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Surfactant properties and emulsifying activity of the gum exudate of *Prosopis juliflora* (Sw.) DC.

Propiedades tensoactivas y actividad emulsionante del exudado gomoso de *Prosopis juliflora* (Sw.) DC.

Propriedades surfactantes e actividade emulsificante do exsudado gomoso de *Prosopis juliflora* (Sw.) DC.

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Abstract

Gums exudates are macromolecules consisting of carbohydrates (majority fraction), proteins and lipids (minority fraction), with variable concentrations of minerals, polyphenols, flavonoids, tannins and other bioactive phytochemical compounds. These natural products are used as emulsifying agents in multiple industries. The surfactant properties of a new source of gum exudate produced by *Prosopis juliflora* (Sw.) DC. were evaluated. Additionally, water-oil dispersions prepared with this natural polymer were tested for their emulsifying capacity and stability. A Du Noüy ring tensiometer was used to determine the amphipathic behavior of the investigated gum. The gum exudate of *P. juliflora* tested at 0.5 % m/v, decreases the values of surface tension (49.35 dyne.cm^{-1}) and interfacial tension (12.78 dyne.cm^{-1}), which evidences the potential emulsifying activity (EA) of this polysaccharide. EA values of 95 % and emulsion stability of 95.8 % were obtained, suggesting that *P. juliflora* gum contributes to improve the capacity and speed of adsorption of molecules between the dispersed phase and continues to form a stable emulsion. The surfactant and emulsifying activity of the investigated gum is associated with the high protein content (16.89 %) and the presence of methyl groups in its structure. Therefore, the gum exudate of *P. juliflora* constitutes a promising source of hydrocolloids as an emulsifier that could be evaluated in the manufacture of pharmaceuticals, food and even cosmetics. Additionally, it constitutes an unexploited natural resource that would contribute to the development of the South American regional economies where this species grows.

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Resumen

Los exudados gomosos son macromoléculas constituidas por carbohidratos (fracción mayoritaria), proteínas y lípidos (fracción minoritaria), con concentraciones variables de minerales, polifenoles, flavonoides, taninos y otros compuestos fitoquímicos bioactivos. Estos productos naturales son usados como agentes emulsionantes en múltiples industrias. Se evaluó las propiedades tensoactivas de una nueva fuente de exudado gomoso producido por *Prosopis juliflora* (Sw.) DC. Adicionalmente, a las dispersiones preparadas agua-aceite con este polímero natural, se les determinó la capacidad emulsionante y su estabilidad. Se empleó un tensiómetro con anillo Du Noüy para determinar el comportamiento anfipático de la goma investigada. El exudado gomoso de *P. juliflora* ensayado a 0,5 % m/v, disminuye los valores de tensión superficial (49,35 dinas.cm^{-1}) e interfacial (12,78 dinas.cm^{-1}), lo cual evidencia la potencial actividad emulsionante (EA) de este polisacárido. Se obtuvieron valores de EA del 95 % y de estabilidad de la emulsión del 95,8 %, lo cual sugiere que la goma de *P. juliflora* contribuye a mejorar la capacidad y velocidad de adsorción de las moléculas entre la fase dispersa y continúa, formando una emulsión estable. La actividad tensoactiva y emulsionante de la goma investigada se asocia con el alto contenido proteico (16,89 %) y la presencia de grupos metilos en su estructura. Por lo tanto, el exudado gomoso de *P. juliflora*, constituye una fuente promisoria de hidrocoloides como emulsionante que podría evaluarse en la fabricación de fármacos, alimentos e incluso cosméticos. Adicionalmente, constituye un recurso natural sin explotar que contribuiría al desarrollo de las economías regionales sudamericanas donde crece está especie.

Palabras clave: actividad superficial, actividad interfacial, emulsionante, biopolímero, hidrocoloides.

Resumo

Exsudados gomosos são macromoléculas que consistem em carboidratos (fração maioritária), proteínas e lipídios (fração minoritária), com concentrações variáveis de minerais, polifenóis, flavonóides, taninos e outros compostos bioativos fitoquímicos. Estes produtos naturais são usados como agentes emulsificantes em muitas indústrias. Estes produtos naturais são usados como agentes emulsificantes em múltiplas indústrias. Foram avaliadas as propriedades surfactantes de uma nova fonte de exsudato gomoso produzido por *Prosopis juliflora* (Sw.) DC. Além disso, as dispersões de água-óleo preparadas com este polímero natural foram testadas quanto à sua capacidade de emulsificação e estabilidade. Um tensiómetro de anel Du Noüy foi utilizado para determinar o comportamento anfíbio da goma investigada. O exsudado de *P. juliflora* testado a 0,5 % m/v, diminui os valores de tensão superficial (49,35 dyne.cm^{-1}) e tensão interfacial (12,78 dyne.cm^{-1}), o que evidencia a potencial actividade emulsificadora (EA) deste polissacarídeo. Foram obtidos valores de EA de 95 % e estabilidade de emulsão de 95,8 %, sugerindo que a goma *P. juliflora* contribui para melhorar a capacidade e velocidade de adsorção das moléculas entre a fase dispersa e continua a formar uma emulsão estável. A actividade surfactante e emulsionante da goma investigada está associada ao elevado conteúdo proteico (16,89 %) e à presença de grupos metilo na sua estrutura Por conseguinte, o exsudado de goma de *Prosopis juliflora* constitui uma fonte promissora de hidrocolóides como emulsionante que poderia ser avaliada no fabrico de produtos farmacêuticos, alimentares e até cosméticos. Além disso, constitui um recurso natural inexplorado que contribuiria para o desenvolvimento das economias regionais da América do Sul onde esta espécie cresce.

Palavras-chave: actividade superficial, actividade interfacial, emulsificante, biopolímero, hidrocoloides.

Introduction

Gum exudates are high molar mass and structurally complex macromolecules, consisting of carbohydrates (majority fraction), proteins and lipids (minority fraction), with variable concentrations of minerals, polyphenols, flavonoids, tannins and other bioactive compounds (Licá *et al*., 2018; Vasile *et al*., 2016).

These polysaccharides are widely used as natural additives in various industries, they exhibit multiple functionality such as: emulsifiers, foaming agents, film formers, fillers, coating material, even as a vehicle to optimize nutrient absorption (Prajapati *et al*., 2013).

Gum arabic produced by *Acacia senegal* is the additive used par excellence in the formulation and preparation of emulsions in multiple industries. The excellent emulsifying activity exhibited by this natural polymer has been associated with the content of arabinogalactan-protein complexes (AGP) in its structure (Williams and Phillips, 2021).

The scarcity and price instability of gum arabic has prompted the search for novel sources of gum. The functionality of gum exudates from *Acacia catechu* (Sharma *et al*., 2021), *Pithecellobium dulce* (Bushan *et al*., 2020); *Prosopis* spp. (Mubgil and Barak, 2020), *Prosopis alba* (Vasile *et al*., 2019; Vasile *et al*., 2016), *Soymida febrifuga* (Bhushett and Annapure, 2018), *Prosopis* spp. (Lopez-Franco *et al*., 2015; López-Franco *et al*., 2012), *Acacia mearnsii* de Wild (Grein *et al*., 2013), have been investigated with the aim of assessing their potential emulsifying capacity, microencapsulant and as raw material in film formation.

In recent years, the potential use of gums obtained from the *Prosopis* genus has been evidenced. The antioxidant activity of the gum exudate of *Prosopis alba* has been reported, which would extend its use as a preservative, especially in the processing of foods, drugs and cosmetics, containing easily oxidizable oils, evaluating these natural products as encapsulants and/or emulsifiers (Vasile *et al*., 2019).

On the other hand, the low viscosity presented by the exudate of *Prosopis* spp., suggests its use in the elaboration of coating films for fresh fruits, coadjuvant in the fruit dehydration process, stabilizing the foam formed during this process; as a binder in the manufacture of tablets and emulsifier/stabilizer of suspensions (Mubgil and Barak, 2020). Additionally, the gums of the genus *Prosopis* have a high dietary fiber content, which could be used in the formulation of functional foods (Mubgil and Barak, 2020; Rincón *et al*., 2020).

Prosopis juliflora (Sw.) DC. (Mimosaceae), a species disseminated in the state of Zulia-Venezuela, produces a brownish-colored, water-soluble exudate at the trunk level with high yield (Clamens *et al*., 2000). The objective of this study was to evaluate the surfactant properties and emulsifying activity of the gum exudate of *P. juliflora* as a potential emulsifying agent.

Materials and methods

Location and identification of *Prosopis juliflora* specimens

The gum exudate of *Prosopis juliflora* (Sw.) DC. (Mimosaceae) was obtained from 30 trees located at 10º40'33'' north latitude and 71º38'29'' west longitude at 32 masl in Maracaibo, Zulia, Venezuela. The botanical identification of the species was certified by MSc. Carmen Clamens, Botanist Taxonomist of the University of Zulia.

Obtaining and purification of the gum exudate of *P. juliflora*

Wounding practices were performed at the trunk level of *P. juliflora* trees in order to stimulate the gum exudation process, which was collected in the non-rainy period (January-April, 2019). Subsequently, it was cleaned of tangible impurities (sand and remaining bark) and subjected to heating (40 ºC, 5 h) in an oven (Model 348, Fisher Scientific brand), in order to minimize moisture and facilitate grinding. A pulverizer (Model Tittan 100) was used, equipped with a motor (3 HP) and two sieves with pore sizes of 0.31 mm and 0.60 mm. The method described by Muñoz *et al*. (2007) was applied to obtain the pure polymer, which was dried in a freeze dryer (LABCONCO Freezone 6), at -40 ºC and 133 x 10^{-3} mBar. The yield obtained from the purified polymer is 49.4 %.

Protein content

It was determined by applying the Kjeldahl method. The protein value was obtained with: N x 6.25.

Determination of the surfactant properties of the gum exudate of *Prosopis juliflora*

Aqueous dispersions of the gum exudate *P. juliflora* were prepared at different concentrations (0.03 - 1.2 % m/v), and their surface and interfacial activity was measured. A tensiometer (CSC Scientific Company) was used. The Du Noüy ring method was applied. The measurements were carried out 30 min after the sample was added to the vessel at a temperature of 25 °C. Isopropyl myristate was evaluated as a hydrophobic medium (Chávez-Narváez *et al*., 2009). The results were expressed in dynes.cm^{-1} and are the average of three (3) measurements.

Preparation of the emulsified water-oil dispersions

The emulsions (200 mL) prepared contained gum exudate of *P. juliflora* at the following concentrations (0.1; 0.25; 0.5; 1.0 % 1.5 and 2 % m/v), the pH of the dispersions was adjusted to 7 with a buffer-bicarbonate solution. Subsequently, 20 mL of corn oil was added. A homogenizer (BOECO, Model OSD-20) was used with the following conditions (agitation 2 min.10.000 rpm) to form the emulsions.

Emulsifying Activity (EA) and Emulsion Stability (ES)

The prepared emulsified dispersions were subjected to different temperatures (60 and 80 °C), for 30 min, then centrifuged (4000 rpm, 8 min), in order to measure the effect of temperature on ES. The EA and ES were determined by applying the methodology described by Naji *et al*. (2012).

Stability of emulsions prepared with *P. juliflora* gum during storage

Water-oil emulsified dispersions containing gum exudate of *P. juliflora* were prepared at 0.5 % m/v, concentration that evidenced the highest emulsifying power of the evaluated gum, were hermetically stored at 25 ºC in glass jars with lids. The absorbance measurements were carried out through turbidimetric tests every 48 hours for 15 days, using a spectrophotometer (Thermo Fisher Scientific, model Genesys-10S). The measurements were made at 650 nm in glass cells with a capacity of 2.5 mL.

Results and discussion

Surfactant properties of *P. juliflora* gum

Figure 1, shows that increasing the concentration of *P. juliflora* gum decreased the surface tension reaching its saturation point at 0.5 % m/v.

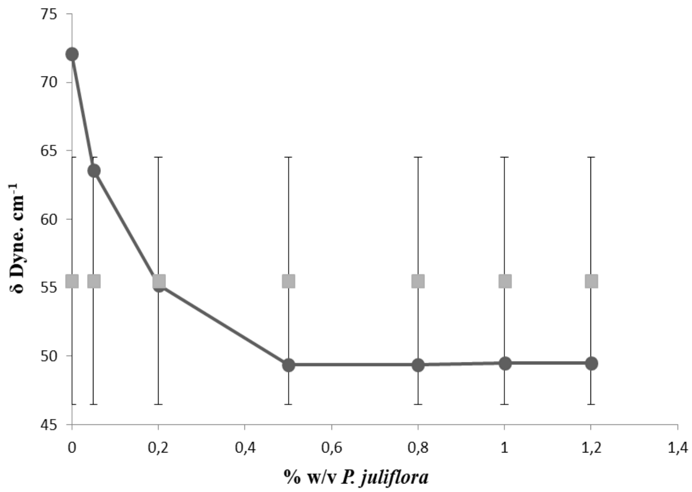

Figure 1. Effect of *Prosopis juliflora* gum exudate concentration on surface tension.

The result obtained in the present study (49.35 dynes.cm^{-1}, 0.5% m/v) is comparable to those reported by Garti *et al*. (1999) for *Portulaca oleracea* (47 mN.m^{-1}, 0.6% m/v) and *Acacia senegal* (45 mN.m^{-1}, 0.5 % m/v) gums, as well as by Muñoz *et al*. (2007) for *Acacia tortuosa* (42.6 mN.m^{-1}, 0.5% m/v). However, they are lower than the values published by Huang *et al*. (2001) for carrageenans (65.0 dyne.cm^{-1}, 0.5 % m/m), xanthan gum (60.8 dyne.cm^{-1}, 0.5 % m/m), guar gum (55.2 dyne.cm^{-1}, 0.5 % m/m), fenugreek gum (53.6 dyne.cm^{-1}, 0.5 % m/m), methylcellulose (52.9 dynes.cm^{-1}, 0.5 % m/m), and to those obtained by Pérez-Mosqueda *et al*. (2013) on *Sterculia apetala* (56.10 mN m^{-1}) and by Grein *et al*. (2013) on *Acacia mearnsii* from Wild (56.70 mN.m^{-1}).

Prosopis juliflora gum evaluated at different concentrations showed interfacial activity (figure 2), evidencing the ability to adsorb at the water-oil interface reducing the interfacial tension (12.78 dynes.cm^{-1}, 0.5% m/v).

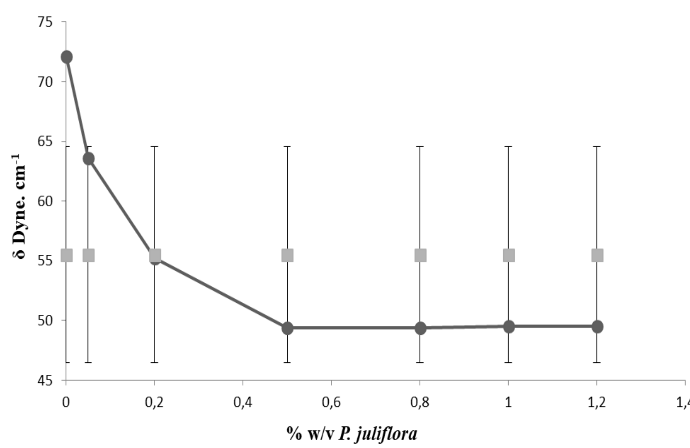

Figure 2. Effect of *Prosopis juliflora* gum exudate concentration on interfacial tension.

This value is analogous to those published for *Sterculia apetala* gum (12.80 dyne.cm^{-1}, 0.5 % m/v) (Rincón *et al*., 2018) in xanthan produced by *Xanthomonas campestris*, (12.0 dyne. cm^{-1}, 0.5 % m/m), pectins (12.40 dyne.cm^{-1}, 0.5 % m/m) and in synthetic polymers such as methylcellulose (12.1 dyne.cm^{-1}, 0.5 % m/m) (Huang *et al*., 2001); but relatively higher than those observed by Huang *et al*. (2001) for *Trigonella foenum-graecum* gum (7.9 dyne.cm^{-1}, 0.5 % m/m), for *Cyamopsis tetragonolobus* (8.3 dyne. cm^{-1}, 0.5 % m/m), and in microcrystalline cellulose (9.5 dyne.cm^{-1}, 0.5 % m/m). The result obtained in this study suggests the potential ability of *P. juliflora* gum in the formation of protective films around the oil droplets guaranteeing the homogeneity and stability of the emulsions. The interfacial ability of these natural polymers has been associated with their amphipathic nature, with hydrophilic groups (OH^- and $COOH^-$) with partially negative charges belonging to neutral and acidic sugars, which interact with water molecules by breaking hydrogen bonds. While the mostly hydrophobic groups of the amino acid side chains present in its protein fraction interact with the hydrophobic groups of the lipids present in the oil (Al-Assaf *et al*., 2009).

The excellent surfactant activity observed for the gum exudate of *P. juliflora* evidences the potential emulsifying capacity of this natural polymer. Analogous results have been published for gums exudates from *Prosopis* spp. (López-Franco *et al*., 2015; López-Franco *et al*., 2012), *Prosopis alba* (Vasile *et al*., 2016), *Acacia catechu* (Sharma *et al*., 2021) and *Pithecellobium dulce* (Busham *et al*., 2020).

The surfactant properties exhibited by the studied gum are probably due to the presence of arabinogalactan-protein complexes in its structure, which has been widely described for gum exudates of *Prosopis* spp. (López-Franco *et al*., 2015; López-Franco *et al*., 2008). The emulsifying activity of gum arabic produced by *Acacia senegal*, an additive widely used as an emulsifier at the industrial level, has been mainly associated with the protein fraction present in the structure of these heteropolysaccharides (arabinogalactan-protein type II) (Lee and McClements 2010, Dickinson, 2011; Williams and Phillips 2021).

Emulsifying activity (EA) and emulsion stability (ES)

Figure 3, shows that 95 % emulsification values were obtained at a concentration of 0.5 % m/v, evidencing no significant difference ($p > 0.05$) when compared to 1 %. However, at a higher concentration (1.5 % m/v) there were statistical differences ($p < 0.05$), obtaining values of 98 %. A suitable concentration (0.5 % m/v) was selected for testing in gum exudates as emulsifying agents.

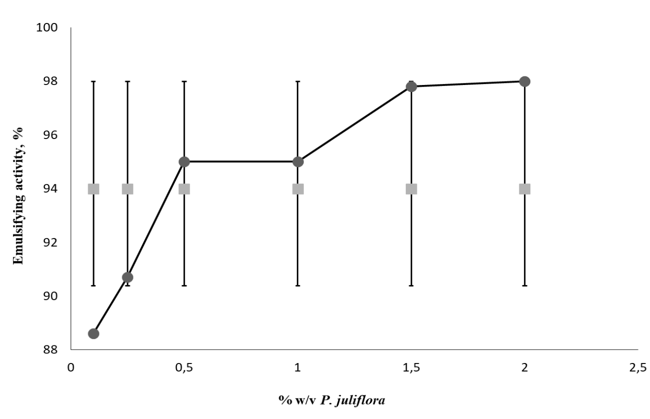

Figure 3. Effect of *Prosopis juliflora* gum exudate concentration on emulsifying activity.

The EA (95 %) obtained by the studied gum is higher than that observed for gum Arabic (92 %, 0.5 % m/m) (Vasile *et al*., 2016) and comparable to the range reported for *Prosopis* spp. gums (93-96 %, 0.5 % m/v) (Vasile *et al*., 2016; López-Franco *et al* 2015; López-Franco *et al*., 2008). This property has been linked to the ability to increase viscoelastic properties at the water-oil interface (Sharma *et al*., 2021, Bhushan *et al*., 2020; Vasile *et al*., 2016; Pérez-Mosqueda *et al*., 2013; López-Franco *et al*., 2012), which contributes to improve the adsorption capacity and speed of molecules at the interface and as a consequence, increases emulsion stability. Functionality that has been mainly associated to the formation of AGPs complexes in the structure of these natural polymers (Al-Assaf *et al*., 2009), as well as, to the presence of methyl groups in the periphery of these hydrocolloids (López-Franco *et al*., 2015; López-Franco *et al*., 2012).

The emulsions prepared with *P. juliflora* gum, present excellent emulsion stability (ES) (95.8 %), at low concentration (0.5 % m/v), at 60 °C, comparable with the rest of the evaluated concentrations (Figure, 4). It has been reported that emulsions prepared with *Prosopis* spp. gum have ES values of 97 % higher than those obtained with those prepared with gum arabic and other species of the genus *Acacia*, which range between 93 and 94 % (López-Franco *et al*., 2015; López-Franco *et al*., 2008). The high molar mass of the complex (AGPs), the degree of polydispersity and the accessibility or structural conformation of the amino acid group of these complexes, constitute factors that affect the decrease in interfacial tension, which is determinant in the stability of emulsions (Dickinson, 2009; Dickinson, 2011).

However, at 80 °C temperature, a significant decrease ($p < 0.05$) of the ES values is observed in comparison with 60 °C temperature (figure 4), which at the concentrations of 0.5; 1.0 and 1.5 % m/v are close to 90 %, suggesting that the prepared emulsions tend to destabilize, and this behavior is mostly marked in the emulsions prepared with *P. juliflora* gum at concentrations lower than 0.5 % m/v (85.8 %).

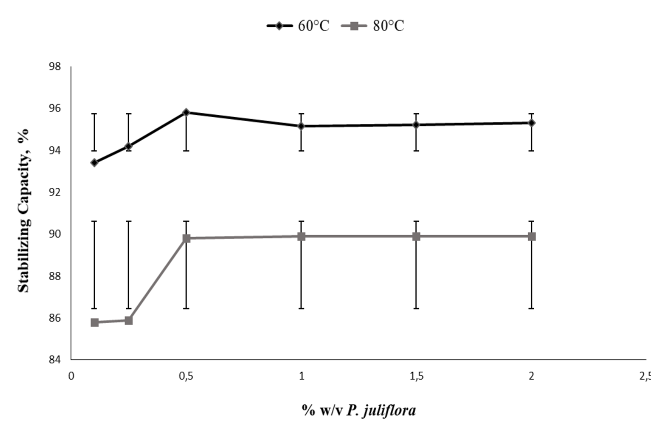

Figure 4. Effect of temperature on the stability of emulsions prepared at different concentrations of *Prosopis juliflora* gum.

The high kinetic energy generated at high temperatures allows the established intermolecular attractions (hydrogen bridges) to be easily overcome, which affects the stability of heterogeneous dispersed systems (Dickinson, 2011). It has been evidenced that emulsions prepared with gum arabic and subjected to temperatures higher than 75 °C, significantly decrease their stability; reported ES values are in the order of 85 % (López-Franco *et al*., 2015; Vasile et al., 2016). This effect has also been evidenced in emulsions prepared with commercial galactomannans (Wu *et al*., 2009) and obtained from *Prosopis* spp. (López-Franco *et al.*, 2013).

Stability of emulsions as a function of storage days.

Figure 5 shows a progressive decrease in absorbance values as a function of days of storage. A significant decrease in absorbance (0.84) was observed on day 10, with respect to those obtained at 0 hours (1.10) and 48 hours (1.02), respectively; suggesting emulsion destabilization causing emulsion rupture.

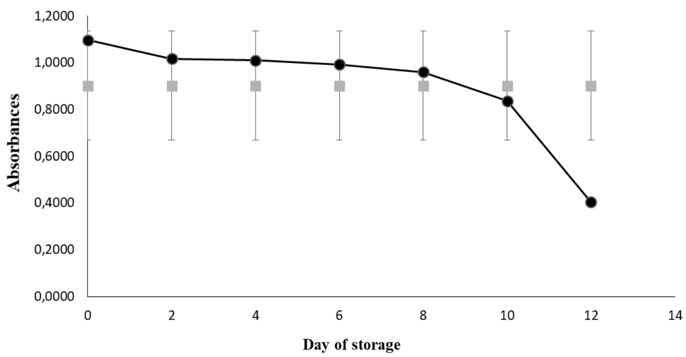

Figure 5. Emulsifying activity of *Prosopis juliflora* gum exudate as a function of storage time.

The behavior obtained in the present study (figure 5), is comparable to those reported for emulsions prepared with *Prosopis* spp. gum and formulated with gum arabic (0.5 % m/v) (López-Franco *et al*., 2015; Vasile *et al*., 2016), which corroborates the potential emulsifying capacity of the gum studied.

The protein content in the gums/hydrocolloids is determinant in the potential emulsifying capacity of these natural polymers. The protein present in the gum exudate of *P. juliflora* (16.89 + 0.51 %) is higher than that reported for the *Prosopis* spp. gum (6.98 + 0.13 %) (López-Franco *et al*., 2012), *Prosopis alba* (13.81 + 0.33 %) (Vasile *et al*., 2016), and significantly higher than that presented by *Acacia senegal* (1.37 + 0.04 %) (López-Franco *et al*., 2012). The protein fraction associated with the polysaccharide acts as a hydrophobic group (anchoring or binding point), responsible for the emulsifying activity (Dickinson, 2011; Dickinson, 2012). On the other hand, preliminary studies of the polysaccharide isolated from the exudate of *P. juliflora*, showed a large proportion of methyl groups (methylated glucuronic acid residues) at the periphery of the structure of this polymer, as has been reported for most *Prosopis* spp. gums (Vasile *et al*., 2016; López-Franco *et al*., 2015; López-Franco *et al*., 2012; López-Franco *et al* 2008), which would increase its emulsifying power.

Conclusions

The gum exudate of *P. juliflora* studied at 0.5 % m/v, decreases the surface and interfacial tension values, which evidences the potential emulsifying activity (EA) of this polysaccharide. The obtained values of EA and emulsion stability indicate that *P. juliflora* gum contributes to improve the capacity and speed of adsorption of molecules between the dispersed phase and continues to form a stable emulsion. The surfactant and emulsifying activity of the gum studied is associated with the high protein content and the presence of methyl groups in its structure. Therefore, the gum exudate of *Prosopis juliflora* constitutes a promising source of hydrocolloids as an emulsifier that could be evaluated in the manufacture of pharmaceuticals, food, and even cosmetics. Additionally, it constitutes an unexploited natural resource that would contribute to the development of the South American regional economies where this species grows.

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