Surface morphological and wetting characterization of the hydrophobic and superhydrophobic leaves of *Pistia stratiotes* L., *Salvinia molesta* D.Mitch., *Ananas comosus* (L.) Merr. and *Dyckia platyphylla* L.B. Smith for bioinspired oil adsorbent materials

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Abstract. In this paper, the surface morphology and wetting properties towards deionized water and pure oil samples with varying carbon chain lengths of adaxial and abaxial leaf surfaces of *Pistia stratiotes* L., *Salvinia molesta* D.Mitch., *Ananas comosus* (L.) Merr. and *Dyckia platyphylla* L.B. Smith were characterized. The surface morphological characterization showed that *P. stratiotes* L. has uniseriate trichomes on adaxial (ad) and abaxial (ab) surface and *S. molesta* D.Mitch. has multifaceted egg-beater shaped trichomes and achlorophyllous filaments. Both surfaces of the bromeliads, *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith have peltate scutiform trichomes. Overall, *P. stratiotes* L. has the greatest trichome density (no. of trichomes/mm²) of ad 36.77 and ab 40.10 among *A. comosus* (L.) Merr. > *D. platyphylla* L.B. Smith > *S. molesta* D.Mitch. Contact angle measurement showed that *P. stratiotes* L. has the best water repellency having (154.39 ± 3.26) ad > *S. molesta* D.Mitch. > *A. comosus* (L.) Merr. > *D. platyphylla* L.B. Smith and (147.90 ± 3.17) ab > *A. comosus* (L.) Merr. > *D. platyphylla* L.B. Smith > *S. molesta* D.Mitch. Lastly, *P. stratiotes* L. showed the best common pure oil adsorption capacity among the four species. Therefore, the understanding on the fundamental concept on how the leaf surface of *P. stratiotes* L. adsorbs the oil and reacts in response to various solvents adsorbed on the leaf surface was established.

1. Introduction

Structural evolution caused by mutation and natural selection over millions of years provide a large diversity of structures and capabilities on various biological surfaces particularly on plants [1]. One of the capabilities of plant surfaces is superhydrophobicity which have been studied as potential models for self-cleaning and water repellent surface that are useful for solving environmental and industrial issues. This is characterized by the repellency of water on a surface with extremely high-water contact angles and various adhesion properties [2].
Several hydrophobic and superhydrophobic plant surfaces such as adaxial surface of *P. stratiotes* L. and *S. molesta* D.Mitch. have been reported to be useful for bioinspired materials where water repellency and fluid flow are important [3][4]. Also, both *P. stratiotes* L. and *S. molesta* D.Mitch. possess selective wetting property which can be useful for cleaning up accidental oil spills while preventing secondary pollution in the environment. These are the few known plants with high potentials for biomimetics which can inspire the advancement of developing superhydrophobic materials because of their capacity to absorb oil while repelling water [5]. Yet, the entire leaf surface of both plants has been less explored in terms of its wetting capacity.

Evidences have shown that trichomes on the adaxial surface of leaves from pineapple (*A. comosus* (L.) Merr.), can repel water [6]. It has been observed that within 3 to 6 hours, water droplets were not absorbed by the leaves of pineapple but remained spherical in shape on the surface. Leaves of several species of plants under genus *Dyckia*, exhibited trichome-mediated interfaces with surface water wherein both the abaxial and adaxial surfaces possess hydrophilic and hydrophobic microstructures [7]. Proper identification of which leaf surface is hydrophobic or hydrophilic has not been well established.

Despite some plants are already known to possess both oleophilic and hydrophobic trichomes on their leaf surfaces, the mechanisms involved in their adsorptions of oil and repellence of water are not well established. Therefore, this study examines the surface roughness of leaves from four reported hydrophobic plants: *P. stratiotes* L., *S. molesta* D.Mitch., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith using scanning electron microscope. The effect of trichomes on water repellency and oil attraction of the leaves is investigated. This is done by analysing the leaves’ surface wettability based on calculated work of adhesion and contact angle measurements. Oil adsorption capacity of the leaves is also computed by simulating oil spills using different pure oil solvents. By examining the microstructural features of leaves from the four plants and quantitatively analysing some of their physical properties, this study will aid envision synthetic biology to create new opportunities in advancing our knowledge about natural hydrophobic and oleophilic materials. It can also help conceptualizing new designs of hydrophobic and oleophilic systems in the future such as hydrophobic or oleophilic materials incorporated in systems used in electrodeposition, colloidal systems, nano- and photolithography [8, 9, 10, 11].

2. Methodology

2.1. Collection and preparation of leaf samples

Five individual plant samples of *P. stratiotes* L. were collected along the margins of Taal Lake, Municipality of Agoncillo, Batangas Province while five plant samples each of *S. molesta* D.Mitch. and *D. platyphylla* L.B. Smith were commercially purchased from plant cultivation centres located in Quezon City Circle and, Farmer’s Market, Metro Manila and in the Municipality of Lucban, Quezon Province. Five plant samples of *A. comosus* (L.) Merr. were purchased from an agricultural farm located in Silang Municipality, Cavite Province. Plant identifications were certified by the National Museum of the Philippines. Five sets of representative leaves were harvested from each plant samples.

2.2. Examination of surface micro/nanostructures

The first set of leaves were examined in three-dimensional (3D) perspective to characterize and compare the surface microstructures of *P. stratiotes* L. with that of *S. molesta* D.Mitch., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith. The leaves were treated properly to preserve the integrity of the dimensions of the trichomes and other microstructures found on the leaf surfaces [12]. To do so, the leaves were fixed in formol acetic alcohol (FAA) solution overnight and then cut into small square-sections measuring about 0.5 cm x 0.5 cm. The fixed leaf sections were mounted on glass slides and were examined and micrographed using Nikon Stereo Microscope (Model: SMZ745T) with Nikon DS-L3 Camera (Goldquest Biotechnologist, Inc.) [12, 13].

The second set of leaves were handed-over to the Microlab (Micro-Biological Laboratory Inc.), Makati, Philippines for permanent slide preparation. Leaf cross-section was prepared at about the
middle portion of each leaf using a razor blade. The cut sections were dehydrated in an ethanol dilution series (50%, 70% x 3, and 95%) and treated with isoxylene (50% Isopropanol and 50% xylene) for 20 minutes and thrice in xylene (20 min). After dehydrating, the leaf cross-sections were embedded in Paraffin Wax (McCormick: Paraplast) overnight. The embedded sections were cut further into thinner sections (~10 µm) using a microtome (Thermo/Microm HM 355 S) and then mounted on glass slides. The mounted sections were gently heated at 60°C on a slide warmer and stained with Safranin and Fast Green FCF [14]. Coverslips were placed over the mounted leaf cross-sections and were examined under optical photomicroscope (Nikon DS-Fi2 K14517, Brownstone Asia-Tech 10A; Goldquest Biotechnologist, Inc.) at 40x HPO.

Small epidermal strips were carefully isolated from the third set of leaves and were prepared following the procedure for stereomicroscopy to preserve the foliar trichomes [14]. These epidermal strips were removed from three locations (base, middle and tip) of both adaxial and abaxial surfaces of each leaf using a razor blade and were lyophilized overnight. The lyophilized strips were mounted over the stubs and sputter coated with gold [15]. The sputtered strips were examined under the SEM (JEOL JSM-5310; operating voltage: 10 kV) at x75, x150, x200, x350 and x500 magnifications [16] and then micrographed. The software integrated in JEOL JSM-5310 was used to measure certain dimensions of the trichomes, such as length, diameter and surface area [15].

To compute for trichome density, the x75 SEM micrographs taken from the three locations on the adaxial and abaxial surfaces of each leaf were used. On each micrograph a 9x9 grid was drawn. The first row of the grid was used to represent the first trial, second row for the second trial and third row for the third trial. In each grid, trichomes were manually counted and divided by the grid’s surface area (2.20 mm²).

2.3. Surface wettability and oil adsorption capacity

Epidermal strips (including cells of the cortex) were isolated from the fourth set of leaves [17]. One epidermal strip each were removed using a razor blade from the adaxial and abaxial surface of each sample. Each strip was mounted on a glass slide using double sided tape to achieve a flat and even surface during examination [12]. The mounted strips were brought to i-Nano Laboratory of STRC, De La Salle University for static measurement of the contact angle (θ) of three leaf locations (base, middle and tip) of the adaxial and abaxial surface using the tensiometer (ThetaLite 100) apparatus. Four 5 µL droplets of deionized water were applied on three locations over the surface of each strip using syringe [18]. Each droplet was micrographed using a microscope aligned for sideways observation of the droplet on the surface of interest. The micrograph of each droplet was then analysed by a program integrated in the apparatus to get an exact value of θ. A total of 20 replicates for each plant sample taken the fourth set of leaves were analysed in a randomized experimental design. Means and standard deviations for static θ of each plant samples were calculated and interpreted [12].

In addition to θ, Work-of-adhesion values (Wₘₜ) were also calculated using the fourth set of leaves. To measure the adherence and strength of interaction between solid surfaces (represented here by the abaxial and adaxial surfaces of leaf) to water, drop adherence were determined using the following formula:

\[ Wₘₜ = (1 + \cos \theta)γ_l \]

The fifth set of leaves was used for oil spill simulation to compute for the oil adsorption capacity of plant samples [5]. The simulation was done in triplicates, using 50 mL of warm tap water for four pure oil solvents: lauric acid, myristic acid, palmitic acid and stearic acid melted at 60 °C using water bath. Each solvent weighed 0.21 g for each leaf triplicate.

A uniform line square with a surface area of 40 mm² was drawn over the adaxial and abaxial surfaces of each leaf. The leaves were air-dried for 3 minutes and the initial mass (M₁, g) was measured using an analytical balance. Each leaf was soaked in the oil spill simulation for 20 seconds, air dried for 3 min to remove excess water before noting the final mass (M₂, g). The amount of adsorbed oil (g) was determined by:

\[ \text{Oil adsorbed} = M₂ - M₁ \]
Since each pure oil solvent has different carbon chains, the amount of molecules adhered on the leaf surface also differs. Therefore, to compute for this amount, the formula for stoichiometry using the Avogadro’s number (6.022 x 10^23) was used:

\[
\text{Amt. of molecules} = \frac{\Delta \text{mass (g)}}{\text{mol mass of pure oil (g)}} \times \frac{1 \text{ mole}}{6.022 \times 10^{23} \text{ molecules}}
\]

Similarities among *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith, *P. stratiotes* L. and *S. molesta* D. Mitch. based on obtained values for surface morphologies, wettability (work-of-adhesion and contact angle measurements) and oil adsorption capacity values were determined using the hierarchical clustering analysis.

2.4. Statistical analyses

One-way analysis of variance (ANOVA) through SPSS ver. 10.0 for Windows was used to compare the contact-angle measurements of the abaxial and adaxial surfaces of *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith, *P. stratiotes* L. and *S. molesta* D. Mitch. toward water as well as for the amount of the molecule of pure oil solvents adhered on each leaf surface. Paired sample T-test was used to determine if there is significant variation on the wettability of the adaxial versus the abaxial surfaces of each plant species towards deionized water. The means of θ values toward water and the amount of adhered common pure oils were further analysed and statistically compared by Tukey’s Test at 5% probability. Cluster tree diagram showing the relationship of all the gathered data was analysed and summarized by single-linkage hierarchical euclidean distances using Statistica™ (TIBCO Software Inc.) [15, 16, 19, 20].

3. Results and Discussion

3.1. Surface morphologies of hydrophobic leaves

The leaf surfaces of *P. stratiotes* L. on both the adaxial and abaxial sides were found to consist of simple, uniseriate foliar trichomes arranged in a single row (Figure 1a). Each is composed of a large basal cell upon which 25 to 30 epidermal cells arise from. The trichome in *P. stratiotes* L. is tapering from basal up to the tip due to the decreasing diameter of the epidermal cells extending acropetally. Hence, terminal portion of each trichome always end with a half-spindled apical cell. The cross-section photomicrographs showed the presence of uniseriate trichomes on both adaxial and abaxial surface (Figure 1c).

The complex hairy leaf surface of *S. molesta* D. Mitch is found to be covered by egg-beater shaped trichomes on the adaxial surface (Figure 1b) and by achlorophilically filamentous trichomes on the abaxial surface (Figure 1d). The trichomes of *S. molesta* D. Mitch. are considered multifaceted and, like *P. stratiotes* L. trichomes, have complex three-level hierarchical surface morphologies. Those trichomes found on the adaxial side of *S. molesta* D. Mitch. consist of single stalks with four uniseriate branches that reconnect at their last apical cells, hence, forming into egg beater-shaped structures. [21]. In adult leaves of *S. molesta* D. Mitch., the apical cells of each trichome is collapsed. Apart from these four cells, the entire leaf including the remaining part of the trichome is covered by wax-like crystals [22]. As a result, only the four apical cells of the trichomes appear ‘smooth’ while the rest of the leaf surface appear ‘rough’.

Trichomes both found in *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith (Figure 2) are almost identical. Their type is referred to as scale or peltate hair which appear to be target-shaped or shield-like attached to the leaf surface by its central lower surface. All peltate trichomes consist of group of discoid cells which are usually united from a stalk or directly attached to the leaf surface [23]. Trichomes of bromeliads (i.e., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith) are classified as peltate scutiform trichomes since these trichomes comprise of multicellular shield and an epidermal stalk consisting of one to five cells [24]. *A. comosus* (L.) Merr. trichomes (Figure 2a-b) appeared to be mushroom-shaped scaly outgrowths surrounding the stomata and are reported to moderate loss of
water [25]. Their trichomes are more abundant on abaxial than adaxial surfaces which give shiny silvery color on normal condition.

Morphological properties of foliar trichomes found in *P. stratiotes* L. as compared to *S. molesta* D.Mitch., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith are shown in Table 1. Note that trichome heights of *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith were not determined due to the positions of these trichomes when the micrographs were taken which render height measurements impossible (Figure 2). In terms of height, trichomes on the adaxial leaf surfaces of *P. stratiotes* L. (1.26-2.39 mm) are shorter than those found in *S. molesta* D. Mitch. (2.46-4.48 mm). In contrast, heights of trichomes found on the leaf abaxial surfaces of *S. molesta* D. Mitch. (1.75-3.02 mm) > *P. stratiotes* L. (2.64-3.36 mm). Despite *S. molesta* D.Mitch. having dissimilar trichomes on both adaxial and abaxial leaf surfaces in terms of morphology, width/diameter (D) of trichomes on the adaxial surfaces in *S. molesta* D.Mitch. (stalk 0.37-1.38 mm; head 0.82-1.95 mm) are relatively greater compared to *P. stratiotes* L. (0.10-0.34 mm) > *A. comosus* (L.) Merr. (0.09-1.31 mm) > *D. platyphylla* L.B. Smith (0.08-1.23 mm), respectively. On the abaxial leaf surfaces, trichomes of *S. molesta* D.Mitch. (0.32-1.08 mm) are also greater in terms of width/diameter (D) compared to *P. stratiotes* L. (0.19-0.43 mm) > *A. comosus* (L.) Merr. (0.12-1.39 mm) > *D. platyphylla* L.B. Smith (0.07-1.05 mm). While quantitative measurements in Table 1 have shown that trichomes in *S. molesta* D.Mitch. are dimensionally greater compared to the trichomes of *P. stratiotes* L., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith, calculations of trichome density revealed otherwise. Regardless on which leaf surface, the number of trichomes/mm² are highest in *P. stratiotes* L. followed by *A. comosus* (L.) Merr. > *D. platyphylla* L.B. Smith > *S. molesta* D.Mitch. (Table 1). Seemingly, the shortness of quantitative dimensions of foliar trichomes in *P. stratiotes* L. are superseded by its high trichome density which consequently affects its leaf surface wettability. For *P. stratiotes* L., high trichome density is directly proportional to high water repellence of its surface. Same principle also applies for surfaces covered with hydrophilic trichomes.

![Figure 1](image1.png)

**Figure 1.** Photomicrographs of the adaxial and abaxial (c & d) of *P. stratiotes* L. (a & b) showing the uniseriate trichomes and *S. molesta* D. Mitch. (b & d) showing the egg-beater trichome.
Figure 2. SEM images of the adaxial (a, c) and abaxial (b, d) of *A. comosus* (L.) Merr. (a-b) and *D. platyphylla* L.B. Smith (c-d) showing the similar peltate scutiform trichomes.

Table 1. Comparison of the morphological properties and description of trichomes found on the adaxial and abaxial surface of *P. stratiotes* L., *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch.

| Species                  | Height (mm) | Width (mm) | Density (no. of trichomes/mm²) | Description                                                                 |
|--------------------------|-------------|------------|--------------------------------|-----------------------------------------------------------------------------|
| *P. stratiotes* L.       | 1.26-2.39   | 0.10-0.34  | 36.77                         | Both adaxial and abaxial surface have simple, multicellular, uniseriate trichomes. Tapering shape from basal up to apical cell in acropetal direction. |
| *A. comosus* (L.) Merr. | ND          | 0.09-1.31  | 27.72                         | Similar peltate trichomes for *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith. Scale, target-shaped or shield-like attached at central surface. Consist of discoid cells. |
| *D. platyphylla* L.B. Smith | ND          | 0.08-1.23  | 24.18                         | Adaxial surface has complex, multicellular, egg-beater shaped trichomes with stalks and head consisting of four simple uniseriate branches reconnected at their last two apical cells. |
| *S. molesta* D.Mitch.   | 2.46-4.88   | 0.32-0.82  | 8.26                          | Abaxial surface has multicellular achlorophyllous trichomes, needle-shaped or acicular. |

a = n=20; b = n=3 per leaf portion; c = diameter; ND = not determined.
3.2. Surface wettability analysis using contact angle (θ) measurement and work-of-adhesion (Wa)

The mean values of contact angle (θ) measurements on three locations (base, middle and tip) of both adaxial and abaxial surfaces of *P. stratiotes*L., *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith and *S. molesta*D.Mitch. using deionized water are summarized in Table 2. Graphical representation for comparing the mean values of θ from these three locations for both adaxial and abaxial leaf surfaces of the four plants are shown in Figure 3.

In general, both the adaxial and abaxial leaf surfaces of *P. stratiotes*L. have the highest θ values compared to *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith and *S. molesta*D.Mitch. (Table 2). Its adaxial leaf surfaces, due to high θ values (154.39 ± 3.26) is superhydrophobic (>150°) [22] while its abaxial leaf surfaces with similarly high θ values (147.90 ±3.17) is hydrophobic (90°-150°) [26]. In *S. molesta*D.Mitch., however, only its adaxial leaf surfaces (θ values = 153.56 ± 0.37) is hydrophobic [22, 26] while its abaxial leaf surfaces (θ values = 42.04 ± 4.55) is hydrophilic [26]. For both *A. comosus*(L.) Merr. and *D. platyphylla*L.B. Smith, their adaxial leaf surfaces are hydrophilic [26] with θ values of 52.01 ± 4.96 and 37.17 ± 2.90, respectively while their abaxial leaf surfaces are hydrophobic [22] with θ values of 130.61 ± 4.33 and 101.98 ± 3.10, respectively.

Among the three different locations (base, middle and tip) of adaxial leaf surfaces in *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith, *P. stratiotes*L. and *S. molesta*D.Mitch., the basal locations have the highest θ values (Figure 3a). In these locations, *P. stratiotes*L. and *S. molesta*D.Mitch. have θ values >150° or mean θ = 156.88° and 153.96°, respectively while *A. comosus*(L.) Merr. and *D. platyphylla*L.B. Smith both have water θ values < 90° or mean θ = 57.12° and 40.43°, respectively. Although it’s the basal locations of adaxial leaf surfaces having the highest θ values for *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith, *P. stratiotes*L. and *S. molesta*D.Mitch., there are no statistical differences among basal locations for every plant examined. This is most evident with *P. stratiotes*L. which has the highest value of p-value > 0.05 compared to *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith and *S. molesta*D.Mitch. Similarly, all other locations on the adaxial leaf surfaces of all four plants measured also reveal no statistical differences. In terms of surface wettability, therefore, there is no variation among these three locations on the adaxial leaf surfaces of the four plants examined.

The θ values from three different locations on the abaxial leaf surfaces of *A. comosus*, *D. platyphylla*, *P. stratiotes* and *S. molesta* is shown in Figure 3b. The middle locations of *P. stratiotes*L. leaves (θ values = 150.77°) and *S. molesta*D.Mitch. leaves (θ values = 46.71°), the tip of *A. comosus*(L.) Merr. leaves (θ values = 135.61°) and base of *D. platyphylla*L.B. Smith leaves (105.45°) have the highest mean θ. As in the adaxial leaf surfaces, there is no statistical difference among these locations on the abaxial leaf surfaces of *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith, *P. stratiotes*L. and *S. molesta*D.Mitch. Therefore, there is no variations on surface wettability among these locations from the four plants examined.

Work-of-adhesion (Wa) values for the liquid adherence of water to the adaxial and abaxial leaf surfaces of *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith, *P. stratiotes*L. and *S. molesta*D.Mitch. were calculated using the measured contact angles (mean values). The calculated values are summarized in Table 2. On the adaxial surface, *D. platyphylla*L.B. Smith showed highest value for work-of-adhesion (Wa) followed by *A. comosus*(L.) Merr. > *S. molesta*D.Mitch. > *P. stratiotes*L. In contrast, *S. molesta*D.Mitch. has highest value for Wa on the abaxial surface and followed by *D. platyphylla*L.B. Smith > *A. comosus*(L.) Merr. > *P. stratiotes*L. Comparing Wa values of the two leaf surfaces, both *P. stratiotes*L. and *S. molesta*D.Mitch. have higher Wa values on the abaxial surface as to the adaxial surface. The Wa values of *D. platyphylla*L.B. Smith and *A. comosus*(L.) Merr., on the other hand, are much higher on the adaxial surface compared to the abaxial surface.

Looking back on the contact-angle measurements of water on both adaxial and abaxial leaf surfaces of *A. comosus*(L.) Merr., *D. platyphylla*L.B. Smith, *P. stratiotes*L. and *S. molesta*D.Mitch. in Table 2, all θ mean values are shown to be inversely proportional to the calculated work-of-adhesion (Wa). The θ mean values are high on the adaxial leaf surfaces of both *P. stratiotes*L. and *S. molesta*D.Mitch. but low on the adaxial leaf surfaces of *A. comosus*(L.) Merr. and *D. platyphylla*L.B. Smith. In contrast, the θ mean values are high on the abaxial leaf surfaces of *A. comosus*(L.) Merr., *D.
platyphylla L.B. Smith and P. stratiotes L. but low on the abaxial leaf surfaces of S. molesta D.Mitch. On the other hand, $W_a$ values are high on the adaxial leaf surfaces of A. comosus (L.) Merr. and D. platyphylla L.B. Smith but low on the abaxial leaf surfaces of P. stratiotes L. and S. molesta D.Mitch. The $W_a$ values are similarly high on the abaxial leaf surfaces of S. molesta D. Mitch. but low on the abaxial leaf surfaces of A. comosus (L.) Merr., D. platyphylla L.B. Smith and P. stratiotes L.

![Graphs](image)

**Figure 3.** The mean values of the $\theta$ of base, middle and tip portions of the adaxial (a) and abaxial (b) surface of P. stratiotes L., A. comosus (L.) Merr., D. platyphylla L.B. Smith and S. molesta D.Mitch using deionized water ($p>0.05$).
Table 2. The $\theta$ mean ± standard deviation values and the work-of-adhesion ($W_a$) of the adaxial and abaxial surface of *P. stratiotes* L., *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch. using deionized water.

| Plant species | Contact angle ($\theta$) | $W_a$ (mJm$^{-2}$) |
|---------------|-------------------------|------------------|
|               | Adaxial | Abaxial | Adaxial | Abaxial |
| *P. stratiotes* L. | 154.39 ± 3.26 | 147.90 ± 3.17 | 7.15 | 11.13 |
| *A. comosus* (L.) Merr. | 52.01 ± 4.96 | 130.61 ± 4.33 | 117.61 | 25.41 |
| *D. platyphylla* L.B. Smith | 37.17 ± 2.90 | 101.98 ± 3.10 | 130.81 | 57.69 |
| *S. molesta* D. Mitch. | 153.56 ± 0.37 | 42.04 ± 4.55 | 7.61 | 126.87 |

$n=60$

The measured $\theta$ values describes the wetting behaviour of the leaf surface based on the tilt angle the applied liquid creates as it roll off upon contact of the leaf surface. The calculated $W_a$, on the other hand, measures the strength of interaction between water and the leaf surface in the process of wetting. Hence, if the value of $\theta$ is high and $W_a$ is low, the liquid forms a semi-spherical or spherical droplet over the leaf surface and if the value of $\theta$ is low and $W_a$ is high, the liquid spreads on the leaf surface.

Paired sample T-test for the mean $\theta$ values revealed that there is a significant difference between the adaxial and abaxial surfaces of *P. stratiotes* L. with p-value ≤ 0.005 and p-value ≤ 0.0001 compared to *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch. (Figure 4). In terms of water repellency, the adaxial leaf surfaces of *P. stratiotes* L. and *S. molesta* D.Mitch. have higher values compared to their abaxial surfaces. However, while the adaxial leaf surfaces of *S. molesta* D.Mitch. are hydrophobic (<150°-90°) its abaxial leaf surfaces are hydrophilic (<90°-10°) which are unlike the adaxial and abaxial leaf surfaces of *P. stratiotes* L., both of which are hydrophobic. One the other hand, the abaxial leaf surfaces of *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith have higher $\theta$ mean values than their adaxial leaf surfaces. Both are categorized as hydrophobic (<150°-90°) having $\theta$ mean values = 130.61° and 101.98°, respectively. These $\theta$ mean values for abaxial leaf surfaces of *A. comosus* (L.) Mitch. and *D. platyphylla* L.B. Smith are significantly higher compared to the abaxial leaf surfaces of *S. molesta* D.Mitch. However, both adaxial leaf surfaces of *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith are confirmed hydrophilic with $\theta$ mean = 52.01° and 37.17°, respectively.

Analysis using the one-way ANOVA revealed a significant difference between the $\theta$ mean values of the adaxial and abaxial surfaces of *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith, *P. stratiotes* L. and *S. molesta* D.Mitch. Results of the Tukey’s T-test showed the adaxial leaf surfaces of *P. stratiotes* and *S. molesta* (p<0.0001) having higher water repellency compared to *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith with the abaxial leaf surfaces of *P. stratiotes* L. (p<0.001) being the highest. Interestingly, these findings reveal *P. stratiotes* L. as having the highest value of water repellency using $\theta$ measurements among other hydrophobic or superhydrophobic leaf surfaces. The $\theta$ mean values measured for *P. stratiotes* L. has been consistently high (>150°) thus, making it a candidate new model for the conception of bioinspired superhydrophobic materials for various applications such as oil-water separation, microfluidic devices, anti-corrosion and anti-bacterial coatings, and anti-moisture devices.
Figure 4. The mean values of the θ of the adaxial and abaxial surface of *P. stratiotes* L.* (p ≤ 0.005), *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch.** (p ≤ 0.0001) using deionized water.

3.3. Oil adsorption capacity using pure oils with varying carbon chain length

Results of the oil spill simulation in tap water using lauric acid, myristic acid, palmitic acid and stearic acid are shown in Figures 5 and 6. In terms of the weight of adsorbed pure oil (mL/g), *P. stratiotes* L. has the highest amount of adsorbed lauric (0.26 mL/g), myristic (0.19 mL/g), palmitic (0.25 mL/g) and stearic acid (0.26 mL/g) compared to *S. molesta* D.Mitch., *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith (Figure 5). In this manner, *P. stratiotes* L., regardless on which leaf surfaces, can be considered as oleophilic as common pure oils tend to adhere and get attracted to its surfaces. The high amount of oil adhering on leaf surfaces of *P. stratiotes* L. is inversely proportional to its computed values for water repellencies but directly proportional to its mean θ values and trichome density. For *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith, their trichome morphologies are directly proportional to trichome densities. Hence, both could be factors affecting difference in wetting properties of their adaxial (hydrophilic) and abaxial (hydrophobic) leaf surfaces. In the case of *S. molesta* D.Mitch., although having the least trichome density compared to *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *P. stratiotes* L., the multifaceted architecture of its egg-beater trichomes on the adaxial surface play an important role in water repellency and oil adsorbency. *P. stratiotes* L. and *S. molesta* D.Mitch. are somewhat similar in terms of their wetting properties towards deionized water and common pure oils. However, these similarities between *P. stratiotes* L. and *S. molesta* D.Mitch. are limited to their adaxial leaf surfaces only as the abaxial leaf surfaces of *S. molesta* D.Mitch. is hydrophilic while both leaf surfaces are hydrophobic in *P. stratiotes* L..

The mean amounts of molecules from lauric acid, myristic acid, palmitic acid and stearic acid adhered to the leaf surfaces of *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith, *P. stratiotes* L. and *S. molesta* D.Mitch. is shown in Figure 6. As the carbon chain length increases from lauric to stearic acids, the amount of molecules of each common pure oil adhered on each leaf surface also increases. This is apparent since there are significant differences (p < 0.05) among the common pure oils with regards to the amounts of adsorbed molecules and the plants to which they adhered.

Based on Tukey’s test, the results showed that the mean amounts of molecules adsorbed over 10.00 x 10^{23} are lauric acid, myristic acid and stearic acid for *A. comosus* (L.) Merr.; only palmitic acid for *D. platyphylla* L.B. Smith; and all four (lauric, myristic, palmitic and stearic acids) for *P. stratiotes* L.
and *S. molesta* D.Mitch. For stearic acid, the amounts of molecules adsorbed are highest in *P. stratiotes* L. and lowest in *D. platyphylla* L.B. Smith. For palmitic acid, the amounts of molecules adsorbed are also highest in *P. stratiotes* L. but lowest in *A. comosus* (L.) Merr. For lauric acid, *P. stratiotes* L. adsorbs the amounts of molecules most while *D. platyphylla* L.B. Smith is the least. Finally, for myristic acid, the amounts of molecules are adsorbed most by *P. stratiotes* L. and least by *D. platyphylla* L.B. Smith. Furthermore, the amounts of molecules adsorbed for palmitic and stearic acids are highest in *P. stratiotes* L. and *S. molesta* D.Mitch. as compared to *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith. There is no significant difference in the amounts of molecules adsorbed for myristic acid by all four plants and so do in the amount of adsorbed palmitic acid molecules between *S. molesta* D.Mitch. and *A. comosus* (L.) Merr. and for stearic acid molecules between *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith.

Overall, *P. stratiotes* L. has the highest adsorption capacity for lauric acid, myristic acid, palmitic acid and stearic acid as compared to the three other plants while *D. platyphylla* L.B. Smith has the least.

**Figure 5.** The amount of pure oils (mL/g) adsorbed by *P. stratiotes* L., *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch.

**Figure 6.** The amount of molecules of each pure oil adsorbed by *P. stratiotes* L.** (**p ≤ 0.002), *A. comosus* (L.) Merr.**(*p ≤ 0.001), *D. platyphylla* L.B. Smith** and *S. molesta* D.Mitch.*** (**p ≤0.005).
3.4. Hierarchical clustering analysis
The linkage tree resulting from the hierarchical clustering analysis of *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith, *P. stratiotes* L. and *S. molesta* D.Mitch. based on obtained values of surface morphologies, wettability and oil adsorption capacity values are shown in Figure 7. In the linkage tree, *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith are clustered together indicating both plants share comparable characteristics in terms of trichome type, surface wettability and oil adsorption capacity. *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith are bromeliads and both have been reported to have a similar peltate scutiform trichomes [24, 27]. Although, both plants have almost similar trichome type and densities on both leaf surfaces, the adaxial surfaces are hydrophilic while the abaxial surfaces are hydrophobic. These characteristics significantly lowers the oil adsorption capacities of both plants since the adaxial surfaces of their leaves have low oil adherence.

*S. molesta* D.Mitch. significantly show no affinity to *A. comosus* (L.) Merr. and *D. platyphylla* L.B. Smith but comes close to *P. stratiotes* L. in terms of trichome morphology, surface wettability and oil adsorption capacity. Both *S. molesta* D.Mitch. and *P. stratiotes* L. are considered superhydrophobic on their adaxial leaf surfaces with respect to its trichome morphology and surface contact angle measurement except trichome density. In *S. molesta* D.Mitch., both leaf surfaces are less dense compared to *P. stratiotes* L. Despite the noted low trichome density, however, *S. molesta* D.Mitch. still exhibit superhydrophobicity on its adaxial leaf surfaces due to its multifaceted hierarchical eggbeater-shaped trichomes with hydrophobic surfaces known for their unique long-standing air retention mechanism and hydrophilic pins that make water molecules hardly spread on its surface [28].

On the abaxial leaf surfaces, *P. stratiotes* L. is considered hydrophobic while *S. molesta* D.Mitch. is hydrophilic. While *P. stratiotes* L. has similar type of trichomes on either side of its leaf surfaces, its adaxial leaf surfaces are certainly superhydrophobic while the abaxial sides are hydrophobic. In contrast, trichomes of *S. molesta* D.Mitch. are dissimilar on either side of its leaves. The type of trichomes found on the adaxial leaf surfaces of *S. molesta* D.Mitch. are shaped like eggbeaters which are hydrophobic while those located on its abaxial surface are achlorophyllic filamentous type which are hydrophilic. The former type has high water repellency while the latter are engaged in water anchorage and stability.

![Cluster tree diagram](image-url)

**Figure 7.** Cluster tree diagram of *P. stratiotes* L. comparing with *A. comosus* (L.) Merr., *D. platyphylla* L.B. Smith and *S. molesta* D.Mitch. using single-linkage hierarchical Euclidean distances based on trichome density, wettability and oil adsorption.

4. Conclusions
The uniform hydrophobic properties exhibited by adaxial and abaxial leaf surfaces of *P. stratiotes* L. is certainly influenced by leaf surface’s chemistry due to hydrophobic coatings and microstructures like trichomes. These hydrophobic properties make *P. stratiotes* L. a prime candidate for bioinspired
materials requiring high water repellency which can be applied to develop highly engineered materials that repels water to avoid the growth of molds and proliferation of various microorganisms which are dependent to moist and rusting.

This new discovery of *P. stratiotes* L. hydrophobic qualities is both advantageous and effective because of the plant’s abundance in tropical freshwaters. Regarded as a pest due to its invasive nature in local water system, the latest information presented about *P. stratiotes* L. will make this plant a necessity while benefiting from its abundance. Meanwhile, the plant’s high oil adsorption capacity can help envisage innovative ideas for oil-water separation and other applications. It is possible to mimic its biological surface, for instance, to make materials capable of halting secondary pollutions and for efficiently controlling accidental oil spills. However, more studies are needed for fully understand the working mechanism behind its surface microstructures and its interaction with other liquids. Conception of hydrophobic materials is increasingly becoming more evident these days by simply studying the potentials of biological surfaces such as what this research have uncovered about *P. stratiotes* L.

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