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Artículo Científico

Capacidad antioxidante y potencial toxicológico de la planta Ibervillea sonorae

Antioxidant capacity and toxicologic potential of Ibervillea sonorae plant

Marco A. Peña-Chávez[1], Jorge Antonio Zacatecas-Ibáñez[4], Jorge Sáenz-Mata[2], María José Rivas-Arreola[3], Mónica Azucena Ramírez-Cabrera[4], Rafael Minjares-Fuentes[1] y María de los Ángeles Sáenz-Esqueda[1]*

[1] Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, Av. Artículo 123 S/N, Fracc. Filadelfia, C.P. 35010, Gómez Palacio, Durango, México

[2] Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Av. Universidad S/N, Fracc. Filadelfia, C.P. 35010, Gómez Palacio, Durango, México

[3] Facultad de Medicina, Instituto Tecnológico de Estudios Superiores de Monterrey, Campus Guadalajara, Av. General Ramón Corona #2514 Col. Nuevo México. Zapopan, Jalisco, México

[4] Laboratorio de Farmacología Molecular y Modelos Biológicos, Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León, Av. Universidad s/n Cd. Universitaria, San Nicolas de los Garza, Nuevo León, México

*Correspondencia: angelesujed@hotmail.es (María de los Ángeles Sáenz Esqueda)

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Abstract

The main objective of this study was to evaluate the antioxidant capacity and toxicological potential of the infusions prepared from *Ibervillea sonorae*. Thus, infusions were prepared using *I. sonorae* roots, either skin or pulp. Total phenolic compounds and antioxidant capacity of the infusions were evaluated through the Folin-Ciocalteu method and ABTS and DPPH tests, respectively, whereas the *Artemia salina*, the MTT using VERO and MCF-7 cell lines, and the Ames assays were used to evaluate the toxicological effect. The total phenolic compounds as well as antioxidant capacity by DPPH and ABTS assays of the *I. sonorae* infusions varied from ~15 to ~24 meq GA/g, 14 to 16 %, and 1,147 to 1,191 meq Trolox/g, respectively. Further, the results showed that *I. sonorae* skin extracts were more lethal (60-80 %) than pulp (<50 %). Interestingly, *I. sonorae* pulp showed higher toxicity on VERO cell line (IC50 = 222 µg / mL) than infusions prepared from skin (IC50 = 379 µg/mL). *I. sonorae* concentrations higher than 500 µg/mL exhibited high potential mutagenic. The intake of infusions prepared from the *I. sonorae* plant could promote health injuries.

***Keywords*: *Ibervillea sonorae*, infusions, antioxidant capacity, cytotoxicity, genotoxicity**

Resumen

El objetivo de este estudio fue evaluar la capacidad antioxidante y potencial toxicológico de las infusiones preparadas a partir de *Ibervillea sonorae*. Para esto, se prepararon infusiones a partir de la raíz de *I. sonorae*, usando la cáscara o la pulpa. Los compuestos fenólicos totales y la capacidad antioxidante fueron determinados mediante el método de Folin-Ciocalteu y las pruebas ABTS y DPPH, respectivamente, mientras que, los ensayos con *Artemia salina*, MTT usando células VERO y MCF-7, y de Ames fueron usados para evaluar el potencial toxicológico. Los compuestos fenólicos totales y la capacidad antioxidante por DPPH y ABTS de las infusiones de *I. sonorae* variaron de ~15 a ~24 meq GA/g, ~14 a ~16 % y ~1147 a ~1191 meq Trolox/g, respectivamente. Las infusiones con cáscara de *I. sonorae* fueron más letales (60 – 80 %) que con pulpa (<50 %). Por el contrario, las infusiones con pulpa exhibieron mayor toxicidad sobre células VERO (IC_{50} = 222 μg/mL) que con cáscara (IC_{50} = 379 μg/mL). Concentraciones >500 μg/mL de *I. sonorae* mostraron elevado potencial mutagénico. La ingesta de infusiones preparadas a partir de la planta de *I. sonorae* podría ocasionar graves daños a la salud.

***Palabras clave*: *Ibervillea sonorae*, infusiones, capacidad antioxidant, citotoxicidad, genotoxicidad**

1. Introduction

Plants have widely been used in traditional medicine to treat different diseases (Sharif *et al.*, 2017). In fact, most of the population has used herbal products as a primary source of healthcare (Ekor, 2014). Nevertheless, around 15 % of clinical studies using herbal medicine as an alternative treatment have reported information about its safety or side effects (Boullata and Nace, 2000). The safety and efficacy of herbal medicine has become a public health concern, mainly due to the increased use of herbal medicinal products, either as primary or complementary treatment (Neergheen-Bhujun, 2013).

México, considered as one of the most biodiverse countries in the world, possesses more than 23,400 plants but only a small group of plants considered medicinal have been studied for their pharmacological, phytochemical and toxicological effects, as well as for their pharmacokinetics (Bye *et al.*, 1995; Alonso-Castro *et al.*, 2017). In the traditional Mexican medicine, the root of *Ibervillea sonorae* (S. Watson) Greene (syn. *Maximowiczia sonorae* S. Watson.; Cucurbitaceae), commonly known as "Wereque" (Déciga-Campos *et al.*, 2007), has been widely used as a topical antibiotic, cathartic, antirheumatic and antidiabetic (Rivera-Ramírez *et al.*, 2011; Sinagawa-García *et al.*, 2015; Torres-Moreno *et al.*, 2015). In fact, *I. sonorae* has become one of the most widely used plants in controlling diabetes mellitus (Estudillo and García, 1988), a metabolic disorder with the highest incidence and mortality rate in México (Guariguata *et al.*, 2014). Most of the beneficial effects associated to *I. sonorae* plant have been attributed of curcumin on the one hand, and phenolic compounds such as gallic acid on the other (Zapata-Bustos *et al.*, 2014; Torres-Moreno *et al.*, 2015). However, to the best of our knowledge, the information about the toxicological effects of *I. sonorae* is very limited. Thus, the main aim of this study was to offer a first report of the mutagenic and cytotoxic potential of infusions prepared from *I. sonorae*.

2. Materials and methods

2.1. Plant material

Plants of *I. sonorae* were collected at the communal land Esperanza (Cd. Obregón, Sonora, Mexico) (27° 36′ 09.35″ N latitude, 109° 54′ 10.7″ W longitude). The identification of *I. sonorae* plants was confirmed by DNA barcoding. A specimen of *I. sonorae* (Voucher: JAAA-00001) was deposited in the JAAA Herbarium (Facultad de Ciencias Biologicas, Universidad Juárez del Estado de Durango). Infusions of *I. sonorae* were prepared using 10 g of *I. sonorae*, either pulp (WP) or skin (WS), suspended in 100 mL of boiling water for 10 min. The supernatant was separated and lyophilized in a laboratory scale freeze dryer LABCONCO FreeZone Triad Cascade Benchtop (LABCONCO, Kansas City, Missouri, USA) operated at 0.01 mBar with condenser and shelf temperatures of −80 °C and −20 °C, respectively. Lyophilized extracts were stored in anhydrous conditions until analysis.

2.2. Phenolic compounds and antioxidant activity of *I. sonorae* infusions

Total phenolic compounds were determined according to the Folin−Ciocalteu method previously described by González-Centeno *et al.* (2014) in a MultiSkan FC spectrophotometer (Thermo Scientific, Waltham, MA USA). Antioxidant activity was tested by using 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2'-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assays following the methodology proposed by Rosales-Castro *et al.* (2012) and Medina-Torres *et al.* (2016), respectively. All determinations were performed in triplicated.

2.3. *Artemia salina* lethality test

In order to assess the possible lethal effect of *I. sonorae* infusions, the *Artemia salina* assay was performed according to the method proposed by Déciga-Campos *et al.* (2007). Briefly, dried brine shrimp eggs were hatched by incubation in saline medium for 48 h at room temperature. Approximately 100 μL of saline medium containing 10 larvae were transferred into a 96-well plate containing 100 μL of *I. sonorae* at different concentration (10, 50, 250, 500 and 1000 μg/mL). Survivors were counted after 24 h. The toxicological effect was expressed as mortality percentage and interpreted as follow: 0 – 10 % non-toxic, 11 – 50 % moderately toxic, 51 – 90 % highly toxic and 100 % extremely toxic. The average lethal concentration (LC_{50}) was calculated using PROBIT analysis and expressed in μg/mL. The LC_{50} values higher than 1000 μg/mL of extract were classified as no toxic (Déciga-Campos *et al.*, 2007). $K_2Cr_2O_7$ was used as a positive control (LC_{50}: 12.5 μg/mL). All determinations were carried out in triplicate.

2.4. Cytotoxic effect of *I. sonorae*

2.4.1. Cell culture

Breast cancer (MCF-7) and African green monkey kidney (VERO) cell lines were supplied by Monterrey Institute Technology of Higher Education (ITESM) and Autonomous University of Nuevo León (UANL), respectively. MCF-7 and VERO cell lines were cultured in Dulbeco's Modified Eagle Medium (DMEM) supplemented with 10 % heat-inactivated fetal bovine serum (FBS), 50 μg/ml streptomycin and 50 UI/mL penicillin and incubated in a ThermoScientific at 37 °C with 5 % of CO_2 and 95 % relative humidity to attain a confluent monolayer. Cells were suspended in 0.05 % trypsin-

EDTA solution for 5 min and centrifuged at 1000 rpm for 10 min at 25 °C in an Eppendorf 5804R centrifuge. Finally, the cells were suspended in DMEM and manually counted using the Neubauer's method.

2.4.2. MTT assay

Cell viability was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay was conducted using a 96-well flat bottom cell culture plate and a confluent layer of MCF-7 and VERO cells. The cytotoxic potential of the *I. sonorae*, either WP or WS, were performed at different concentration (125, 250, 500 and 1000 μg/mL). Briefly, 250 μL of supplemented DMEM and 50 μL of *I. sonorae* infusion were placed in 96-well plate and incubated for 72 h, for triplicate. After incubation, 10 μL of MTT solution and 90 μL of supplemented DMEM were added and incubated for 4 h in culture conditions. The media containing MTT was removed and 100 μL isopropanol was added to remove the color produced due to the reaction. The absorbance was measured at 550 nm in a microplate reader (Bio-Rad iMark). The results were expressed as IC_{50} values which were calculated by regression analysis. The percentage of viability was calculated using the following equation:

$$Percentage\ of\ viability\ (\%V) = \frac{mean\ optical\ density\ of\ test\ chemical}{mean\ optical\ density\ of\ control} \times 100$$

2.5. Genotoxic effect of *I. sonorae* infusions

Genotoxic potential was performed *in vitro* through one bacteria assay based on the modified Ames test modified for liquid culture and 96-well plate scale, with and without metabolic activation, referred as S-9 by using one histidine-dependent auxotrophic mutant of *S. typhimurium* TA100. The assay was purchased from EBPI bio-detection products (Mississauga, Ontario) known as Muta-Chromo Plate. On each Muta-Chromo test plate, infusions of *I. sonorae* infusions (WP and WS) were combined with a reaction mixture. The *I. sonorae* infusions were tested at 500 and 1000 μg/mL due to being the bioactive concentrations in the brine shrimp lethality test. 2-aminoanthracene (2-AA) and sodium azide (NaN_3) were included as positive control for treatments with and without S-9 mixture, respectively. Background plates containing sterile water, reaction buffer and *S. typhimurium* TA100 were used to determine spontaneous mutations occurring during incubation. All plates were incubated for 5 days at 37 ºC (GI2-2 SheL Lab Digital Incubator). The number of revertant colonies in the samples was scored and compared to the number of positive wells in background plates. All results were analyzed using Bio-Informatics Toolkit software provided by EPBI.

2.6. Statistical analysis

The phenolic compounds, antioxidant capacity and cytotoxicity of the *Ibervillea sonorae* extracts, either WP or WS, were statistically analyzed using analysis of variance (ANOVA) with a *p*-value of 0.05. Tukey test was used as post-hoc analysis using a significant value of 0.05. All calculations were performed using Minitab 18 statistical software.

3. Results and discussions

3.1. Total phenolic compounds and antioxidant capacity

The total phenolic compounds of the *I. sonorae* infusions are shown in Table 1. As can be seen, the total phenolic compounds accounted for about 24 meq GA/g in WS infusions whereas for WP infusions it was ~15 meq GA/g. On the other hand, the antioxidant capacity and percentage inhibition of *I. sonorae* infusions were tested by ABTS and DPPH assays, respectively. As can be seen in Table 1, the antioxidant capacity of both infusions, WP and WS, measured by ABTS assay was 1,146.9 and 1,191.4 meq Trolox/100 g, respectively, whereas the DPPH radical scavenging capacity was 13.79 % for the WP extract and 16.39 % for the WS extract.

Table 1. Total phenol content (TPC) and antioxidant activity measured by the DPPH and ABTS assays of *I. sonorae.*
Tabla 1. Contenido de compuestos fenólicos totales y capacidad antioxidante evaluada por los métodos DPPH y ABTS de *I. sonorae*

Sample	TPC	DPPH	ABTS
	(meq GA/g)	(% inhibition)	(meq Trolox/100 g)
WS	24.14 ± 0.50	16.39 ± 1.36	1,191.40 ± 46.8
WP	15.24 ± 0.05	13.79 ± 1.87	1,146.90 ± 89.1

The results obtained in the present study, either WS or WP, showed moderately low and low TPC, respectively, according to the categories proposed by Chew *et al.* (2011). Interestingly, similar TPC results have been reported by Zapata-Bustos *et al.* (2014) and Núñez-Gastélum *et al.* (2018) for extracts of *I. sonorae*. In particular, Zapata-Bustos *et al.* (2014) reported that *I. sonorae* extracts contained about 1.4 g GAE/kg of phenolic compounds, while Núñez-Gastélum *et al.* (2018) found about 10 mg GAE/g. Interestingly, gallic acid has been identified as the most abundant phenolic compound in *I. sonorae* extracts which has also been considered as the main responsible for the anti-diabetic properties of *I. sonorae* (Zapata-Bustos *et al.*, 2014).

Although the information about the antioxidant capacity of *I. sonorae* extracts is scarce, several authors have observed that *I. sonorae* extracts exhibit a poor antioxidant capacity, in particular free radical scavenging capacity, which has been related to the low content of phenolic compounds (Ramírez-Ortíz *et al.*, 2017; Núñez-Gastélum *et al.*, 2018).

3.2. *Artemia salina* lethality test

A wide variety of biologically active chemical compounds are toxic to brine shrimp, the death of this organism when exposed to varying concentrations of these compounds is the basis for this toxicity test. The results of the lethality test are shown in Figure 1. As can be seen, the lethality of *I. sonorae* infusions increased as the concentration increased. Interestingly, the WS extract showed higher lethality (80 %) than WP extract (47 %) at the maximum concentration (1000 μg/mL). According to the percentage lethality, those infusions prepared from *I. sonorae* skin are considered as highly toxic, while infusions from *I. sonorae* pulp are moderately toxic.

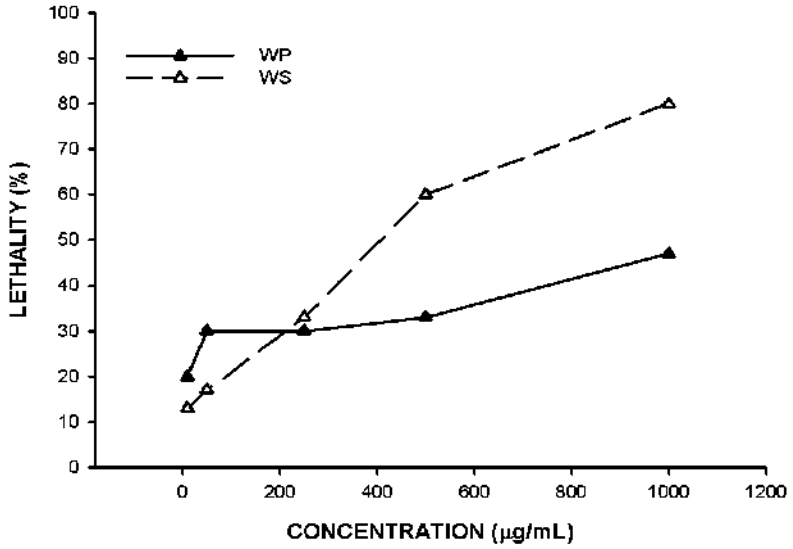

Figure 1. Results of the lethality test of *I. sonorae* infusions on *Artemia salina*.
Figura 1. Resultados del ensayo de letalidad de las infusiones de *I. sonorae* sobre *Artemia salina*

The *A. salina* lethality test is often used as a first toxicological indicator for plant extracts. Also, the toxicity to *A. salina* has been correlated with possible antitumor activity (Naidu *et al*., 2014; Leite *et al*., 2015). Those extracts with LC_{50} values below 1000 µg/mL have been considered as a harmful and noxious to the organism (Meyer *et al*., 1982; Déciga-Campos *et al*., 2007). Based on this premise, WP infusions could be considered as safe, since LC_{50} was higher than 1000 µg/mL whereas infusions of *I. sonorae* skin could not (LC_{50} of 359 µg/mL). According to Meyer *et al.* (1982) only those extracts obtained from WP could be safely used for human consumption. The results obtained in this study were similar to those reported by Aarland *et al.* (2015) who reported a LC_{50} >1000 µg/mL for the hexane extract *I. sonorae*. To the best of our knowledge, this is the first report using in *A. salina* larvae model to evaluate the toxicology of aqueous extracts prepared from *I. sonorae*.

3.3. Cytotoxicity of *I. sonorae* infusions by MTT assay

The results of the cytotoxicity of *I. sonorae* infusions using MCF-7 and VERO cell lines are shown in Figure 2 and Table 2. As can be seen, *I. sonorae* infusions, either WP or WS, promoted a significant decrease in the viability of MCF-7 as well as VERO cells (p<0.05). The viability of MCF-7 was reduced from ~91 % up to ~46 % when the WP concentration increased from 125 to 1000 µg/mL whereas a 90 % to 61 % decrease was estimated for WS infusion. Interestingly, no significant differences in MCF-7 viability were observed when WP and WS extracts were tested at concentrations of 125 and 250 µg/mL (Figure 2a).

Figure 2a

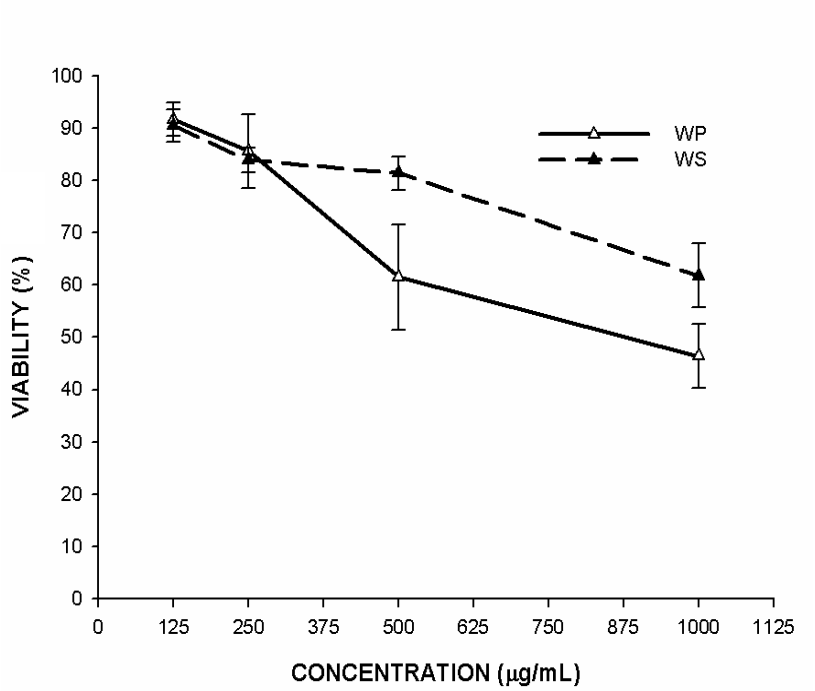

Figure 2b

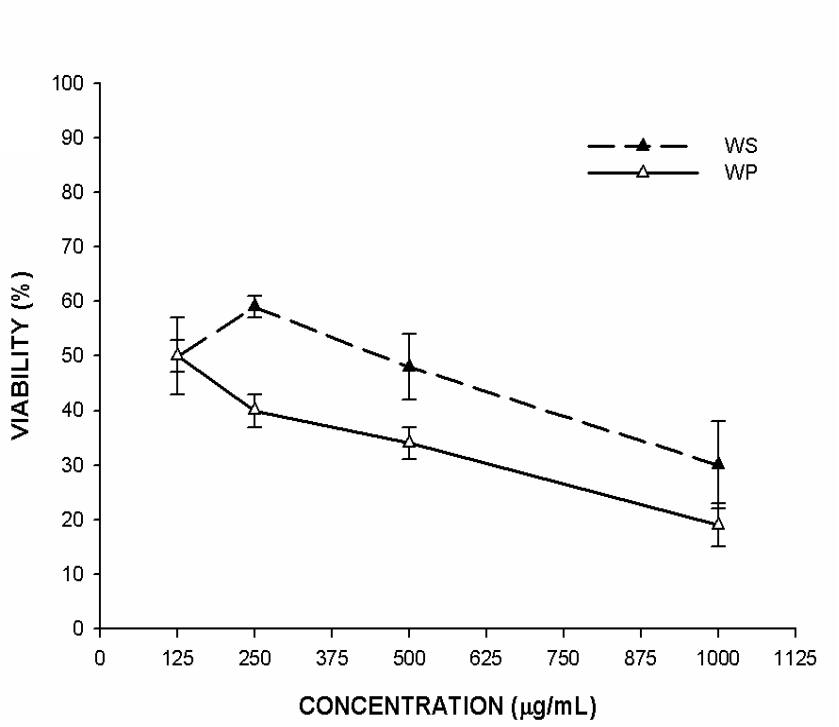

Figure 2. Cell viability of (a) MCF-7 and (b) VERO lines in the presence of *I. sonorae* infusions.
Figura 2. Viabilidad celular de las líneas (a) MCF-7 y (b) VERO in presencia de las infusiones de *I. sonorae*.

Table 2. Cytotoxic activity of *I. sonorae* infusions on VERO and MCF-7 cell lines.
Table 2. Actividad citotóxica de las infusiones de *I. sonorae* sobre las líneas celulares VERO y MCF-7.

Sample	IC50 (μg/mL)					
	VERO			MCF-7		
WP	222	±	32	902.8	±	51.97
WS	379	±	33	ND*		

*ND: not determined

Regarding to VERO cell line, an initial viability of 50 % was observed which decreased to 30 % and to 20 % using WS and WP, respectively (p<0.05) (see Figure 2b). Interestingly, VERO cells (IC_{50} <400 μg/mL) were more sensitive than MCF-7 cells (IC_{50} <1000 μg/mL) to the *I. sonorae* infusions, either WP or WS. It is important to note that the MCF-7 and VERO viability exhibited a concentration-dependent behavior since the viability decreased as the concentration increased.

Cytotoxicity is defined as an alteration of basic cellular functions that can result in cell damage (Arencibia *et al*., 2019). The MTT assay is a colorimetric methodology considered as a useful tool for the determination of the cytotoxic potential of various chemicals, drugs, environmental pollutants and plant extracts (Sharif *et al*., 2017). The antiproliferative activity of *I. sonorae* has been shown in several studies (Vega-Avila *et al*., 2009; Torres-Moreno *et al*., 2015; Quintanilla-Licea *et al*., 2016).

Interestingly, cucurbitacin, the main compound present in the Cucurbitaceae family, has been considered as the main responsible, not only of the bitterness and toxicity, but also, for the antiproliferative effect associated with these plants (Achenbach *et al*., 1993; Tannin-Spitz *et al*., 2007; Patel and Krishnamurthy, 2013). In the last decade, *I. sonorae* has gained great interest in the cancer treatment due to the presence of cucurbitacin which has demonstrated biological activity against glioblastoma (Yuan *et al*., 2014), human multiple myeloma (Yang *et al*., 2017) and human breast cancer (Duangmano *et al*., 2012).

3.4. Genotoxicity of *I. sonorae* infusions by Ames test

The genotoxic effect of the *I. sonorae*, either WP or WS, was assessed by the modified Ames method using a 96-wells microplate. The results of the modified Ames method are shown in Figures 3a and 3b. As can be seen, WP and WS extracts, at concentrations of 500 and 1000 μg/mL, with the S-9 mixture showed a lower number of positive wells compared to the background and 2-AA in the first three days of incubation. After this time, the number of positive wells increased as incubation time increased regardless of the extract (Figure 3a).

On the contrary, the number of positive wells (CFU *hys*+) observed using WP and WS extracts was higher than that of the background when these were tested without S-9 mixture. It is important to note that the number of positive wells of the WP and WS extracts did not exceed the positive wells of the positive control (NaN_3) during the incubation time (see Figure 3b).

Figure 3a

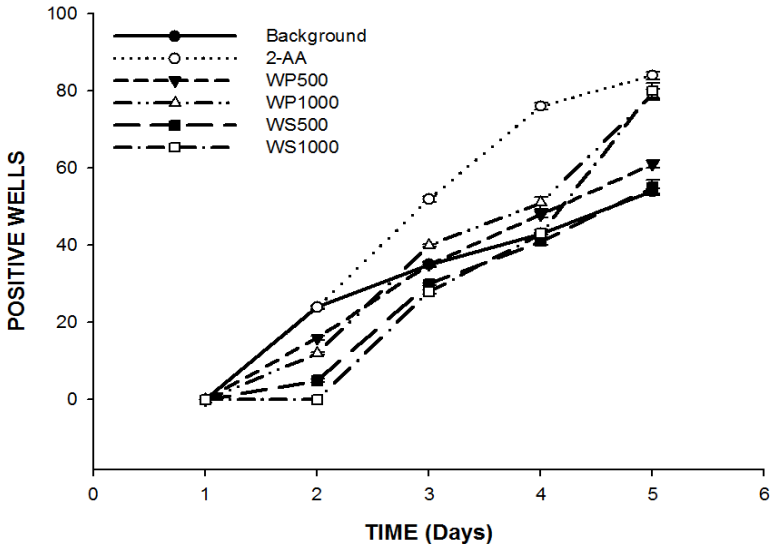

Figure 3b

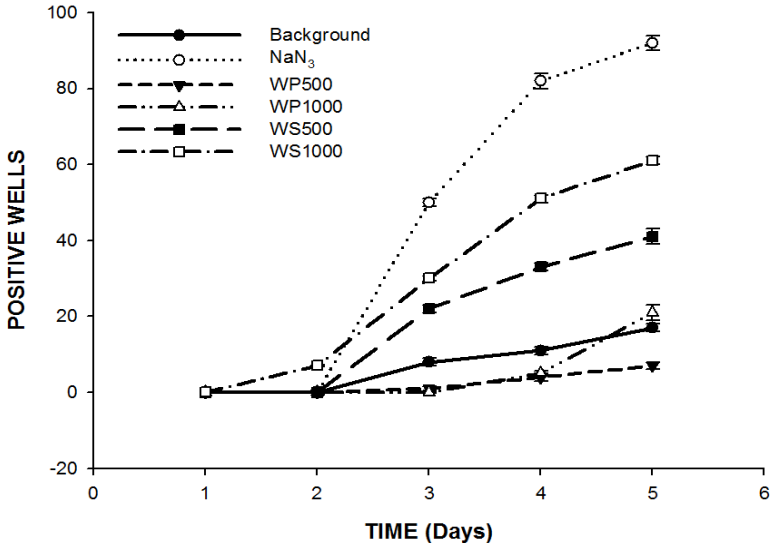

Figure 3. Genotoxic effect induced by infusion of *I. sonorae* on *S. thyphimurium* TA100 (a) with and (b) without S-9 mixture

Figura 3. Efecto genotóxico inducido por la infusión de *I. sonorae* sobre *S. thyphimurium* TA100 (a) con y (b) sin mezcla S-9.

On the other hand, it was observed that both extracts WP and WS, promoted genotoxic effects in the *S. thyphimurium* TA100 (*His-)* strain, without S-9 mixture. The mutation rate was evaluated by comparison with "Background" which shows the level of spontaneous mutation of *S. thyphimurium*. Interestingly, the presence of the S-9 mixture promoted a lower number of positive wells compared with the background and 2-AA at the first three days of incubation. The S-9 mixture is commonly used to simulate the metabolic activity of liver, which is the main organ involved in the metabolism of xenobiotics [30]. It is important to highlight that the genotoxic effect of aqueous extracts of *I. sonorae* has not been reported previously. The present *in vitro* study showed that the aqueous extract of *I. sonorae* could cause gene mutations by substituting base pairs. In theory, a single hit on DNA may be sufficient to start genomic stability (Zhou *et al*., 2013).

4. Conclusions

The toxicological potential of *I. sonorae*, a plant used in traditional medicine in the north of Mexico, was studied. Thus, those infusions prepared from the pulp (WP) of *I. sonorae* might be considered as non-lethal whereas the intake of infusions prepared from skin (WS) could promote health damages. Interestingly, extracts from both plant tissues demonstrated to reduce not only the cancer cells, such as MCF-7, but also healthy cells, such as VERO. This could be related to the genotoxic effect observed by the Ames test. Interestingly, *I. sonorae* showed to be a plant with a moderate content of phenolic compounds that possess low radical scavenging capacity. Thus, the intake of infusions prepared from the *I. sonorae* plant could promote health injuries. Further studies are required to understand the different biological mechanisms involved in the toxicological effects observed in the present study.

Conflict of interest

The authors declare that there is no conflict of interest of any kind in the preparation and publication of this article.

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