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Physico-Chemical Properties of the Biosurfactant Obtained from Fruit Extract of *Genipa americana* L. and *Tamarindus indica* L. and its Application in Oil Removal

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In the last years, the industrial application of natural surfactants of origin of fruits, vegetables, nuts and grains has increased due to its potential physicochemical properties (foaming, emulsification, solubilization, sweetener, bitterness) and biological activities (hemolytic, antimicrobial, insecticide). These natural alternatives are considered economically and environmentally viable and explored to various purposes including the bioremediation of soils contaminated by oils. The aims of the present study were to evaluate the surfactants properties of the ethyl acetate extracts obtained from the fruits Genipa americana L. (Genipap) and Tamarindus indica L. (Tamarinds) and their applications in oil removal. The physico-chemical analyzes performed were surface tension, emulsification activity using different hydrocarbons and vegetable oils, surface tension stability in different temperatures, pH and addition of salt, and test of dispersion. To evaluate the capacity of this extracts (biosurfactants) on oil removal in engine parts, small-scale tests were conducted using glass blade. The results showed that this ethyl acetate extracts obtained from fruits extract exhibited surfactant properties due to the reduction of the surface tension of water from 72mN/m to 31.57mN/m and 34.71mN/m with the critical micelle concentration of 0.95g/L e 1.9g/L, with the increase values of the emulsification activity ranging from 32% and 100%. The results also showed a considerable stability of these biosurfactants derived from fruit extract submitted to different temperature, pH and salt concentrations as well as their compatibility with chemical surfactants. The biosurfactants obtained from fruit extract of *G. americana* and *T. indica* can be considered new perspectives on oil removal from engine parts with future potential to be marketed and replace the application of chemical surfactants.

1. Introduction

The industrial need for surfactant is constantly growing. Surfactants possess both hydrophilic and hydrophobic structural moieties, which in turn impart many unusual properties, including an ability to lower the surface tension (Brasileiro et al., 2015). The commercial importance of surfactants is evidenced from the increasing trends in their production and the number of industrial applications. The vast majority of commercially available surfactant is synthesized from petroleum. Because of its environmental advantages, there are large commercial interests in replacing natural by synthetic surfactants (Souza et al., 2014). Biosurfactants usually exhibit emulsifying capacity, recently received much more attention because of their potential to become an environment-friendly alternative to conventional chemical surfactants due to their biodegradability, low toxicity, ability to be produced from renewable resources, and functionality of hydrophobic substrates depends on their biosurfactant efficiency, and most efforts are focused on the isolation of novel active biosurfactants (Sarubbo et al., 2015).

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The plant-derived biosurfactant wide belong to a diverse group of naturally occurring surface-active compounds. They occur in different tissues of a large number of plant species belonging to nearly 100 families, such as *Agavaceae, Alliaceae, Asparagaceae, Costaceae, Dioscoreaceae, Liliaceae, Ruscaceae, Solanaceae*, beyond *Aspilia montevidensis* (Asteraceae), *Trigonella foenum-graecum* (leguminosae), *Musa paradisiaca* (musáceas), *Psidium guajava* (Myrtaceae), *Citrullus lanatus* (Cucurbitaceae), *Terminalia catappa* (Combretaceae), *Morinda citrifolia* (Rubiaceae), among other (Augustin et al., 2011).
They can be found in different plant parts including roots, shoots, flowers, fruits and seeds. Their common feature is the formation of a soapy lather when shaken in water solution. This property has for a long time been used in the identification of these biosurfactants containing plant species as well as for their quantification. The foaming, emulsifying, medicinal, and antimicrobial activities have been demonstrated in biosurfactants, which lead to a range of applications in area of foods, detergents, pharmaceuticals, cosmetics and environmental. Due to the foaming abilities, biosurfactants may also be used as emulsifiers helping in degradation of xenobiotics like polycyclic aromatic hydrocarbons (PAHs). The increased solubility of PAH in the presence of these biosurfactants make them easily available for degrading bacteria (Zhou et al., 2013).
The presence work describes the properties physico-chemical along with environmental application of the biosurfactants produced by Genipa americana L. (Jenipapo) and Tamarindus indica L. (Tamarinds).

2. Material and methods

2.1 Collect and preparation of vegetables

The fruits *Genipa americana* L. (Jenipapo) and *Tamarindus indica* L. (Tamarinds) were purchased commercially (Central Supply and Logistics of Pernambuco - CEASA). After collection, the fruits were taken to the laboratory for the washing process under water. Samples were placed in the oven for 48 h at a temperature of 50 ° C for dehydration. After dehydration, the samples were ground to a fine powder.

2.2 Obtaining the extractive solution vegetable

Powdered vegetable matter (50 g) from *Genipa americana* L. and *T amarindus indica* L. were transferred separately to Becker and 100 ml of 1% aqueous sodium hydroxide solution was added and subjected to heating 60 ° C for 30 minutes under constant stirring. The extractive solution was filtered under reduced pressure using Whatmann n°1 filter paper. Then the filtered solution was acidified with citric acid slowly added and with constant stirring until the solution reached pH 2. The acid solution was placed in a separation funnel for the extraction of the biosurfactant using ethyl acetate in a ratio of 1: 2 (v / v). The procedure was repeated until the ethyl acetate became colorless. The extractive solution was frozen at -4 ° C and lyophilized. The vegetable extract lyophilized containing the biosurfactant was submitted to analysis.

2.3 Surface activity

The surface tension of the aqueous solution (distilled water as control and crude vegetable extract as test and Tween 80 as test of in marketed surfactant) using a Du Nouy Tensiometer model Sigma 70 (KSV Instruments LTD, Finland) at room temperature. The surface measurement was carried out at 25 ± 1 ºC after dipping the platinum ring in the solution for a while in order to attain equilibrium conditions. The measurement was repeated three times and an average value was obtained.

2.4 Emulsification activity

Emulsification activity was measured using the method described by Cooper and Goldenberg (1987), whereby 2 mL of a oils (Kerosene, canola oil, soya bean oil, corn oil, vegetable fat, oil motor, oil motor powders use petroleum, diesel oil, n-Hexadecan, mineral oil, vegetable oil, olive oil and castor oil) were added to 2 mL of the solution of extract vegetable in a graduated screw-cap test tube and vortexed at high speed for 2 minutes, the commercial surfactant Tween 80 as test. The emulsion stability was determined after 24 h, and the emulsification index was calculated by dividing the measured height of the emulsion layer by the mixture's total height and multiplying by 100. The assays were carried out in triplicate and did not vary more than 5 %.

2.5 Critical micelle concentration (CMC)

The concentration at which micelles began to form was represented as the Critical Micelle Concentration (CMC). The CMC of the extracts vegetable containing the biosurfactant was determined for the concentration 0.001–3 % (w / v) in distilled water and followed by the measurement of surface tension. The surface tension of each concentration was determined in triplicate. The maximum standard deviation associated with the surface activity measurements was ± 0.2 mN / m. The CMC of the extracts brute vegetable was estimated from constant value of surface tension.

2.6 Oil displacement test

The oil displacement test is a method used to measure the diameter of the clear zone, which occurs after dropping a surfactant-containing solution on an oil-water interphase. The binomial diameter allows an evaluation of the surface tension reduction efficiency of a given biosurfactant. The oil displacement test was done by adding 20 ml of distilled water to a petri dish with a diameter of 15 cm. After that 20 µl moto oil was dropped onto the surface of the water, followed by the addition of 10 µl of solution of extract vegetable. The diameter and the clear halo visualized under visible light were measured after 30 s (Rodrigues et al., 2006).

2.7 Stability studies

Stability studies were performed using the extracts vegetable heated at 50, 100 and 121 ºC for 1 h and cooled to room temperature, and exposure to a low temperature (5 °C) after which the surface tension were determined. The pH of the vegetable extract was adjusted to different values (2.0 to 14.0) for the determination of stability regarding surface tension. The effect of the addition of NaCl (at concentrations of 2.0 to 50.0 %) was also determined. All assays were carried out in triplicate and did not vary by more than 5 %.

2.8 Surface Wash Test

For washing test was used metal blade the size of 26x76 mm and part of its area was uniformly contaminated with 250 µl of motor oil. The contaminated section of the slide was submerged in the solution of the extract containing the biosurfactant under constant stirring for 3 minutes. At the end, the slide was dipped in distilled water, removing any excess from the test solution. This was oven dried 40 °C for 30 minutes and its weight noted. The removal rate shall be calculated by the weight of the contaminated sheet minus the weight of the sheet after washing on the weight of the contaminated sheet minus the weight of the clean sheet x 100.

3. Results and discussion

3.1 Assessment of physiochemical properties of vegetable biosurfactants

The biosurfactant of origin vegetable, include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains. Their structural diversity is reflected in their physicochemical and biological properties, which are exploited in a number of traditional test. The oil displacement test, and the evaluation of the surface tension and Emulsification activity are indicative of the surfactants (Xu et al., 2011). In the present study the surface activities of the crude extracts vegetable containing the biosurfactant of *Genipa americana* L. (Genipap) and *Tamarindus indica* L. (Tamarinds) were investigated and compared with the Tween 80. The oil displacement test was highly positive for extracts vegetables than the commercial surfactant, Tween 80, which indicated high surface activity (Table 1).

Table 1: Physicochemical properties of vegetables biosurfactants produced by Genipa Americana L. and Tamarindus indica L.

Characterization	Surface tension (mN/m)	CMC (g/L)	Oil displacement (cm^2)
Distilled water	72. 03 ± 0. 20	Negative	Negative
Genipa americana L.	31.39 ± 0. 15	0.650	0.650
Tamarindus indica L.	30.02 ± 0.17	0.870	0.870
Tween 80	37. 76 ± 0.19	1.250	1.250

The ability of the vegetable extracts as well as Tween 80 to reduce the surface tension of distilled water was compared (Table 1). The extracts of *Genipa americana* L. and *Tamarindus indica* L. reduced the surface tension of distilled water to a minimum value with low value of CMC. As shown in Table 1 Tween 80 reduce the surface tension but with high CMC values. The results suggested that the biosurfactants of the extracts vegetable from provided excellent properties in terms of reduction of surface tension and a low value of CMC.

The emulsification activity of biosurfactants can be clearly understood through investigation of the emulsification index. Once the emulsification index is determined, this information can beapplied to estimate the level of biosurfactant to be utilized by environmental pollution treatment plants (Sarubbo et al 2015). The biosurfactant vegetable exhibited emulsification effects on all threen hydrocarbon compounds, and the emulsification index reached 37–100% (Figure 1).

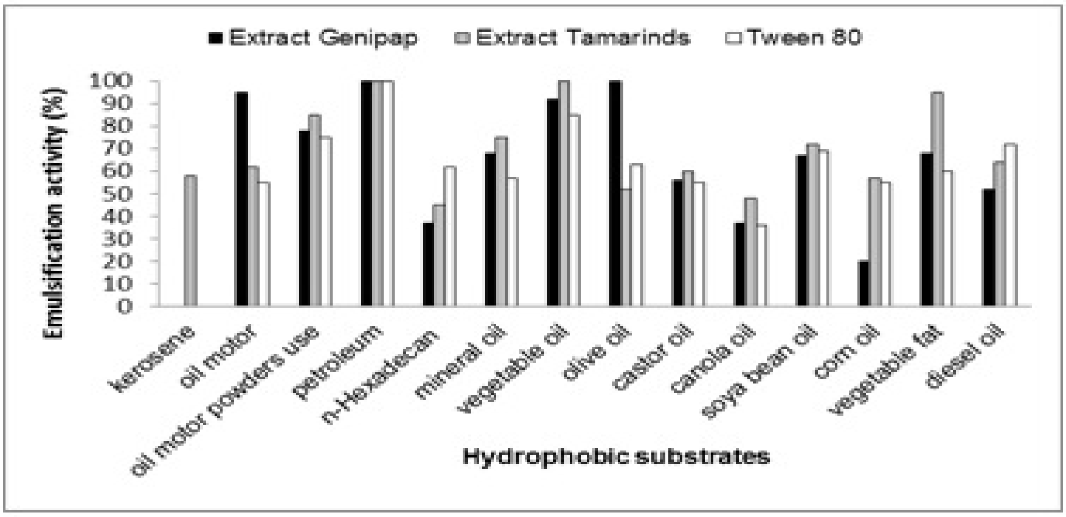

Figure 1: Emulsification activity (E24) of the crude biosurfactants vegetables produced by Genipa americana L. and Tamarindus indica L. towards different hydrocarbon substrates compared with Tween 80

Thus, the vegetable biosurfactant showed surface activities comparable with the commercial surfactant (Tween 80). The stability of plant biosurfactants at various pH, temperature and NaCl concentrations was studied using surface tension measurements. The surface tension of the plant extracts containing the biosurfactant remained very stable during exposure to a wide range of temperatures (5 - 121 ° C, Figure 3), pH (2-10, Figure 4) and salt (2-50% w / v , Figure 2). A similar stability of the biosurfactant was reported when a salinity range was applied (Banat, 2010). Results showed that vegetables biosurfactant are stable in alkaline conditions for the related applications in the future.

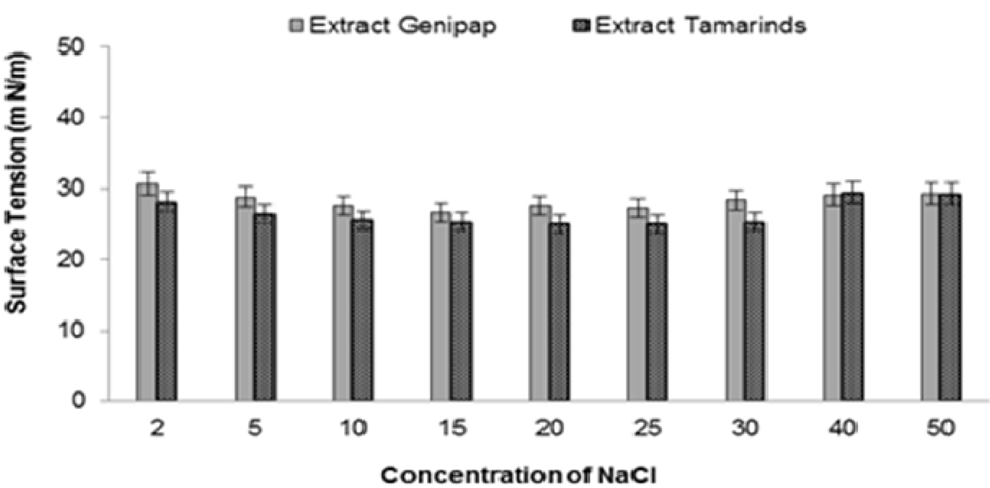

Figure 2: Effect of concentration NaCl on the surface tension of vegetable biosurfactants produced by Genipa americana L. and Tamarindus indica L.

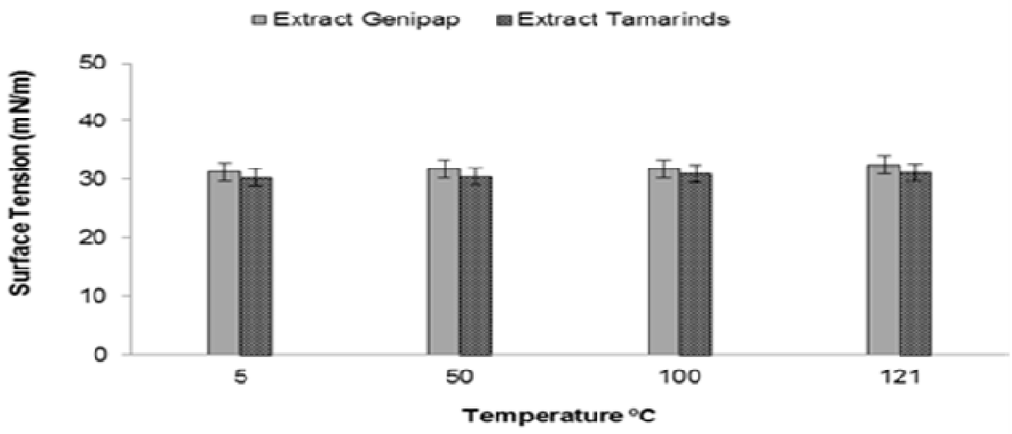

Figure 3: Effect of temperature on the surface tension of vegetable biosurfactants produced by Genipa americana L. and Tamarindus indica L.

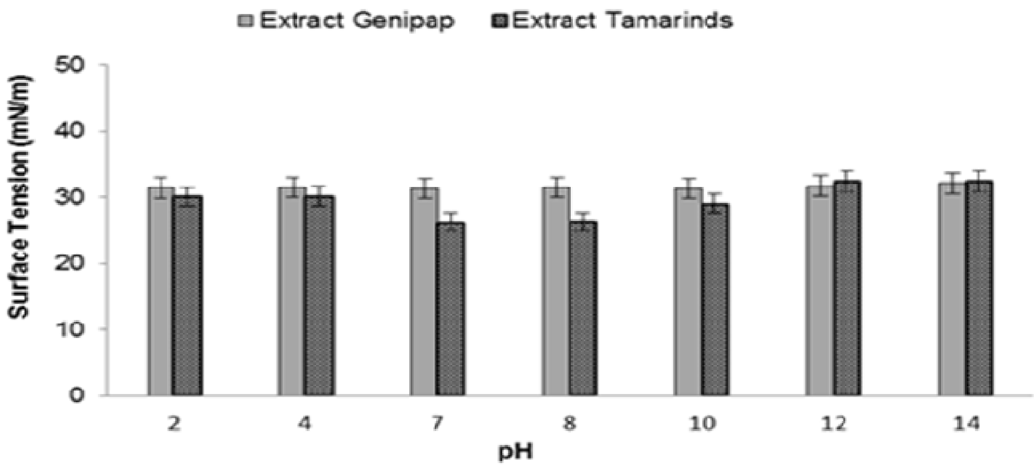

Figure 4: Effect of pH on the surface tension of vegetable biosurfactants produced by Genipa americana L. and Tamarindus indica L.

3.2 Oil removel by washing with vegetable biosurfactant

The application of biosurfactants is one of the most promising techniques to remove and recover a significant amount of the residual oil in environment (Sarubbo et al., 2012). The results of test of washing demonstrated that the biosurfactants vegetables were able to remove 82% and 78% the motor oil, while the distilled water (control) not removed and the synthetic surfactant removed 54% of the contaminated oil respectively (Table 2). And therefore, the biological surfactants have advantages over chemical surfactants in being more efficient, effective and ecofriendly, because they remove oil contaminants by mobilization without modifying the chemical nature of soil (Silva et al., 2014).

Table 2: Removal of motor oil by the biosurfactants from Genipa americana L. and Tamarindus indica L. and distilled water (as the control)

Solutions	Removal (%)
Distilled water	0%
Genipa americana L.	82%
Tamarindus indica L.	78%
Tween 80	44%

4. Conclusions

The biosurfactants produced by *Genipa americana* L. (Jenipapo) and *Tamarindus indica* L. can be considered potential alternatives to be applied in the remediation of environments contaminated by oil and oil products. The physicochemical characteristics of the biosurfactants obtained confirmed their tensoactive and emulsifying properties, and consequently their potential for use in industrial and environmental applications.

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