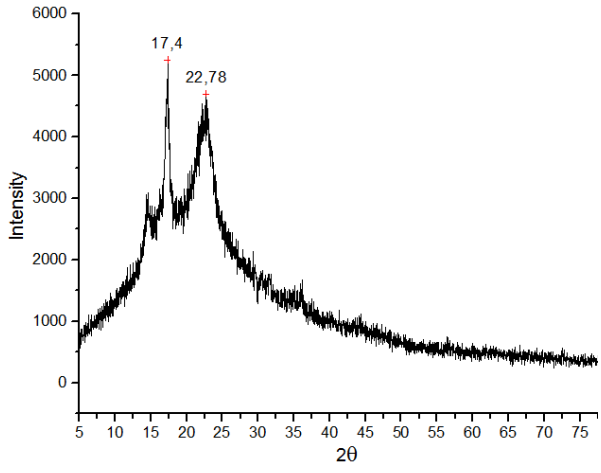


Figure 5. XRD pattern of *V.affinis* nest

CONCLUSION

This study demonstrated that the *V. affinis* nest has an interesting morphology. The main components of the nest are organic compounds, which are likely to be a mixture of cellulose, hemicellulose, and lignin. As is the case with other wasps, it can be predicted that the

nests are made using a special adhesive produced by *V. affinis*. The availability of this material in its natural form offers an opportunity for further investigations into the biology and chemistry of natural materials.

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