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Research Article
Assessment of the *Salvia officinalis* and *Myrtus communis* Aqueous Extracts Effect on Cell Surface Tension Parameters and Hydrophobicity of *Staphylococcus aureus* CIP54354 and *Bacillus subtilis* ILP142B

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Abstract

Background and Objective: The surface physicochemical characteristics play a crucial role in adhesion and biofilm formation. Adhesion process is central to many environmental, industrial and medical applications. Medicinal plants extracts are commonly used in these applications and can potentially influence the bacterium/surface interaction. Two bacterial strains, *Staphylococcus aureus* CIP54354 and *Bacillus subtilis* ILP142B and two medicinal plants aqueous extracts types *Salvia officinalis* and *Myrtus communis* were examined upon bacterial cell surface physicochemical properties. **Methodology:** The effect of medicinal plants extracts on bacterial cell surface physicochemical properties was examined using a combination of contact angle measurements, Lifshitz-Van Der Waals (LW) and acid-base (AB) surface free energies calculations. **Results:** The study demonstrated that plants aqueous extracts treatment could modify cell surface tension parameters including Lifshitz-Van Der Waals (γ^{LW}), electron-donor (γ^{-}) and electron-acceptor (γ^{+}) and thereby the bacterial cell hydrophobicity, depending on the aqueous extracts type and concentration and the bacterial surface characteristics. **Conclusion:** A possible application of these findings in the pharmaceutical industry for the production of compounds supporting antibiotics for treating oral diseases seems to be worth exploring.

Key words: Bacterial cell hydrophobicity, bioadhesion, surface free energy, contact angles, plants extracts

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bacterial adhesion on surface is ubiquitous. Most often, biofilms are unwanted and related to diverse problems as food and drinking water contamination, dental carries and periodontal diseases and bio-deterioration process[1-3]. The bacterial adhesion to the surface is a complicated process that is affected by various physicochemical properties of both substrata and microbial surfaces. These interactions can be classified into: Lifshitz-Van Der Waals interactions, electrostatic interactions[3-6] and polar or Lewis acid-base interactions (i.e., electron donor and electron acceptor)[7,8]. Reports in the study have shown that parameters such as hydrophobicity, surface charge and donor/acceptor electron (acid-base) properties may have a significant effect on microbial adhesion[5,7].

In recent years, the exploitation of natural medicinal and aromatic plants has been reported in several scientific studies as a new biological approach to fight against the biofilms formation on different surfaces[9-12]. Moreover, divers works have reported the effect of medicinal plants extracts on the hydrophobicity of many microorganisms cell surfaces using different methods like salt aggregation test[13], cell surface hydrophobicity[14,15]. In contrast, despite the crucial role of the acid-base interactions in adhesion phenomenon and their high importance than the other interactions[16], the effect of medicinal plants extracts on surface tension proprieties has not been reported. Thus, the purpose of the present study was first to determine the influence of the *Salvia officinalis* and *Myrtus communis* aqueous extracts on *S. aureus* CIP54354 and *B. subtilis* ILP142B surface hydrophobicity by water contact angles and the approach of Van Oss. In addition, the study also investigates their effect on the electron donor-electron acceptor properties and surface tension using contact angle measurements.

MATERIALS AND METHODS

Plant material and aqueous extracts preparation: The aerial parts (leaves and stems) of cultivated *Salvia officinalis* (Labiateae) and *Myrtus communis* L. (Myrtaceae) were freshly harvested on March, 2014, in the National Institute of the Medicinal and Aromatic Plants of Taounate, Morocco. The freshly-cut plants were air-dried and then the samples were packed in paper bags and stored until the extraction.

The aqueous extracts were prepared as follow: About 50 g of dried powdered plant were suspended and extracted by refluxing with boiling distilled water (10% w/w) for 10 min. After cooling, the samples were then filtered through Whatman paper No. 1. Finally, the crude extracts were recovered and dried in a rotary vacuum evaporator (Temperature $\leq$40°C).

Bacterial strains and cell preparation: *Staphylococcus aureus* CIP54354 and *Bacillus subtilis* ILP142B strains were used in this study. Each bacterial strain was grown independently in liquid Luria Bertani medium containing the following (per litre of distilled water): 10 g of tryptone, 5 g of yeast extract 10 g of NaCl. After 24 h of incubation, cells were harvested by centrifugation for 15 min at 8400×g and washed twice with and re-suspended in 0.1 M KNO_3 solution.

Contact angle measurement: Bacterial lawns were prepared following the procedures described by Busscher *et al.*[16]. Microbial cell suspended in KNO_3 sterile solution were deposited on a cellulose acetate membrane filter (0.45 µm). Usually the state of drying of a microbial lawn lasts 30-60 min and indicates that only bound water is present on the surface[17,18]. Thereby, prior to measuring contact angles, the filters were air dried at room temperature for 30 min[19,20]. The contact angles were determined using a goniometer (GBX instruments, France) by the sessile drop method using three pure liquids with known energy characteristics (γ^{LW}, γ^- and γ^+) (Table 1): Distilled water, formamide (>99%) and diiodomethane (>99%). For contact angle measurements, a drop of 2 µL of the test liquid was dispensed on the filter surface[21]. The contact angles were taken 15 sec after drop deposition at room temperature (25±2°C). Contact angles were measured in triplicate with separately cultured microbes. Each reported contact angle is a mean of the three independent measurements from bacterial lawns.

Hydrophobicity and surface free energy calculation: Once the contact angles were performed using three diagnostic liquids, the non-polar Lifshitz-Van Der Waals (γ^{LW}) component and polar electron-donor (γ^-) and electron-acceptor (γ^+) parameters of the bacterial (B) surface tension were

Table 1: Surface tension properties of contact angle liquids[a]

Liquid	γ^{LW}	γ^+	γ^-
	----------(mJ m^{-2})----------		
Water	21.8	25.5	25.5
Formamide	39.0	2.3	39.6
Diiodomethane	50.5	0.0	0.0

[a]This result was obtained by Van Oss[23]

calculated by the extended Young's equation[22]. In this approach the pure liquid (L) contact angles (q) can be expressed as in Eq. 1:

$$\gamma_L(\text{Cos}\theta+1) = 2(\gamma_B^{LW}\gamma_L^{LW})^{1/2}+2(\gamma_B^{+}\gamma_L^{-})^{1/2}+2(\gamma_B^{-}\gamma_L^{+})^{1/2} \quad (1)$$

The Lewis acid-base surface tension component is defined by Eq. 2:

$$\gamma_B^{AB} = 2\left(\gamma_B^{-}\gamma_B^{+}\right)^{1/2} \quad (2)$$

Contact angle measurements and the approach of Van Oss *et al.*[22] and Van Oss[23] were used to evaluate the cell surface hydrophobicity. In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material when immersed in water (w): ΔGiwi. Indeed, if the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic (ΔGiwi<0), conversely for a hydrophilic material, ΔGiwi>0. This later is calculated through the surface tension components of the interacting entities, according to the following Eq. 3:

$$\Delta\text{Giwi} = -2\gamma_{iw} = -2\left[\begin{array}{l}\left(\left(\gamma_i^{LW}\right)^{\frac{1}{2}}-\left(\gamma_w^{LW}\right)^{\frac{1}{2}}\right)^2+\\ 2\left((\gamma_i^{+}\gamma_i^{-})^{\frac{1}{2}}+\left(\gamma_w^{+}\gamma_w^{-}\right)^{\frac{1}{2}}-\left(\gamma_i^{+}\gamma_w^{-}\right)^{\frac{1}{2}}-\left(\gamma_w^{+}\gamma_i^{-}\right)^{\frac{1}{2}}\right)\end{array}\right] \quad (3)$$

Effect of aqueous extracts on the cell surface proprieties: The influence of *Salvia officinalis* and *Myrtus communis* aqueous extracts on cell surface tension parameters and hydrophobicity of *Staphylococcus aureus* CIP54354 and *Bacillus subtilis* ILP142B was studied as described by Fathilah with some modifications[24]. Briefly, 10 mL of the bacterial suspensions studied (10^8 UFC mL^{-1}) were dispensed into tube 1 through 5 and exposed for 15 min, under agitation (225 min) at 37°C to the herb aqueous extracts dissolved in sterile distilled water to different final concentrations of 0, 1, 5, 10 and 20 mg mL^{-1}. After the contact time, the test tubes were centrifuged, washed and suspended in KNO_3 (0.1 M) sterile solution and then deposited on a cellulose acetate membrane filter (0.45 μm) to proceed to contact angle measurement as described above.

RESULTS AND DISCUSSION

Qualitative and quantitative cell surface hydrophobicity: Cell surface hydrophobicity is recognized as one of the key determining factors in bacterial adhesion to surfaces. Several techniques are usually employed to assess cell surface properties. Cell surface hydrophobicity was evaluated by hydrophobic interaction chromatography[25], bacterial adhesion to hydrocarbon[26], salting out and water contact angle[27]. At present, the use of contact angle hysteresis approach (advancing and receding contact angles) or that of the water contact angle measurements, using surface energy approach is very advisable and favored for determining the hydrophobicity of cell surfaces, which involves comparison to a threshold contact angle to make the assessment.

According to Vogler[28], hydrophobic surfaces exhibit a water contact angle values higher than 65°, whereas hydrophilic ones exhibit water contact angle values lower than 65°. The mean water contact angles measured on *S. aureus* CIP54354 and *B. subtilis* ILP142B are presented in Table 2. In the absence of plant aqueous extracts, *B. subtilis* ILP142B with a water contact angle of 13.0±0.3° is less hydrophilic than *S. aureus* CIP54354 with an angle measured at 31.3±0.1°. The values obtained are similar to those reported by Hamadi and Latrache *et al.*[29]. The water contact angles of *S. aureus* CIP54354 treated by *Salvia officinalis* aqueous extract at the concentration of 5 mg mL^{-1} is increased to 70.4±1.5°. Similar trend was demonstrated in

Table 2: Contact angles with water (θ_w), formamide (θ_F), diiodomethane (θ_D) of bacteria treated with herb aqueous extracts

	θ_{water}		$\theta_{formamide}$		$\theta_{diiodomethane}$	
Aqueous extracts treatment (mg mL^{-1})	*S. aureus* CIP54354	*B. subtilis* ILP1428B	*S. aureus* CIP54354	*B. subtilis* ILP1428B	*S. aureus* CIP54354	*B. subtilis* ILP1428B
Untreated	31.3±0.1	13.0±0.3	21.6±1.8	19.4±0.6	46.7±0.7	63.5±0.8
***Salvia officinalis* a queous extracts**						
1	45.9±1.6	29.5±0.3	54.4±0.5	37.8±1.7	42.9±1.6	62.4±0.3
5	70.4±1.5	27.1±0.2	63.8±1.3	33.7±0.8	44.3±0.6	62.9±0.5
10	51.6±1.0	46.7±3.5	55.6±0.6	53.6±0.3	22.6±0.8	54.8±0.7
20	45.1±0.7	33.1±0.1	51.8±0.6	44.3±0.6	50.7±0.5	65.5±0.1
***Myrtus communis* a queous extracts**						
1	34.4±0.1	28.4±0.2	56.9±0.5	23.0±3.0	44.7±0.6	67.0±0.5
5	49.7±0.7	39.1±0.8	53.9±1.3	40.6±0.8	49.2±0.3	59.2±0.9
10	48.4±1.1	36.4±0.3	61.0±0.6	39.0±0.5	49.8±0.9	51.3±0.8
20	39.9±1.9	38.5±0.3	62.5±0.4	37.0±0.7	61.6±1.5	46.8±0.6

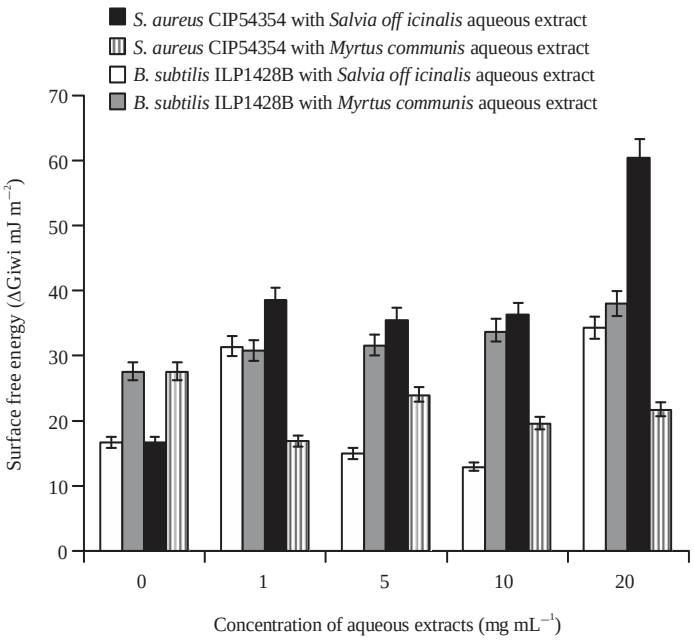

Fig. 1: Surface free energy (ΔGiwi) of bacterial cells. Error bars represent 1 Standard Deviation (SD) with n = 3

the case of *Myrtus communis* aqueous extract treatment that caused the increase of water contact angle to 49.7±0.7° compared to the untreated one (Table 2). In addition as can be noted in Table 2, the water contact angle measurements show that plant aqueous extracts treatment can modify bacterial cell hydrophobicity, depending on aqueous extracts type, concentration and the bacteria cell surface properties.

The use of contact angle measurements in association with the surface free energy calculation can thus provide a physical and mathematical basis for consistent assessment of bacterial cell hydrophobicity. The surface free energy between bacterial cells in water (ΔGiwi) is a quantitative expression of the cell surface hydrophilicity or hydrophobicity. If the interaction between the two entities is stronger than the interaction of each entity with water, the material is considered hydrophobic (ΔGiwi<0), conversely, a hydrophilic material, ΔGiwi>0. This is the first time that the effect of plant extracts on surface free energy of bacteria has been described using contact angle measurements and the approach of Van Oss *et al.*[30]. The quantitative hydrophobicity revealed initial hydrophilic behavior in the two strains with ΔGiwi>0. The *Salvia officinalis* aqueous extract reduced the quantitative cell surface hydrophobicity of *S. aureus* CIP54354 and *B. subtilis* ILP142B strains and the effect increased with increasing extract concentration. However, this results show that the surface free energy of the two strains studied become more or less hydrophilic following *Myrtus communis* aqueous extract concentrations treatment (Fig. 1). As indicated in the study, the plant extracts treatment of bacteria decreases their Cell Surface Hydrophobicity (CSH). Indeed, the study of Razak *et al.*[24] demonstrated that the extracts of *Piper betle* and *Psidium guajava* reduce the cell surface hydrophobicity of *Steptomyces sanguinis*, *Steptomyces mitis* and *Actinomyces* sp. Moreover, Nordin *et al.*[31] has shown that the CSH of *C. albicans*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis* and *C. tropicalis* were remarkably reduced by the extract of *B. javanica* treatment. The crude aqueous extracts of clove reduced the cell surface hydrophobicity of *S. mutans* and the effect increased with increasing extract concentration[32]. In contrast, Voravuthikunchai *et al.*[33] reported that *Punica granatum* pericarps and *Quercus infectoria* nutgalls extracts increased the cell hydrophobicity of 10 clinical isolates of *Helicobacter pylori* and no effect of *Paullinia cupana* on hydrophobicity of *Candida albicans* strain was reported by Matsuura *et al.*[34].

According to Schaer-Zammaretti and Ubbink[35], cell wall constituents such as phosphate, carboxylate groups and proteins impart bacteria with variable surface charge and hydrophobicity. The alteration in the chemical and molecular composition of the cell surface induced by aqueous extracts treatment may be manifested as a change in the cell surface hydrophobicity.

Lifshitz-Van Der Waals surface tension component: The data shown in Table 1 were used to calculate the LW surface tension component (γ^{LW}), according to Eq. 1. In the absence of

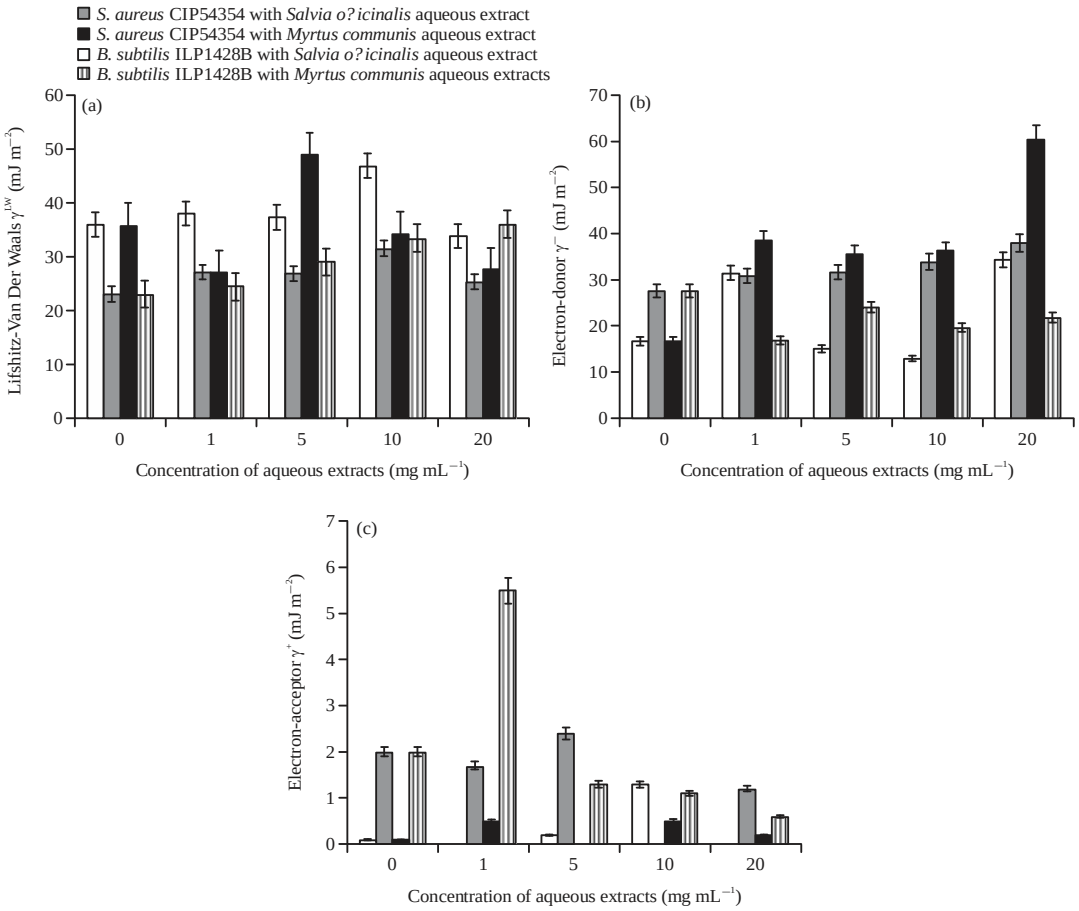

Fig. 2(a-c): Surface tension parameters of the bacterial cells surfaces as a function of aqueous extracts concentration, (a) Lifshitz-Van Der Waals (γ^{LW}), (b) Electron-donor (γ^-) and (c) Electron-acceptor (γ^+). Error bars represent 1 Standard Deviation (SD) with n = 3

plant aqueous extracts, the γ^{LW} values are 36.0±0.9 and 26.5±0.4 mJ m^{-2} for *S. aureus* CIP54354 and *B. subtilis* ILP142B, respectively. These values are typical for bacteria with a reported mean γ^{LW} of Hamadi and Latrache *et al.*[29]. The plant aqueous extracts treatment produces distinct changes in γ^{LW} depending on the bacterial strain, concentration used and aqueous extracts type (Fig. 2a). For *B. subtilis* ILP142B the values of γ^{LW} decreases when the concentration of *Salvia officinalis* and *Myrtus communis* aqueous extracts is increased from 0-10 mg mL^{-1}, where further addition to 20 mg mL^{-1} of *Salvia officinalis* extracts, γ^{LW} values of *B. subtilis* ILP142B increase to 25.4±0.4 mJ m^{-2}. This results further show that γ^{LW} values of *S. aureus* CIP54354 strain increase or decrease following plant aqueous extract concentrations.

Electron-donor (γ^-) and electron-acceptor (γ^+) parameters: The acid-base interactions which also contributes to the interaction between the cells and the surfaces, seems to be an important factor in the adhesion phenomenon. The acid-base interactions are 10-100 times more important compared to others interactions. The electron-donor (γ^-) and electron-acceptor (γ^+) parameters are presented in Fig. 2b and c. In the absence of plant aqueous extracts, the values of γ^- and γ^+ for *S. aureus* CIP54354 are 41.5±0.1 and 2.0±0.0 mJ m^{-2} and those of *B. subtilis* ILP142B are 40.6±2.0 and 0.0±0.2 mJ m^{-2}, respectively. The values obtained are similar to those reported by Hamadi and Latrache[29]. To the best of our knowledge, the present study seems to pioneer the assessment of the plant aqueous extracts effect on electron donor-acceptor proprieties using contact angle measurements. As aqueous extracts concentration is raised, the values of γ^- indicating higher electron-donor propriety for *S. aureus* CIP54354 strain. Indeed, the values of γ^- vary between 41.5±0.5 and 52.4±0.7 mJ m^{-2} for *Salvia officinalis* aqueous extracts and between 41.5±0.5 and 70.0±0.8 mJ m^{-2} for *Myrtus communis*. For *B. subtilis* ILP142B, the results show

that the values of γ^- depends a dose-dependent manner. This results show also a small variation in acceptor electron properties with the trend differs from that observed with electron donor proprieties (Fig. 2b, c). As reported in the study, the microbial surface properties depend fundamentally on the chemical composition of cell surface. In fact, the basic groups like carboxyl groups (COO^-), lipopolysaccharides, lipoproteins, amines (NH_2 and phosphate (PO_4)[36] or sulfate groups (SO_3)[37] exposed on the microbial cell surface are the ones which determine their electron-donor property. While, the cell surface electron acceptor is attributed to the presence of amino and acidic groups such as R or R-NH-OH and NH_3 groups[38]. Thereby, the effect of plant aqueous extracts on the electron donor-acceptor proprieties of the bacterial cells studied could be due to the alteration in the chemical and molecular composition of their cell surfaces.

CONCLUSION

The findings presented in this study, demonstrated that all aqueous extracts tested have shown their influence on the physicochemical properties of bacterial cell surfaces studied including cell surface hydrophobicity and electron donor-electron acceptor properties. Also, the results show that this effect is depending on the aqueous extracts type, concentration and the bacterial surface characteristics. A possible application of these findings in the pharmaceutical industry for the production of compounds supporting antibiotics for treating oral diseases seems to be worth exploring.

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