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Evaluation Of *Jatropha gaumeri* Latex In Bucal Ulcers: Toxicological, Phytochemical And Antioxidant Profile.

Floribeth León Pérez[1]*, Saydé Pérez Olivares[1], Rafael Mex Álvarez[2], Josefina Graciela Ancona León [2], Verónica Macedo Reyes[3], and Victor Monteón[4].

[1]Faculty of Dentistry, Autonomous University of Campeche, Mexico
[2]Faculty of Biological Chemistry Sciences Autonomous University of Campeche, Mexico
[3]State Center of Oncology of San Francisco of Campeche, Mexico
[4]Center for Biomedical Research, University of Campeche, Mexico

ABSTRACT

Jatropha gaumeri Greenm latex is widely used in traditional medicine in Mexico. In this work, we studied its cicatrizant activity, toxicity, phytochemical composition, and antioxidant effect. Phytochemical screening was performed in ethanol extract of latex. Acute and subacute oral toxicity was determined in rats with doses of 1mg/kg by means of transaminases, urea and creatinine before and after the study, followed by histological sections stained with hematoxylin and eosin. The clinical evaluation was carried out in 39 patients with mouth ulcers, the control group received oleozon®, and the other group 350µL of latex. Our result showed similar cure rate in oleozon® and latex treated patients with significant remission of pain within the first 24 hours. Acute and subacute oral toxicity in the rat model, showed no statistically significant differences in seric level of alanine and aspartate amino transferase, urea and creatinine, neither at the histology level. The phytochemical analysis revealed terpenes, phenols, tannins, lactones, quinones, flavonoids and saponins. In conclusion, the latex presented anti-inflammatory, analgesic properties and cure rate comparable with oleozon® in the treatment of recurrent aphthous stomatitis and traumatic ulcers.

Keywords: *Jatropha gaumeri*, oral ulcers, acute toxicity, antioxidant.

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Corresponding author

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INTRODUCTION

The most frequent disease in the buccal mucosa is ulcer. These are injuries resulting from the destruction of the epithelial lining and part of the underlying tissue. Among the main ulcers present in the oral mucosa are those of traumatic origin, acute herpetic gingivo stomatitis (GEHA) and recurrent aphthous stomatitis (RAS). Regardless of the type of ulcers, those who suffer from them manifest intense pain and hinders feeding, which subsequently affects personal performance, family and work relationship. The lesion usually disappears approximately 5 to 14 days, but sometimes may last even weeks. It is known that RAS does not have an exact etiology, so the treatments used are multiple and varied; in addition, there is currently no specific drug that completely cures this condition. The most successful therapeutic trials have been able to shorten the acute period of the disease and prolong the time of recurrences [1]. In this sense, alternative treatments such as ozone therapy, laser therapy, *Rosa damascena* and Berberine gelatin have been used.

Another natural product with expectations for the treatment of oral ulcers is the latex *Jatropha gaumeri* Greenm, a quasi-endemic plant of the Yucatan Peninsula, Mexico where it is known mainly with the Mayan word pomolché, it is widely used in traditional medicine for its properties of healing skin lesions, oral mucosa and digestive ulcers. Recent work described its anti-inflammatory, cytotoxic activity in epithelial cancer cells, treatment of intestinal infections and digestive disorders [2]. Although numerous studies have shown that species of the genus *Jatropha* have diverse applications from ornamental plants to biofuels and mainly as remedies in the traditional medicine, however, the *gaumeri* species has been poorly studied [3-5]. In other study, it was found that diterpenes in extracts of leaves and roots showed antimicrobial activity against *B. subtilis* and compounds such as β-sitosterol, triterpenes and taraxasterol exhibited antioxidant activity [6].

However, systematic studies are lacking in the evaluation of cicatrizant, cytotoxic and antioxidant activity of the latex. The importance of this work lies in demonstrating the effectiveness and safety of *J. gaumeri* Greenm latex in the treatment of oral ulcers.

MATERIALS AND METHODS

Clinical study: A controlled, double-blind clinical trial was conducted over a 1 year period. The sampling was of convenience. The participants of the study were all those patients diagnosed with canker sores, recurrent aphthous stomatitis (RAS), acute herpetic gingivostomatitis (GEHA) , traumatic ulcers in the oral cavity, who came to the clinics of the dentistry faculty for their treatment.

Inclusion criteria were patients of both sexes with a diagnosis of canker sores, RAS, GEHA, and traumatic ulcers, between 3 and 65 years of age and who agreed to participate in the study. Patients with a diagnosis of diabetes mellitus, peptic ulcer, blood dyscrasias and systemic diseases that could affect the recovery period of oral tissues were excluded, including patients receiving chemotherapy or radiotherapy, those with mental retardation, alcoholics and with psychiatric treatment. Patients who interrupted their treatment or stopped attending the evaluations were eliminated from the study.

Patients were enrolled in two study groups. One group received topically 116 µL (0.005mg/Kg) of undiluted latex in the lesion three times a day (the latex was stored in a refrigerator for not more than 24 hr). The second group received 3 drops of ozone solution in each lesion three times a day and it was called the control group. Both groups received treatment after meals and oral hygiene. The treatment was given only while the lesions persisted and for no more than 10 days. Patients were monitored at 24, 48, 72 hours and on the 5th, 7th and 10th days. The variables investigated were pain using analogue scale (mild=from 1 to 4, moderate=from 5 to 7 and severe=from 8 to 10), level of erythema or redness and healing time of the ulcer. Two independent specialized dentist surgeons carried out the diagnosis and follow-up.

All participants in the study were volunteers, who provided the necessary information regarding the study, and signed an informed consent letter.

The work was performed in compliance with Official Mexican Standard NOM-012-SSA-201, Official Mexican Standard NOM-062-ZOO-1999, and institutional guidelines and approved FOMIX-CONACYT No.144468

Latex Collection: The latex was obtained by cutting the secondary stems of the *Jatropha gaumeri* Greenm plant. It was collected in sterile material, transported and stored at 4° C until its use. This plant was located at the coordinates: 19° 48' 50.14'' -90° 33' 12.51''. A specimen was deposited in the UCAM herbarium of the Historical and Social Research Center of the Autonomous University of Campeche, with the registration number 25005.

Determination of acute and subacute toxicity: Forty female Wistar rats weighing approximately 200 G were used. They were divided into 2 groups of 10 rats each. A latex-free control group and two experimental groups: acute and subacute toxicity group. Both received a daily dose of 200 µL (1mg/Kg) administered orally for 7 days (acute group) and for 1 month (subacute toxicity group). The animals were kept with water and food ad libitum. Blood samples were obtained before and at the end of the experiment. The seric level of ALT, AST, creatinine and urea was determined. At the end of the studies, the rats were sacrified and the kidney and liver were processed for histology by paraffin inclusion and stained with hematoxylin and eosin.

Phytochemical screening: The latex of each plant was diluted in absolute ethanol (1:10).

Content of polyphenols and total tannins: Identification was carried out by the Folin-Ciocalteu method and tannins by methods cited elsewhwere [7-9].

Antioxidant activity: The antioxidant activities are estimated by the capturing capacity of 2, 2-diphenyl-1-picrylhydrazole (DPPH) as previously described [10-13].

Statistical analysis: Descriptive statistics as means standard deviation and the two-way Anova test was used to compare the decrease of pain, erythema and healing after treatment in treated groups, and the paired student's t-test to compare levels of seric markers (PRISMA version 4.0 program).

Ethical matters: The animal study was taken after the prior approval of Institutional Animal Ethics Committee following the Principles of Laboratory Animal Care. The clinical investigation was aproved and the informed consent was obtained (proposal No. 144468)

RESULTS

Clinical study:

We examined 39 patients between 3 and 61 years old, who were attended to at the clinics of the Faculty of Dentistry at the Autonomous University of Campeche. Patient groups were matched by age, sex, and type of injury. Forty nine percent (19 patients) were treated with pomolché latex and 51% (20 patients) with exclusive ozone therapy (Oleozon®). The most frequent types of ulcers were recurrent aphthous stomatitis (48.7%), followed by traumatic ulcers (35.9%), 4 patients with acute herpetic gingivostomatitis (10.2%) and only 2 cases of simple aphthous ulcers (5.1%). The lesions on the lips represented 28.2% and 25.6% showed lesions in several parts of the oral mucosa (Table 1). One hundred percent of patients were cured in the latex experimental group on the 10^{th} day whereas 95% in the control group. Regardless of the type of ulcer, both groups showed significant remission of pain within the first 24 hours of treatment application. We did not find significant differences when comparing the reduction of redness and healing time. But when we compared only the two most frequent pathologies (RAS and Traumatic Ulcer) those treated with latex showed a significant statistical difference in the reduction of redness ($p < 0.001$) (Figure 1 and 2). No intolerance to latex or oleozon was observed.

Toxicity:

Our results indicate no adverse effects to latex along the study. The seric marker of tissue lesion at the level of liver and kidney showed no statistical differences among groups. Also, the untreated and treated animals showed

similar seric concentration of ALT, AST, creatinine and urea (Figures 3, 4, 5, 6). At the histology level, there was no evidence of inflammatory infiltrate nor morphological changes indicating tissue abnormalities or lesions.

Phytochemical screening:

The latex gave positive results for phenols, anthocyanidins, tannins, terpenes and lactones and lower intensity but equally positive for quinones, flavonoids, but negative for reducing sugars, amino acids, cardiotonic glycosides and essential oils (Table 2). The flavonoids and quinones were phytochemicals more affected by seasonality, but in general, there were no variations in chemical composition between plant specimens.

Phenol content and antioxidant activity:

The phenol latex stability gradually decreases with time and temperature storage. Most of the total phenols detected are tannins and a small proportion are non-tannin phenolic compounds that decreased in storage condition at 37°C (Table 3). Regarding the different seasons of the year, no significant differences were found in total phenol content and tannin content, nor in the latex obtained from the different plants of the study (data not shown).

However, the antioxidant activity was influenced by seasons, being higher in September (IC_{50} = 8.9 ± 0.6), and lowest in December (IC_{50} = 18.4 ± 0.9) (Table 4).

DISCUSSION

In our study, about 50% of the cases corresponded to the RAS, which coincides with previous works [7-8] (Table 1). When comparing the group of patients treated with latex and with the oleozon, it was found that there were no statistically significant differences in their analgesic properties, cicatrizant activity and pain in both groups. However, inflammation decreased significantly faster in the latex groups compared to the oleozon groups (Fig. 2). These results may suggest that latex has anti-inflammatory components. Therefore, we can infer that regardless of the type of ulcer treated in the study population, the effectiveness of the latex is similar to the oleozon.

Similar results have been described using other natural products for the treatment of oral ulcers such as nystatin and compared to oleozon [9], recover time took between 9 to 15 days whereas in our work it took 9 to 14 days.

About the treatment of recurrent aphthous stomatitis, an aqueous extracts of *Rhizophora mangle* showed a beneficial in the treated group, the signs and symptoms decrease faster than in the placebo group [10]. On the other hand, the use of gel based on glycerin and *Alchemilla vulgaris* to treat RAS showed 60.4% improved in 2 days whereas the placebo group only had 10.4% [11]. The use of a barberine gel and *Rosa damascena*-based mouthwash observed an improvement at days 4 and 7 [8, 12]. In our study, we found a similar improvement in this group of patients.

The anti-inflammatory and analgesic activity observed in patients treated with latex could be due to the fact that the latex has a significant amount of polyphenols. It is known that flavonoids can inhibit enzymes such as 5-lipoxygenase and cyclooxygenase decreasing the production of metabolites derived from arachidonic acid which is a precursor of inflammatory mediators. In addition, it may inhibit extracellular matrix proteases such as elastases and hyaluronidases.

On the other hand, the antioxidant ability to neutralize reactive oxygen metabolites could also influence to decrease inflammation. The presence of tannins may contribute to the healing process, because of their ability to bind to macromolecules such as proteins including collagen and proline-rich proteins present in saliva, which exerts an astringent and constricting effect. In addition, some tannins have a vasoconstricting effect that may potentiate the effect of analgesia [13-15]. The anti-inflammatory and antioxidant activity of *Jatropha* genus latex has also been demonstrated by others [4-5]. The mechanism proposed for this anti-inflammatory activity rely on its inhibitory activity on the transcription factor NF-kB [16].

Although there are no reports of adverse effects of latex in traditional medicine, it is known that roots, leaves and seeds of plants of the genus *Jatropha*, the presence of metabolites such as phorbol esters is responsible for food poisoning. In Mexico, there are *Jatropha* species that contain low concentrations of these metabolites in their seeds and are considered non-toxic [2]. However, there are no published studies in respect to latex composition of *J. gaumeri* neither to the presence of phorbol esters. Therefore, it is necessary far-reaching studies to cover this gap.

Regarding toxicity, several authors have reported that toxicity depends on the dose, the mode of administration and the animal model used in the biological test. In a murine model no toxic effects of the species *J. curcas* Linn y *J. neopasiflora* Pax was noted, neither with latex by others [2, 4-5, 17]. In our study, we did not observe the death of the animals nor lesions in the mucosa or in the organs analyzed, we observed a slight increase in ALT and creatinine, however, the difference was not significant when compared to the control group, which remained within the ALT normal values for female Wistar rats (47.66 +/- 14.27 U/L). This data confirmed previous findings as mention above.

On the other hand, the latex of the species of *J. gossypiifolia* had mutagenic effect in *Allium* cepa L in concentrations higher than 1.25 mL /L. This effects is attributed to cyclic peptides, and terpenes that capture calcium, thus inhibiting the PKC pathway by inhibiting cellular proliferation [18]. However, we did not find the presence of amino acids in the latex of *J. gaumeri*, so studies are required to rule out genotoxic activity.

The results of the phytochemical screening of latex demonstrate the presence of metabolites such as polyphenols, terpenes, quinones, lactones, tannins, anthocyanidins, flavonoids and saponins, so similar to previously reported works for other *Jatropha* species. Including the presence of terpenes such as; 1,2-epi-jatrogrosidione, a diterpenoramnofolane and 15-epi-4 E-jatrogrosidentadione, a diterpenolatirane, triterpenes such as α And β amirine and taraxasterol, flavonoids [2-3, 6, 19-20].

The antioxidant capacity of many phenolic compounds has been studied extensively, which are known to act as free radical scavengers neutralizing dangerous reactive species of oxygen and metal ions protecting cells against oxidative damage; so they can limit the risk of various diseases associated with free radical stress [21-23]. Thus, it is possible that antioxidant property of latex could be to the presence of high content of phenolic compounds.

In this study, we found that the concentration of phenols in latex is high (428±14mg gallic acid/g total solids) and its presence occurs over the year, which is important considering its application in the clinical study. We observed that the concentration of phenols is influenced by conservation conditions such as time and temperature. Because we used only the Folin-Ciocalteau method which is specific only for phenolic compounds, so we can not rule out the presence of non-phenolic compounds (tocopherol, ascorbyl palmitate and citric acid) that comply with the antioxidant activity in the plant [24].

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Table 1. Number of patients grouped according to gender and type of pathology.

Pathology	Patients treated with Latex			Patients treated with OLEOZON®		
	N	SEX		N	SEX	
		M	F		M	F
Simple aphthous ulcers	1	1	0	1	0	1
Traumatic ulcer	7	4	3	7	4	3
Recurrent aphthous stomatitis	9	4	5	10	3	7
Acute herpetic gingivostomatitis	2	1	1	2	0	2
TOTAL	19	10	9	20	7	13

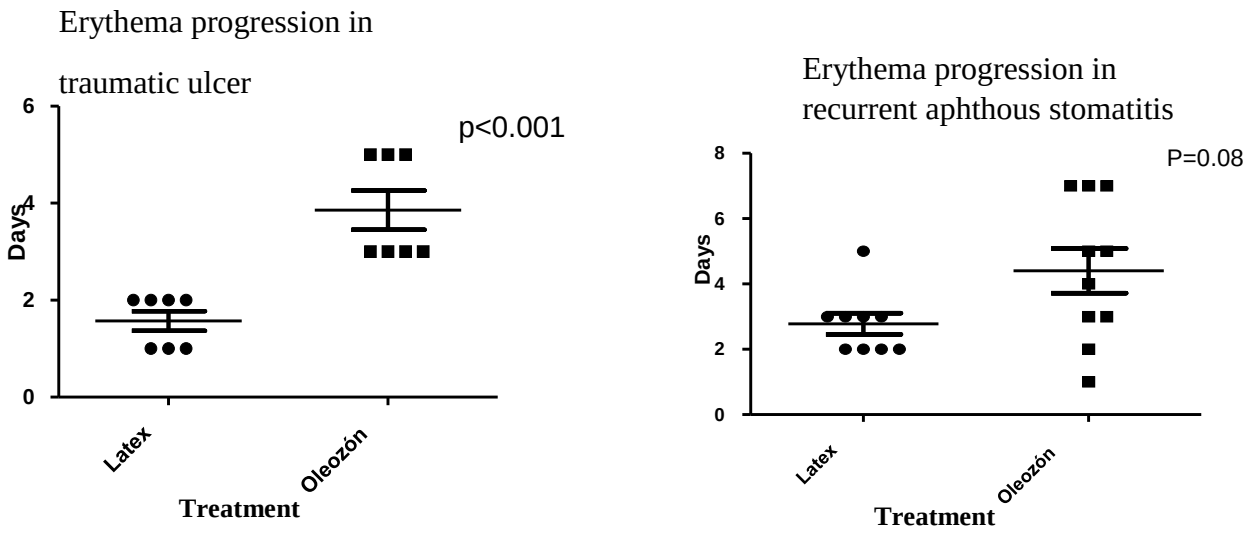

Figure 1 Erythema around the lesion of patients with traumatic ulcers and with RAS treated with latex and with oleozón. The Mann Whitney test (p <0.05)

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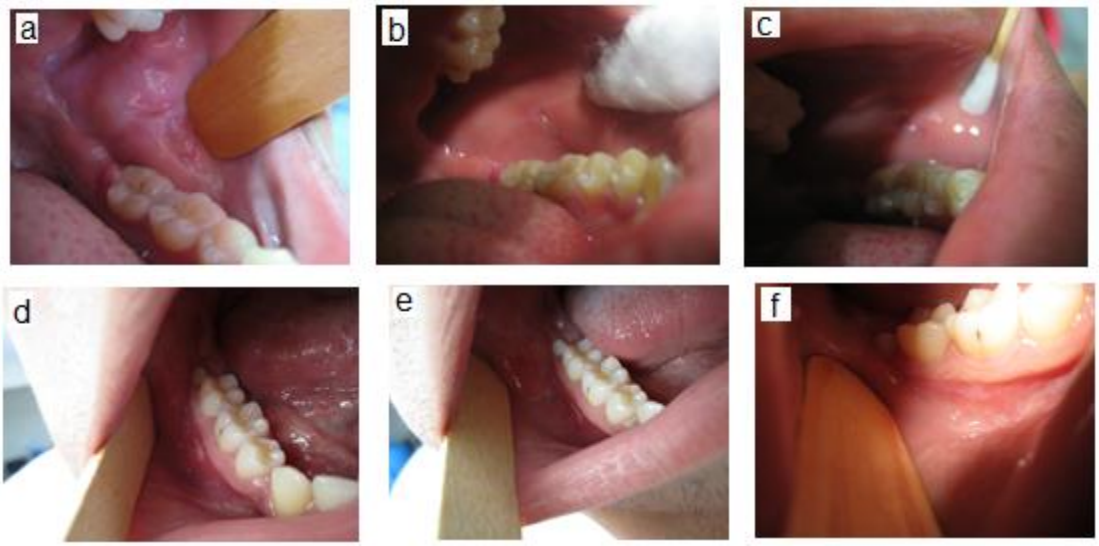

Figure 2. Male patients with a diagnosis of traumatic ulcer treated with latex (a, b, c) and with oleozón (d, e, f). (a and d) start of treatment, (b and e) after 3 days of latex application and (c and f) after 5 days.

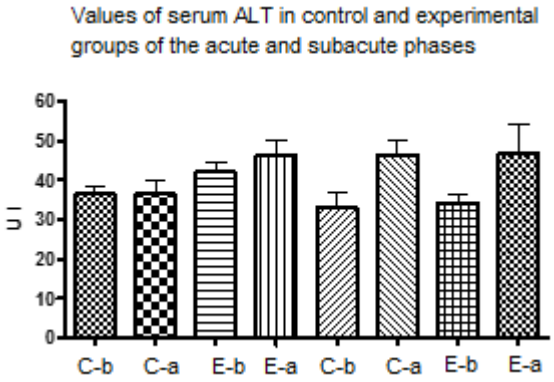

Figure 3. Serum determination of the ALT enzyme in the two study groups: Control (C) and experimental (E) at the beginning and end of treatment (b-before and a-after), during the acute and subacute phases.

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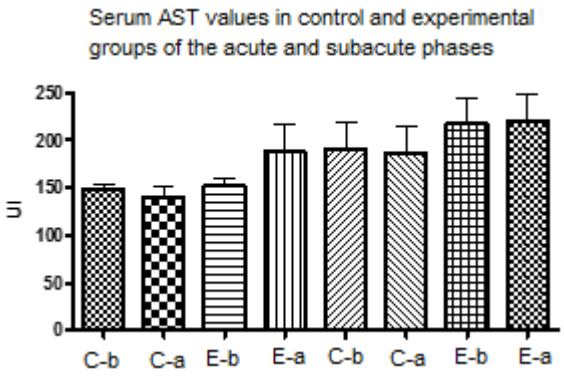

Figure 4. Serum determination of the AST enzyme in the two study groups: control (C) and experimental (E) at the beginning and end of treatment (b-before and a-after), during the acute and subacute phases.

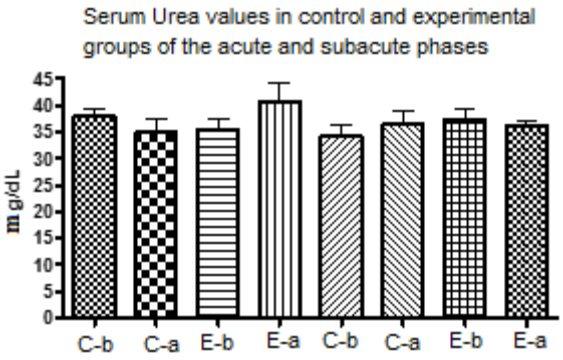

Figure 5. Serum determination of the urea in the two study groups: control (C) and experimental (E) at the beginning and end of treatment (b-before and a-after), during the acute and subacute phases.

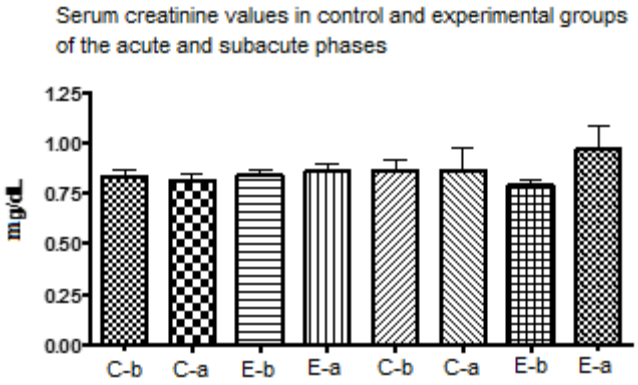

Figure 6. Serum determination of the creatinine in the two study groups: control(C) and experimental (E) at the beginning and end of treatment (b-before and a-after), during the acute and subacute phases.

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Table 2. Phytochemical screening of the latex diluted in ethanol (1:10) of 3 plants of *Jatropha gaumeri* Greenm during the four seasons of the year.

METABOLITES	Spring	Summer	Fall	Winter
	*Plants 1 2 3	Plants 1 2 3	Plants 1 2 3	Plants 1 2 3
Phenols	4+ 4+ 4+	4+ 3+ 4+	4+ 4+ 4+	4+ 4+ 4+
Flavonoids*	2+ 1+ 3+	3+ 2+ 3+	Neg	Neg
Lactones	2+ 2+ 3+	3+ 3+ 3+	2+ 2+ 2+	2+ 2+ 2+
Quinones	1+ 1+ 2+	2+ 2+ 2+	1+ 1+ 1+	1+ ½ ½
Terpenes	3+ 1+ 4+	3+ 3+ 3+	4+ 4+ 4+	3+ 3+ 3+
Reducing sugars	Neg	Neg	Neg	Neg
Anthocyanidins	3+ 3+ 3+	3+ 3+ 3+	3+ 3+ 3+	3+ 3+ 3+
Alkaloids	Neg	Neg	Neg	Neg
Essential oils	Neg	Neg	Neg	Neg
amino acids	Neg	Neg	Neg	Neg
Cardiotonic Gly	Neg	Neg	Neg	Neg
Tannins	4+4+4+	4+4+4+	3+3+3+	3+3+3+
Saponins**	Neg	Neg	Neg	Neg

* The Shinoda test for flavonoids is negative, however, with aluminum chloride solution is strongly positive. ** Saponins gives negative reaction in the ethanolic solution however it is positive when using latex in aqueous solution.

Table 3. Concentration of phenols and tannins during different storage times, expressed as µg of gallic acid and tannic acid / mg of total solids of the extract.

	Concentration of phenols			Concentration of tannins		
0 days		439±0.5µg			294±0.4µg	
	4º C	**37º C**	**-20°C**	**4°C**	**37º C**	**-20°C**
7 days	423±6	364±5		285±4	278±4	
14 days	413±4	295±8		274±4	252±	
21 days	408±5	268±7		265±3	232±	
35 days	390±3	210±4	434±4	236±5	186±6	287±3

Table 4 Latex antioxidant activity according to seasonality, expressed in the amount of ppm of the latex necessary to neutralize 50% of DPPH radical.

Month	IC_{50}
December	14.4±0.6
March	18.4±0.9
June	10.5±0.6
September	8.9±0.6

CONCLUSION

The use of latex in oral ulcers treatment is comparable to treatment with oleozon, it has an important analgesic and anti-inflammatory effect; these findings open an alternative therapeutic. The latex showed a complex mixture of metabolites among these, polyphenols with important antioxidant activity.

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