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Vol. 14(28), pp. 2251-2257, 15 July, 2015
DOI: 10.5897/AJB2014.14365
Article Number: AA32F5054152
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

*Full Length Research Paper*

# Antifungal, acute toxicity and mutagenicity activity of extracts from *Datura stramonium*, *Jacquinia macrocarpa* and *Krameria erecta* on *Fusarium verticillioides*

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Received 11 December, 2014; Accepted 13 July, 2015

**The effect of *Baccharis glutinosa*, *Jacquinia macrocarpa*, and *Krameria erecta* extracts was investigated on the growth and the spore germination of *Fusarium verticillioides* (ATCC 52539). Brine shrimp (*Artemia salina*) was used to evaluate the potential acute toxicity of the fractions obtained from plant extracts. The butanol fraction of *J. macrocarpa* totally inhibited the radial growth for 144 h and up to 95% after 168 h. The ethyl acetate fraction of *B. glutinosa* caused 100% of radial growth inhibition for 96 h. The ethyl acetate fractions of *B. glutinosa* and *K. erecta* caused the higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively. All plant fractions tested at a concentration of 5.0 mg mL$^{-1}$ caused 100% brine shrimp lethality after 24 h. The Ames test did not reveal the presence of an evident mutagenic activity.**

**Key words:** Antifungal activity, plant extracts, brine shrimp bioassay, mutagenicity assay, *Fusarium verticillioides.*

## INTRODUCTION

The plant species in Mexico are more than 26,000 from which 4,000 are estimated to have medicinal use (Mittermeier and Goettsch, 1992). In addition, some of them have exhibited other properties such as antifungal activity and might be considered natural bioactive substances for the control of post-harvest fungal infections. Plant extracts are generally assumed to be more acceptable and less hazardous than synthetic compounds and they might represent an alternative anti-fungal approach (Jobling, 2000).

*Baccharis glutinosa* Pers (syn.: *Baccharis salicifolia* (Ruiz & Pav.) Pers) and *Jacquinia macrocarpa* (syn.: *Jacquinia aurantica*), are traditional medicinal plants that belong to the *Asteraceae* and *Theophrastaceae* families, respectively (Barrows, 1967; Moreno-Salazar et al., 2008). Ethnic groups from Northwest Mexico have been

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using *B. glutinosa* for gastrointestinal disorders whereas they have used *J. macrocarpa* to prepare a mustard-colored dye from the fruits and a tea out of the flowers that strengthens the heart (Yetman and Van Devender, 2002). Also, cytotoxic and anti-inflamatory properties have been reported for *B. glutinosa* extracts (Fukuda et al., 2006; Abad et al., 2006; Abad and Bermejo, 2007). These plants are widely distributed from Southwest U.S.A. to central Mexico (Barrows, 1967; Moreno-Salazar et al., 2008). *Jacquinia* is native from West Indies where it is known as *J. aurantica*. DiSalvo (1974) reported that *B. glutinosa* aqueous extract of dried powdered leaves to inhibit dermatophytes *in vitro*. He mentioned that *B. glutinosa* is recommended in the southwestern desert of the United Sates for the therapy of athlete's foot caused by *Tinea pedis*. In addition, *Datura stramonium* has been reported to have antifungal activity.

Fractions from methanolic extracts of these plants have shown antifungal properties against some phytopathogenic and toxigenic molds such as *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* (Buitimea-Cantúa et al., 2013). Those authors reported radial growth inhibition, hyphal diameter and length, and mycotoxin production. Based on the above, the aim of this study was to evaluate the antifungal properties of fractions of extracts obtained from *Krameria erecta*, *Baccharis glutinosa,* and *J. macrocarpa* on *F. verticillioides,* and to evaluate their mutagenic potential and acute toxicity.

## MATERIALS AND METHODS

### Plant materials

*J. macrocarpa* Cav. spp. pungens and *Krameria erecta* Willd ex Schult were collected in the area of Los Arrieros, Sonora (Latitude N 28° 20.538' W 111° 08.911' altitude 280 feet and latitude N 28° 19.526' W 111° 08.828' altitude 227 feet) during august 2010. Aerial parts of *B. glutinosa* Pers. were collected during February 2011 in the riverside of Tecoripa River near the rural community of Tecoripa, Sonora. A voucher sample of each plant was deposited at the Herbarium of the Scientific Research and Technology Department of the University of Sonora (DICTUS) in Hermosillo, Sonora (Mexico) to confirm its identification. The plant specimens were sealed in plastic bags, and transported to the laboratory.

### Preparation of antifungal extracts

Plants were sun dried (35-40°C) for 2 weeks and milled (Pulvex 200, U.S.A.) to a particle size of 0.5-1.0 mm. Sixty grams of powdered aerial parts of each plant were extracted with 940 ml of 70% methanol by agitation for 1 h with a wrist action Burrel shaker (Burrel Corporation, Pittsburg, PA), and stored at 25°C for 3 days at darkness. The extracts were filtered first through Whatman filter paper No. 1 and then through micropore glass filter. The methanolic extracts (crude extracts) were evaporated to dryness at 40°C with vacuum in a Yamato rotary evaporator RE 300). Crude extracts were evaluated for antifungal activity. The extracts that showed the highest inhibition activity was evaporated to dryness and subjected to fractionation. Twenty grams of dried extracts were suspended in 1 L of water and sequentially partitioned with hexane, ethyl acetate, and n-butanol (Koketsu et al., 1996) and all were tested for antifungal activity. Plates with potato dextrose agar medium, PDA, (DIFCO, USA) were prepared using 5 mg $mL^{-1}$ of each fraction [Ethyl acetate fraction of *B. glutinosa* and *K. erecta* (FAe *Bg*) and (FAe *Ke*), respectively and n-butanol fraction of *J. macrocarpa* (FB *Jm*)]. Petri dishes containing PDA prepared with each of the different solvents used for fractionation were used as controls.

### Antifungal activity assay

A strain of *F. verticillioides* (ATCC 52539) was selected for its high fumonisin production. Fungal strain was activated in PDA and incubated at 25 ± 2°C for 10 days. Spores were harvested by pouring a sterile solution of 0.1% (v/v) Tween 80 into the flask and stirring the suspension with a sterile magnetic bar for 5 min. Spore concentration of the suspension was determined using a Neubauer chamber and adjusted to a final concentration of 1 x $10^5$ spores/mL. Petri dishes containing PDA medium prepared with 5 mg $mL^{-1}$ of plant extract fractions were centrally point-inoculated with 1 x $10^5$ spores/mL and incubated in the darkness at 25°C for 7 days. Two types of controls were prepared, one contained PDA medium plus aliquots of each solvent and the other one containing only PDA media. Colony diameters were measured every 24 h using a caliper and compared to those grown in the control media until the fungal growth in the control reached the plate border. All the measurements were carried out by triplicate. The radial growth inhibition percentage was calculated using the following equation: Radial Inhibition (%) = [(Rc-Ri)/Rc] x 100. Where *Rc* is the mean value of the colony radius in the control media and *Ri* is the colony radius value of the colonies grown in PDA amended with the partitioned extracts.

### Germination of spores

Petri dishes containing PDA amended with 5 mg $mL^{-1}$ of extract fractions (FAe *Bg*, FAe *Ke,* and FB *Jm*) were inoculated by spreading 3 µl of a spore suspension containing 1x$10^4$ spores/mL and incubated at 25°C using a 12 h light/dark cycle (Precision Low temperature Illuminated Incubator 818, U.S.A.). Two types of controls were prepared, one contained PDA medium plus aliquots of each solvent and the other one contained only PDA media. Samples were taken every 4 h of incubation time and 200 spores were counted at random (germinated and non-germinated) using light microscope. Count of spores was performed until the control reached 100% of spores germinated. The number of germinated spores per plate was determined. A spore was considered germinated when the length of its germinal tube reached one-half of the spore diameter. Each germination experiment was made by triplicate. The inhibition of spore germination was determined using Equation 1, in which $S_i$ represents the percentage of germinated spores in the plates treated with the extract fraction, and $S_c$ was the percentage of germinated spores in the control containing each of the solvents (Paul et al., 1993).

$$\text{Inhibition (\%)} = \frac{\%S_c - \%S_t}{\%S_c} \times 100$$

### Brine shrimp bioassay

In order to evaluate the potential acute toxicity of the fractions obtained from plant extracts, brine shrimp larvae assay was used (Jiménez et al., 1997). Dried *Artemia salina* eggs (0.1 g) were

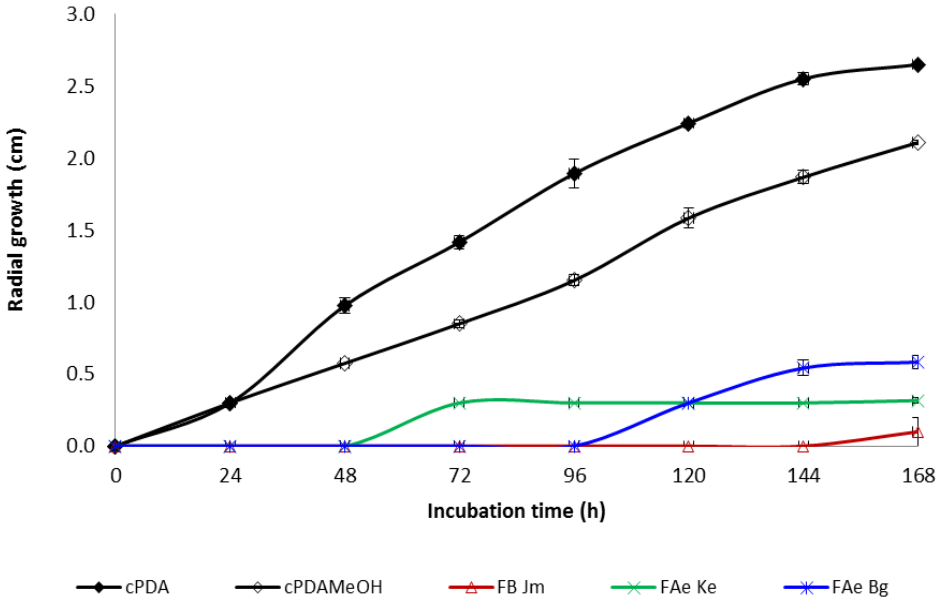


**Figure 1.** Radial growth of *Fusarium verticillioides* in PDA amended with 5 mg $mL^{-1}$ of extracts fractions from *Jacquinia macrocarpa* (FB *Jm*), *Krameria erecta* (FAe *Ke*), and *Baccharis glutinosa* (FAe *Bg*).

deposited in 1 L of sterile marine water with aeration and light during 24 h to hatch. Brine shrimp larvae were exposed to 5.0, 0.5, 0.005, and 0.0005 mg $mL^{-1}$ of the extract fractions for 24, 48, and 72 h. The number of dead larvae was recorded every 24 h to estimate the percentage of mortality. The assay was carried out by quintuplicate.

### Mutagenicity assay

The mutagenic potential of the fractions obtained from plant extracts was determined according to the procedure described by Maron and Ames (1983) using *Salmonella* tester strains TA98 and TA100, with and without bioactivation (S9). Each was placed on nutrient broth (Difco Nutrient Broth) for reproduction during 12 h at 37°C in a circulation water bath at dark. One-hundred microliters of partitioned plant extracts, FAe *Bg*, FAe *Ke* and FB *Jm*, were deposited in test tubes (5, 0.5, 0.005, 0.0005, 0.00005, and 0.00005 mg $mL^{-1}$). Then, each tube was combined with 2.0 mL of bacteriologic agar (Sigma Chemical Co.) supplemented with histidin and biotin, 100 µL of bacterial culture, and 500 µL of S9 mix. This mixture was poured onto minimal glucose agar Petri dishes and incubated for 48 h at 37°C. For mutagenicity, positive control sodium azide was used (without S9) and aflatoxin B1 (with S9). The number of revertants was counted using a colony counter and compared against the controls. The assay was carried out by triplicate.

A completely randomized design of the radial growth and spore germination was carried out. The JMP 2004 software computed the analysis of variance and the means were compared with the Tukey multiple range tests ($P<0.05$) (JMP vs. 5.0, SAS Institute Inc., USA).

## RESULTS AND DISCUSSION

The extract fractions exhibited a moderate to high antifungal activity against *F. verticillioides*. No fungicide effect was observed, only an inhibitory activity was detected in the fungus growth when compared to controls. Controls with solvents and pure PDA control showed the higher radial growth. When inoculated on PDA containing the extracts fractions, the radial growth of the fungi was delayed during the incubation time (Figure 1). The BF *Jm* totally inhibited the radial growth for 144 h and up to 95% after 168 h (Table 1). On the other hand, the FAe *Bg* caused 100% of radial growth inhibition for 96 h, after that the inhibitory effect was reduced to 72%. Treatment with FAe *Ke* also inhibited the radial growth in 100% for 48 h and 65% after 72 h of incubation. These results are in agreements with a previous work (Rosas-Burgos et al., 2009), which reported an inhibition of 67% of *F. verticillioides* radial growth. This result is of relevance for the present study because it confirms that *Baccharis glutinosa* has a fungistatic activity; plant specimens used in the present study were collected in a different year to those used by Rosas-Burgos et al. (2009) and similar results were reached. This might suggest that bioactive compounds are present in *B. glutinosa* independently of the year in which the plant is collected. Nevertheless, a recent study showed that concentration of total phenolic compounds and flavonoids on *B. dentata* showed significant seasonal variation (Sartor et al., 2013). Regarding the chemical constituents found in the genus, coumarins, flavonoids and terpenoids are the most frequently reported (Cifuente et al., 2002; Simoes-Pires et al., 2005; Wachter et al., 1999). On other hand, Kurdelas et al. (2010) isolated three coumarins from *Baccharis darwinii*. The effect of extracts may be

**Table 1**. Radial growth inhibition (%) of *Fusarium verticillioides* in PDA amended with 5 mg $mL^{-1}$ of extracts fractions from *Jacquinia macrocarpa* (FB *Jm*), *Krameria erecta* (FAe *Ke*), and *Baccharis glutinosa* (FAe *Bg*).

| Incubation time (h) | FB Jm | FAe Ke | Fae Bg |
|---|---|---|---|
| 48 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| 72 | 100 ± 0.0 | 65 ± 0.0 | 100 ± 0.0 |
| 96 | 100 ± 0.0 | 74 ± 0.0 | 100 ± 0.0 |
| 120 | 100 ± 0.0 | 81 ± 0.0 | 81 ± 0.0 |
| 144 | 100 ± 0.0 | 84 ± 0.0 | 71 ± 5.1 |
| 168 | 95 ± 8.2 | 85 ± 1.4 | 72 ± 3.6 |

FB Jm = n-Butanol fraction of *J. macrocarpa*; FAe Ke = ethyl acetate fraction of *K. erecta*; FAe Bg = ethyl acetate fraction of *B. glutinosa*.

**Table 2.** *Fusarium verticillioides* spore germination rate on PDA with and without the evaluated extracts fraction (5 mg $mL^{-1}$).

| Treatment | Spore germination rate (% EG $h^{-1}$) | Radial extension rate (cm $h^{-1}$) |
|---|---|---|
| FAe *Bg* | 0.000 | 0.0113 |
| FAe *Ke* | 0.354 | 0.0125 |
| FB *Jm* | 1.438 | 0.0003 |
| cPDAMeOH | 11.406 | 0.0129 |
| cPDA | 11.750 | 0.0185 |

FAe *Bg* = *Baccharis glutinosa* ethyl acetate fraction; FAe *Ke* = *Krameria erecta* ethyl acetate fraction; FB *Jm* = *Jacquinia macrocarpa* n-butanol fraction; cPDAMeOH = PDA plus methanol control; cPDA = Control of PDA; EG $h^{-1}$ = Germinated spores.

due to their chemical composition and probably to the membrane composition of the fungi. *B. dracunculifolia* DC, a native plant of South America, is one of the most studied of this genus and baccharin (3-prenyl-4-(dihydro-cinnamoyloxy) cinnamic acid) is the chemical compound isolated from its aerial parts. Tabti et al. (2014) mentioned that terpene hydrocarbons and phenolic compounds affects the fungi development. Information on the mechanism(s) of action by these type of compounds in *Baccharis* is not available. Velluti et al. (2005) mentioned that other authors have attributed it not only to the presence of terpenes, phenolic compounds, and other components, but also to the chemical structure, such as the presence of hydroxyl groups in their phenolic compounds.

Also, the values of radial extension rate, determined from the slope of the radial growth versus time during the linear growth phase, were reduced (Table 2). The lower value corresponds to the treatment with FB *Jm* which caused 100% of inhibition. Result indicate that spores inoculated on control treatments began to germinate after 4 h and reached the 100% of germination at 14 h. Spores inoculated on media containing FAe *Bg* and FAe *Ke* caused 100 and 95% germination, respectively at 14 h. On the other hand, spores cultivated in the presence of FB *Jm* were poorly affected.

The fractions FAe *Bg* and FAe *Ke* caused higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively (Figure 2). FB *Jm* exerted the lowest effect on spore germination inhibiting only 19.0% and allowed the higher germination velocity compared to the other plant fractions (Figure 2). The first morphological change in spore germination is called swelling in which the diameter of the spore increases. It involves water uptake and a decrease in the micro-viscosity of the cytoplasm. Also, molecules are directed to the cell cortex to enable addition of new plasma membrane and cell wall (Bartnicki-Garcia and Lippman, 1977; Momany, 2002). At later stages of development, the growth speed of the germ tube increases and the functional organization of the hyphal tip area acquires its full potential. The structure of the fungal cell wall is unique to the fungi and it is composed of chitin, glucans, mannans and glycoproteins (Bowman and Free, 2006). Damage on the fungal cell wall produces morphological alterations, inhibition of fungal growth or apoptotic cell death (Escalante et al., 2008; Alonso et al., 2010; Khan and Nasreen, 2010), which are presumably the result of alterations caused to the components of the cell wall, β-glucan and chitin. Recent research has shown that extracts from *B. glutinosa* and *J. macrocarpa* have chitinase activity against polymeric extracts from *A. flavus* and *F. verticillioides* (Buitimea-Cantúa et al., 2013), which helps to explain our findings.

Table 3 shows the data obtained from the negative control of *A. salina* suspended in marine water. *A. salina* exposed to sodium azide (5.0 and 0.5 mg $mL^{-1}$, positive control) reached 100% mortality after 24 h. All plant fractions tested at a concentration of 5.0 mg $mL^{-1}$ caused 100% brine shrimp lethality after 24 h. The extract fraction from *J. macrocarpa* (BF *Jm*) showed similar toxicity effects than sodium azide after 24 h when *A. salina* was either exposed to 5.0 or 0.5 mg $mL^{-1}$. However, when BF *Jm* concentration decreased, the brine shrimp mortality also diminished. This plant extract fraction caused the lowest mortality from the three plant extracts evaluated. The other two extracts showed similar mortality at all of the concentrations evaluated. Our results suggest the presence of toxic compounds in each of the plant extracts, which are able to physiologically affect *A. salina*.

The positive mutagenicity controls, sodium azide and aflatoxin B1, tested in *Salmonella* Thyphimurim strains TA 98 and TA 100, are presented in Table 4. We observed that they were sensitive in this experiment and reproducible results could be achieved. Plant extracts did not induced any mutagenic effect on both *Salmonella* tester strains (Table 5). Mutagenicity exerted by extracts was considered negative since the number of revertant

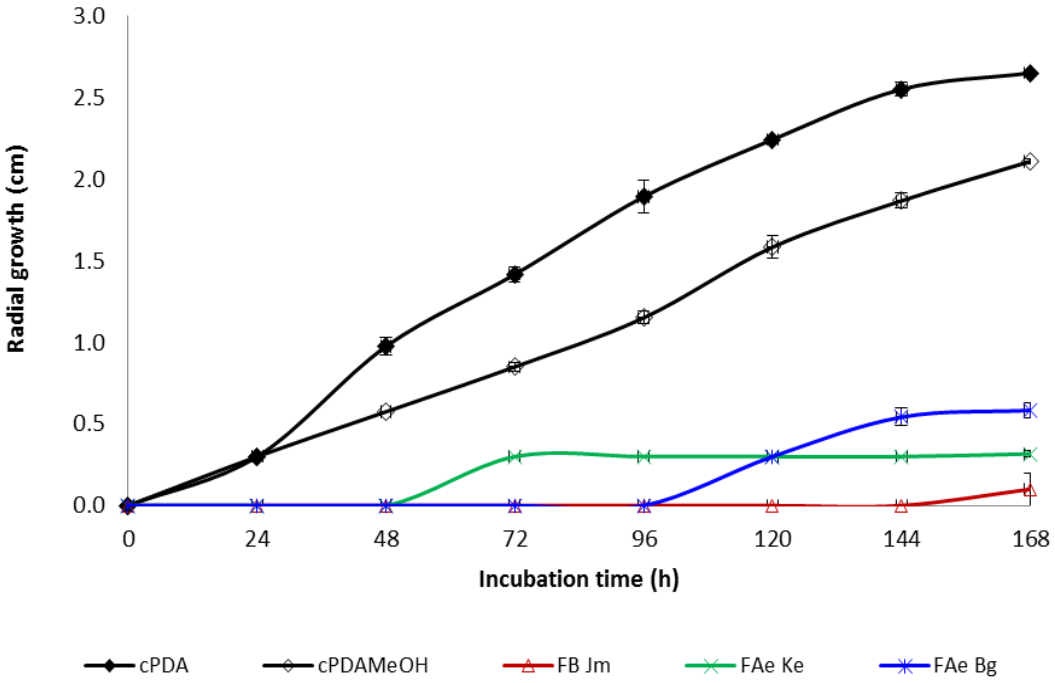


**Figure 2.** Radial growth of *Fusarium verticillioides* in PDA amended with 5 mg mL$^{-1}$ of extracts fractions from *Jacquinia macrocarpa* (FB *Jm*), *Krameria erecta* (FAe *Ke*), and *Baccharis glutinosa* (FAe *Bg*).

**Table 3.** Mortality of *Artemia salina* when exposed to different treatments

| Treatment (mg mL$^{-1}$) | Exposition time (h) | | |
|---|---|---|---|
| | 24 | 48 | 72 |
| Marine water with sodium azide | 1.67 ± 1.7$^{a}$ | 4.71 ± 4.7$^{a}$ | 84 ± 5$^{a}$ |
| 0.005 | 4 ± 4$^{a}$ | 36 ± 12$^{b}$ | 84 ± 2$^{a}$ |
| 0.05 | 24 ± 6$^{b}$ | 58 ± 13$^{c}$ | 93 ± 5$^{b}$ |
| 0.5 | 100 ± 0$^{c}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |
| 5.0 | 100 ± 0$^{c}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |
| **FB *Jmb*** | | | |
| 0.005 | 26 ± 4$^{b}$ | 55.6 ± 12$^{bc}$ | 92.71 ± 3$^{b}$ |
| 0.05 | 55 ± 10$^{c}$ | 70 ± 10$^{d}$ | 97 ± 2b$^{c}$ |
| 0.5 | 99 ± 0.8$^{e}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |
| 5.0 | 100 ± 0$^{e}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |
| **FAe *Ke*** | | | |
| 0.005 | 54.7 ± 10$^{c}$ | 86 ± 5$^{e}$ | 97 ± 2$^{b}$ |
| 0.05 | 88 ± 8.5$^{d}$ | 96 ± 4$^{f}$ | 100 ± 0$^{c}$ |
| 0.5 | 81.1 ± 6.8$^{d}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |
| 5.0 | 100 ± 0$^{e}$ | 100 ± 0$^{f}$ | 100 ± 0 |
| **FAe Bg** | | | |
| 0.005 | 49.1 ± 14$^{c}$ | 89 ± 4.6$^{e}$ | 99.2 ± 0.8$^{c}$ |
| 0.05 | 60.2 ± 12$^{c}$ | 95.5 ± 2$^{ef}$ | 100 ± 0$^{c}$ |
| 0.5 | 87.5 ± 5$^{d}$ | 92.8 ± 3$^{f}$ | 100 ± 0$^{c}$ |
| 5.0 | 100 ± 0$^{e}$ | 100 ± 0$^{f}$ | 100 ± 0$^{c}$ |

FB *Jm* = *Jacquinia macrocarpa* n-butanol fraction; FAe *Ke* = *Krameria erecta* ethyl acetate fraction; FAe *Bg* = *Baccharis glutinosa* ethyl acetate fraction.

**Table 4.** *Salmonella* test strains TA98 and TA100, with and without bioactivation (S9) exposed to different concentrations of mutagenicity positive controls.

| Concentration (mg mL$^{-1}$) | TA 98 | | | TA 100 | | |
|---|---|---|---|---|---|---|
| | RI | RE | RM | RI | RE | RM |
| **Sodium azide without S9** | | | | | | |
| 0.015 | 922 ± 65$^{b}$ | 26 | 35 | 2140 ± 111.4 | 224 | 10 |
| 0.15 | 1147 ± 117$^{c}$ | 26 | 44 | 2388 ± 67.7 | 224 | 11 |
| 1.5 | 2053 ±88$^{d}$ | 26 | 79 | 3474 ± 317.0 | 224 | 15 |
| 15 | 253 ±42$^{a}$ | 26 | 10 | 1626 ± 213.0 | 224 | 7 |
| **Aflatoxin B1 with S9** | | | | | | |
| 5 | 41.0 ± 7$^{a}$ | 26 | 1.58 | 236 ± 12.5 | 224 | 1.16 |
| 50 | 42.0 ± 3$^{a}$ | 26 | 1.63 | 278 ±15.6 | 224 | 1.36 |
| 500 | 932.0 ± 46$^{c}$ | 26 | 35.86 | 2867 ± 415.0 | 224 | 14.1 |
| 5000 | 205.0 ±9$^{b}$ | 26 | 7.88 | 329 ± 66.3 | 224 | 1.6 |

RE = Spontaneus revertants; RI = induced revertants; RM = mutagenicity ratio.

**Table 5.** Antimutagenic potential of the plant extracts fractions. Both *Salmonella* test strains TA98 and TA100, with and without bioactivation (S9), were exposed to different concentrations of the extracts fractions.

| Concentration (mg mL$^{-1}$) | *Bg* FAe TA 100 | *Bg* FAe TA 98 | *Jm* FB TA 100 | *Jm* FB TA 98 | *Ke* FAe TA 100 | Ke FAe TA 98 |
|---|---|---|---|---|---|---|
| **With S9** | | | | | | |
| 0 | 231.5 ± 10.6 | 34.5 ± 12.0 | 162.5 ± 48.8 | 47.5 ± 9.2 | 187 ± 83.4 | 45.5 ± 12.0 |
| 0.00005 | 201.5 ± 17.7 | 33 ± 4.2 | 229 ± 82.3 | 49 ± 11.3 | 272.5 ± 13.4 | 36 ± 5.7 |
| 0.0005 | 217 ± 42.4 | 44.5 ± 12.0 | 221 ± 77.8 | 53 ± 8.5 | 276 ± 9.9 | 36 ± 0 |
| 0.005 | 271.5 ± 10.6 | 40 ± 4.2 | 258.5 ± 51.6 | 43 ± 4.2 | 261.5 ± 37.5 | 34.5 ± 4.9 |
| 0.05 | 245.5 ± 7.8 | 36 ± 4.2 | 257.5 ± 21.9 | 34 ± 1.4 | 271.5 ± 50.2 | 30.5 ± 6.4 |
| 0.5 | 241.5 ± 24.7 | 38 ± 1.4 | 277 ± 15.6 | 38.5 ± 7.8 | 295 ± 46.7 | 53 ± 29.7 |
| 5 | 191 ± 48.1 | 41.5 ± 2.1 | 278.5 ± 16.3 | 42 ± 21.2 | 210 ± 53.7 | 34 ± 41.0 |
| **Without S9** | | | | | | |
| 0 | 231.5 ± 10.6 | 34.5 ± 12.0 | 162.5 ± 48.8 | 47.5 ± 9.2 | 187 ± 83.4 | 45.5 ± 1.0 |
| 0.00005 | 212 ± 4.2 | 35 ± 14.1 | 242 ± 15.6 | 43.5 ± 10.6 | 225 ± 2.8 | 40 ± 0 |
| 0.0005 | 214 ± 5.7 | 32 ± 5.6 | 248 ± 26.8 | 37 ± 1.4 | 256 ± 19.8 | 39 ± 9.8 |
| 0.005 | 225.5 ± 0.7 | 28 ± 0 | 237.5 ± 16.7 | 41 ± 1.4 | 194 ± 39.6 | 36 ± 9.8 |
| 0.05 | 250.5 ± 36.6 | 26 ± 2.8 | 240.5 ± 0,7 | 38 ± 4.24 | 204 ± 42.4 | 42 ± 19.8 |
| 0.5 | 231.5 ± 3.5 | 26.5 ± 0.7 | 242 ± 1.4 | 42 ± 0 | 229.5 ± 24.7 | 44.5 ± 9.2 |
| 5 | 192 ± 11.3 | 28.5 ± 3.5 | 266 ± 7.1 | 33 ± 1.4 | 230.5 ± 24.7 | 56 ± 12.7 |

All values represent mean of triplicate determination ± standard deviation.

per plate observed did not double the number of spontaneous revertants. Similar findings have been were reported (Nogueira et al., 2011); they found that fractions from *Baccharis trimera*, evaluated *in vivo* and *in vitro*, were not mutagenic. Also, other authors reported no genotoxic activity of *Baccharis incarum* on *Drosophila* melanogaster (Berzaín and Rodrigo, 2006). These findings suggest that the genus *Baccharis* might not be of potential toxicity to superior animal organisms; however, further investigation should be performed for a full toxicity assessment.

This study indicates that the plant extracts had anti-fungical activity on *F. verticillioides* and can be exploited in the future to reduce fungal spread. They delayed radial growth during the the incubation time. Ethyl acetate fractions of *B. glutinosa* and *K. erecta* caused the higher inhibitory effect on *F. verticillioides* spore germination, 100 and 95%, respectively. Suppresion on spore production could be the major contribution to limit the pathogen spread. Plant fractions tested at 5.0 mg mL$^{-1}$ caused 100% brine shrimp lethality after 24 h and the Ames test did not reveal mutagenic activity.

## Conflict of interests

The author(s) did not declare any conflict of interest.

## AKNOWLEDGMENTS

The authors are grateful to CONACYT (Mexican Council for Science and Technology) for the grant 58249.

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