Initial investigations on the surface properties of *Hibiscus tiliaceus* Linn.

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Abstract: There is a growing interest in biomimicry that explore nature-inspired principles and properties in the design and development of innovative materials and technologies. Therefore, it is important to investigate the surface properties of plants as inspirations for a wide spectrum of biomimetic applications. In this study, leaves of *Hibiscus tiliaceus* Linn., of the family Malvaceae, and popularly known as malobago (Tagalog), were investigated for their wettability and oleophilicity using the following parameters: (1) microstructures present in the epidermal surface of the leaf (SEM), (2) wettability (tensiometer; contact angle, θ), and (3) oil sorption capacity (OSC). The micrographs of the abaxial leaf surface of *H. tiliaceus* showed a dense network of overlapping and intertwining nonglandular trichomes, consisting of 8-ray stellates. The new proposed contact angle for superhydrophobicity is 145°, as determined by the measured wetting and adhesion interactions between water and a variety of surfaces (Law, 2014). The mean contact angle measured for *H. tiliaceus* was $\theta=142.84\pm3.81°$. For the leaf's oil sorption capacity, the mean value obtained using motor oil was 1.7693 ± 0.0793 g/g. Previous studies showed that hierarchically structured surfaces are associated with high contact angles and sorption capacities (Barthlott et al., 2016). For *H. tiliaceus,* the near superhydrophobic contact angle and OSC>1 may also be attributed to the dense and overlapping stellates and microstructures present on the plant leaf surface. The information reported in this work will be helpful in exploring the biomimetic potential of *H. tiliaceus*, especially its usefulness in remediation (e.g. oil spill clean-up, self-cleaning applications).

Key Words: *Hibiscus tiliaceus* Linn.; trichomes; wettability; sorption; biomimetics

1. INTRODUCTION

Biomimicry is now considered as an exciting route to the development of new materials (Reed et al., 2009; Murray, 2018). The use of biomimetic principles provides a foolproof approach to product design, relying on the unique properties exhibited by living organisms. For plants, a comprehensive review of biomimetic applications has been provided (Barthlott et al., 2016). The structural hierarchy and chemical composition of the plant surface both affect surface roughness which then influence properties (Koch and Barthlott, 2009; Barthlott *et al.*, 2017). Scanning electron microscopy has revealed different micro- and nanostructures that include trichomes or plant hairs (Fernandez et al., 2014; Hardin, 1979).

Hydrophobicity, hydrophilicity, and oleophilicity are some of the important properties of plants. The first two are associated with wettability, the ability of a surface to retain water (Koch and Barthlott 2009). In a completely wettable leaf (hydrophilic), the surface is covered by a thin film of water while in a non-wettable leaf (hydrophobic), water is repelled and forms beads. Contact angle (θ) is the angle formed by the water droplet at the three-phase boundary where liquid, gas, and solid meet. A surface is hydrophobic when its contact angle is $\theta \geq 90°$, and is hydrophilic when $\theta < 90°$. The concept of superhydrophobicity, when the surface has no affinity for water, has been proposed to be adjusted from the more popular consensus of $\theta \geq 150°$ to the more accurate $\theta \geq 145°$, based on measured wetting and adhesion interactions (Law, 2014). The surface structures of water-repellent plants were presented and attributed to hierarchical surface micro- and nanostructures present (Barthlott and Neinhuis, 1997). Perhaps, the most popular plant that demonstrates superhydrophobicity and self-cleaning property (lotus effect) is *Nelumbo nucifera* ($\theta = 162°$). Oleophilicity, on the other hand, refers to the preferential adhesion of a surface to oil particles. Oleophilic plants have the potential for oil-spill clean-up. As summarized above, hierarchical structures influence properties. It is the aim of this study to introduce *Hibiscus tiliaceus* Linn. in this area as preliminary data indicated that it is nearly superhydrophobic and is also oleophilic.

Hibiscus tiliaceus Linn., of the family Malvaceae, commonly called coast cotton wood, is a typical coastal plant in many regions including South-East Asia. *H. tiliaceus* can be found throughout the Philippines where it is popularly known as malobago (Tagalog). It is a small evergreen tree, 4-10 m tall when mature, having a broad crown of widely spreading and crooked branches that form dense thickets. The leaves are suborbicular in morphology, with a pointed apex and heart-shaped base, and a nearly smooth margin. Different parts of *H. tiliaceus* are used in traditional medicine, and are also found to exhibit anti-microbial/cancer properties (stuartxchange.com; keys.trin.org.au). To date, there are no published data yet reporting the leaf structures and associated properties of *H. tiliaceus* Linn. Reported in this paper is the first part of the study providing information on the type of trichome present on the plant surface, as well as the leaf's wettability, and oil sorption capacity. New knowledge from the study can be used in the future to identify the potential biomimetic applications of the plant.

2. METHODOLOGY

2.1 Plant Collection

Hibiscus tiliaceus leaves were collected from De La Salle University (DLSU) Laguna Campus, Biñan, Laguna, Philippines, during the dry period between October 2017 and March 2019. The plant was identified at the Bureau of Plant Industry, and a voucher specimen is deposited at the DLSU Manila Herbarium. The collection of the plant leaves did not pose any ethical concern as evaluated by the DLSU Research Ethics Office (REO).

2.2 Preparation of Leaf Samples

The preparation of the leaves followed published procedures with modifications (Wang, et. al. 2014). Mature and undamaged leaves, of nearly equal leaf areas, were used. For each leaf, three distinct areas were labelled to represent the lower, middle and upper areas, as needed for imaging and wettability studies.

2.3 Experimental Design

2.3.1 Scanning Electron Microscopy

Scanning electron microscopy was done using Phenom XL desktop SEM with Pro Suite software package (Phenom World, Netherlands). Representative parts of the leaf were cut up (5mm x 5mm) and attached to metal stubs. Magnifications of 310x, 500x, and 1000x were used for the 2D images. The 3D images showing roughness reconstruction were also taken.

2.3.2 Wettability

The wettability (contact angle, CA (θ)) of the leaf surface of *H. tiliaceus* was determined following standard procedures with modifications (Aryal and Neuner, 2016). The setup consists of the Theta lite contact angle meter (Attension, Sweden) with a built-in camera. Samples were cut up (1.5cm x 1.0cm) and then mounted onto a glass slide. The water droplet volume used was 5μL.

2.3.3 Oil Sorption Capacity

The oil sorption capacity (OSC) of *H. tiliaceus* leaves was measured following standard procedures with modifications (Zeiger et al., 2016). The entire leaf was submerged for 30 s in two types of oil: Apollo mineral oil (Sonneborn, Inc. PA, USA) and Shell Helix HX-3 SAE-40 mono-grade motor oil (Shell Brands International AG, Malaysia). International AG, Malaysia). The leaf was left suspended for 90 s. The oil sorption capacity (OSC) is the difference in mass before and after immersion in oil divided by the initial mass of dry leaf (Behnood et al., 2013).

2.3.4 Statistical Analysis

Statistical analysis (t-test, α=0.05) was done using R software, version 3.5.1 (CRAN), to compare the oil sorption capacities of *H. tiliaceus* leaves after submersion in mineral oil and motor oil. A $p<0.05$ is considered significant.

3. RESULTS AND DISCUSSION

3.1 Surface Structures

Figure 1 shows the 2D (*a,b*) and 3D (*c,d*) SEM micrographs of the abaxial leaf surface of *H. tiliaceus*. It is evident from image *a* that the epidermal layer consists of a dense network of trichomes that are superimposed over one another. In image *b* (see arrow), the trichomes are nonglandular, 8-ray simple stellates that are characterized by rays fused at the central base. The

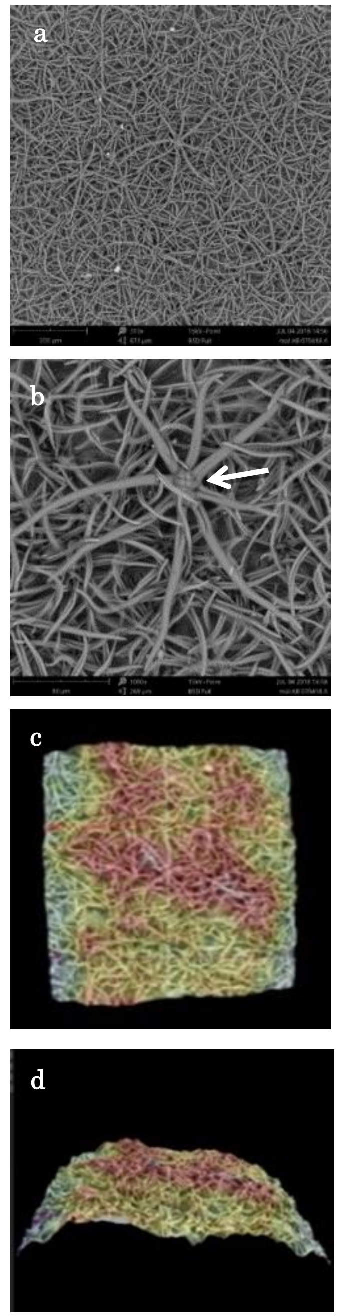

Fig. 1. Scanning electron micrographs of the abaxial leaf surface of *H. tiliaceus*. 2D (scale bar = 100 μm): a-310x and b-1000x. 3D (537 μm field of view): c and d.

trichomes are intertwined, resulting to mesh-like microstructures as likewise revealed by the 3D surface roughness (images *c,d*). It has been reported that micro- and nano-structures influence plant properties. It is hypothesized here that the same will be observed for *H. tiliaceus*. Wettability and oil sorption capacity were studied to evaluate this.

3.2 Wettability

H. tiliaceus leaves gave a mean contact angle of 71.71±4.14° (n=6) and 142.84±3.81° (n=9) for the adaxial and abaxial sides, respectively. This classifies the adaxial side as hydrophilic and the abaxial side as nearing superhydrophobicity as defined previously (θ=145°; Law, 2014). Earlier studies indicate that leaves with trichomes, and particularly those for which trichome density is greater than 25 mm^{-2}, are more hydrophobic (Pandey and Nagar, 2002). Further investigations have shown that the adaxial side of *Callistephus chinensis* leaves, characterized by a CA of 139°, was densely covered with conoid trichomes, while the adaxial side of *Cucurbita pepo* leaves, sparsely covered by villous structures, had a CA of 70° (Wang et al., 2015). As mentioned earlier, *H. tiliaceus* has a dense network of overlapping, stellate trichomes on its abaxial side. It is possible that these complex and dense leaf structures are responsible for the high CA. It has been established that surface roughness and epicuticular waxes give rise to enhanced nonwetting properties (Wagner et al., 2003). As described in the principle of superimposed hierarchical sculptures, tiered structures cause an extreme reduction of surface contact area and thus prove to be mechanically more stable under dynamic conditions than simple structured surfaces (Barthlott, 2016). For example, in *Nelumbo nucifera* (lotus), the leaf surface consists of papillose epidermal cells that form micro-papillae that lead to microscale roughness (Barthlott, et. al., 2017). Observed in the SEM images of *H. tiliaceus* is the complexity of the microstructures as well as a high trichome density which have been reported previously to promote hydrophobicity in other plants (Bhushan and Nosonovsky, 2010).

3.3 Oil Sorption Capacity

The oil sorption capacity of a plant material is of interest as this property can be explored for remediation purposes (i.e., oil spill clean-up). The mean oil sorption capacities of *H. tiliaceus* were 1.4227±0.0622 g/g and 1.7693±0.0793 g/g using mineral oil and motor oil, respectively (Figure 2). Statistical analysis (α=0.05) showed that there is a significant difference in the OSC between mineral oil and motor oil (p=0.0029). The higher sorption observed using motor oil is consistent with reported data that adsorption is more efficient in heavier or more viscous oils (Boleydei et al., 2018). It is likely that the high OSC of *H. tiliaceus* can be attributed to the dense and complex network of overlapping stellates observed in the plant. Other studies indicate that hierarchically structured surfaces are associated to high OSCs. The water fern, *Salvinia molesta*, exhibited both superhydrophobic and superoleophilic properties (*Salvinia* paradox) and these were attributed to the dense coverage of egg-beater shaped *S. molesta* was found to adsorb per leaf area (m^2) an average 630.0 mL of artificial crude oil, 774.8 mL of trichomes (Barthlott et al., 2016; Zeiger et al., 2016). hydraulic oil, 989.3 mL of crude oil OK-679, and 1099.6 mL of crude oil EK-651. In the same study comparing four different *Salvinia* species, it was also observed that those with longer and fused/bent trichomes exhibited the highest OSCs. Though extensive reviews of bio-based materials for oil spill treatment are available (Doshi, et al., 2018, Alaa El-

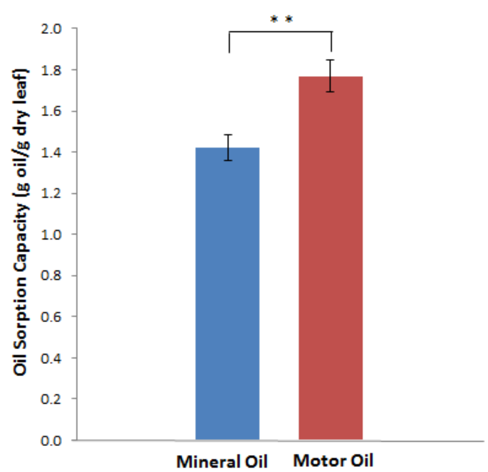

Fig. 2. Oil sorption capacity of *H. tiliaceus* Linn.

Din, et al., 2018), treatment conditions and type of oil used varied: rice husks (OSC of 3.7 kg/kg using gasoline) after pyrolysis (480°C) (Angelova, et al., 2011); potato peel (2.15 g/g, waste lubricating oil) after oven drying (70°C) and crushing (Tontiwachwuthikul, et.al., 2016); banana skin (5-7 g/g, crude oil) after sun-drying, oven drying (70°C), crushing, and sieving (Alaa El-Din, et al., 2018). *H. tiliaceus* leaves used in the study offer an attractive approach to oil spill clean-up because it can be used directly and will not need any leaf pretreatment, hence, will minimize the carbon footprint of the remediation process.

4. CONCLUSIONS

The SEM micrographs of the abaxial leaf surface of *H. tiliaceus* revealed a dense network of overlapping and intertwining 8-ray stellates. The contact angle measured was θ=142.84±3.81°, while the oil sorption capacity using motor oil was 1.7693±0.0793 g/g. The nearly superhydrophobic and high sorption properties can be attributed to the complex microstructures on the leaf surface. This is consistent with previous studies indicating the relationship between surface roughness, wettability, and oleophilicity. To date, this is the first report on the structures and properties of *H. tiliaceus*. Future studies can further evaluate the potential biomimetic applications of the plant.

5. ACKNOWLEDGMENTS

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