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Experimental protocol for the recovery and evaluation of bioactive compounds of tarbush against postharvest fruit fungi

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ABSTRACT

The aim of this study was to recover and evaluate *in vitro* the antifungal activity of bioactive compounds of tarbush *Flourensia cernua* against fruit postharvest fungi and their antioxidant capacity. A yield of 15% of bioactive compounds of tarbush was obtained by infusion method and heating using water as solvent. A concentration of 4000 mg/L showed a higher antioxidant activity against the ABTS radical (3.21 μMol/g) in comparison with the DPPH radical (7.62 μMol/g); however the DPPH radical showed a better correlation with the content of tannins. The BCT showed values of IC_{50} between 1519 and 3310 mg/L against *Rhizopus stolonifer, Botrytis cinerea, Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Antifungal activity is attributable mainly to gallic acid and flavonoids identified by infrared and HPLC analysis. In this study, the BCT have shown to be a possible natural alternative of antioxidant and antifungal compounds for use against postharvest fruit fungi.

1. Introduction

Fruit production around the world is affected especially during harvest, because frequently have long periods of storage prior to their arrival to the market causing great losses due to the decline in quality or quantity of the total production (De León-Zapata et al., 2013). Postharvest diseases are attributable mainly to fungi such as *Rhizopus, Colletotrichum*, (Jasso-De Rodríguez et al., 2011) *Penicillium, Fusarium, Botrytis, Nectria* and *Alternaria* (Rosenberg, 1999).

Chemical fungicides have been used intensively against disease transmission and for postharvest fruit control (Jasso-De Rodríguez et al., 2011). Nevertheless, it has also caused resistance in pathogens, contamination of the environment (Rivas et al., 2005) and complications in human health caused by consumption of food with toxic residues (Palacios-Nava & Moreno-Tetlacuilo, 2004).

The potential use of plant extracts with antifungal properties for control of phytopathogens has been demonstrated at laboratory, greenhouse and at field level (Bergeron, Marston, Hakizamungu, & Hostettmenn, 1995) as a new alternative to the use of synthetic fungicides.

México have a wide variety of plants; it is the fourth richest country worldwide in this aspect (Jasso-De Rodríguez et al., 2011). Particularly the regions of the north of México, with its semiarid climate, have a great number and variety of wild plants grown under extreme climatic conditions, it is thought that some 25,000 species are registered and 30,000 not described (Adame & Adame, 2000).

One of them is tarbush (*Fluorensia cernua* D.C.), which is abundant in arid and semiarid regions of Mexico, where the tea brewed from the leaves of this plant is used in traditional medicine to treat digestive disorders, rheumatism, venereal diseases, herpes, bronchitis, varicella and common cold (Ventura, Gutiérrez-Sánchez, Rodríguez-Herrera, & Aguilar, 2009). It has been reported that components of tarbush extracts have antioxidant (Abou-Gazar, Bedir, Takamatsu, Ferreira, & Khan, 2004; De León-Zapata et al., 2013), anti-HIV (Gnabre, Ito, Ma, & Huang, 1996), antifungal (De León-Zapata et al., 2013; Jasso-De Rodríguez et al., 2011), antitumor (MacRae & Towers, 1984) and antidiabetic properties (Luo et al., 1998).

The biological activity of tarbush is due to its chemical composition mainly by compounds as methyl orsellinate, ermanin, flourensadiol, dehydroflourensic acid, long chain hydrocarbons from tetracosane 4-olide to triacontano-4-olide and lactones (Jasso-De Rodríguez et al., 2007) in addition to saponins (Méndez et al., 2012), terpenes (Estell, James, Fredrickson, & Anderson,

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2013) and condensed tannins equivalent to catechins (Belmares, Garza, Rodríguez, Contreras-Esquivel, & Aguilar, 2009; Castillo et al., 2010; De León-Zapata et al., 2013; Méndez et al., 2012).

For extraction of active phytochemicals compounds against fungal pathogens, the most commonly used solvents are methanol, ethanol, hexane, chloroform and diethyl ether (Guerrero-Rodríguez et al., 2007; Jasso-De Rodríguez et al., 2007).

Ventura-Sobrevilla et al. (2006) reported that the extracts of *F. cernua*, *Jatropha dioica*, *Turnera diffusa* and *Euphorbia antisyphilitica* are effective against some fungi such as *Penicillium purpurogenum*, *Fusarium spp.*, *Alternaria sp.*, *Aspergillus flavus* and *Rhizoctonia sp.* Other authors reported that the *F. cernua* leaves contain substantial levels of phenolic compounds (Estell et al., 2013). Also, exists some reports on growth inhibition of phytopathogenic fungi such as *Rhizoctonia solani* and *Phytophthora infestans* with metanol extracts of tarbush (Gamboa-Alvarado, Hernández-Castillo, Guerrero-Rodríguez, Sánchez- Arizpe, & Lira-Saldívar, 2003), *R. stolonifer*, *C. gloesporoides* and *P. digitatum* with hexane and ethanol extracts of tarbush (Jasso-De Rodríguez et al., 2011) and *Colletotrichum spp.* with hexane, diethyl ether and ethanolic extracts against termites (Tellez et al., 2001).

The use of organic solvents which are safe and less toxic is important. In the context of the Green Chemistry (Cann, 2009), a safe solvent is the one which may be removed easily without needing to use an alternative purification technique, as the water.

Continuing in search of natural compounds with fungicide activity but friendly with the environment, the aim of this study was to recover bioactive compounds of a aqueous extract of tarbush *F. cernua* as a potential source of natural antioxidants and make an *in vitro* evaluation of the inhibitory activity against the postharvest fungi that causes diseases in fruits of commercial interest as *R. stolonifer, B. cinerea, F. oxysporum* and *C. gloeosporioides.*

2. Materials and methods

2.1. Reagents and chemicals

Gallic acid, the radicals ABTS and DPPH, methanol, ethanol, acetonitrile, formic acid, potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), trolox and Folin–Ciocalteau reagent were purchased from Sigma Chemical Co. All the standards had purities above 95%. The culture media of potato–dextrose–agar (PDA) was purchased from Sigma Chemical Co. (St. Louis, USA).

2.2. Plant collection

Leaves of tarbush *F. cernua* were collected from areas nearby to Saltillo, Coahuila, Mexico during March and April of 2014. Plant material was dehydrate at room temperature for 8–10 days and using a conventional oven (Labnet, International, Inc.) at 60 ± 1 °C for 2 days. The leaves were stored in amber bottles or dark plastic bags at room temperature (25 ± 1 °C) until the obtention of the aqueous extract of tarbush.

2.3. Recovery of bioactive compounds of tarbush (BCT)

For the recovery of bioactive compounds from tarbush (Fig. 1), it was selected one sample of 10 g of leaves of tarbush and placed in an amber flask and then 100 mL deionized water was added. The mixture was manually stirred and heated in one hot plate for 2 h at 60 ± 1 °C. The extract was filtered with a Wathman Num. 1 paper, was added in glass Petri plates and then placed in a conventional oven (Labnet, International, Inc.) during 36 h at 60 ± 1°. The yield of the BCT was determined by gravimetry. The BCT were stored in containers covered with aluminium or amber bottles at 5 ± 1 °C. Were prepared samples of the BCT at 500, 1000, 2000, 3000 and 4000 mg/L, for to evaluation of their biological activity.

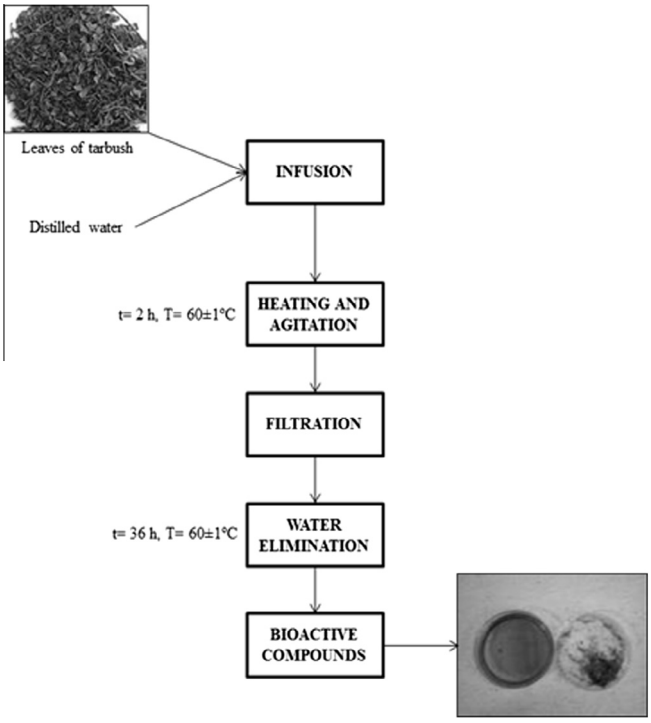

Fig. 1. Schematic diagram for the recovery of bioactive compounds of tarbush (BCT).

2.4. Total tannins

Concentration of total tannins was determined by spectrophotometry using the method described by Makkar (1999). Comercial Folin–Ciocalteau reagent was added (400 μL) in each one of samples of the BCT prepared to different concentrations. Samples were vortexed and allowed to stand for 5 min at room temperature. Then 400 μL of sodium carbonate at 0.01 M and 2.5 ml of distilled water were added. Finally, absorbance was readed at 725 nm. A reference curve was implemented using 400 μL of gallic acid at different concentrations ranging 200–1000 mg/L.

2.5. Determination of antioxidant activity

The cation radical ABTS was synthesized by the reaction of a 7 mM ABTS solution with a 2.45 mM $K_2S_2O_8$ solution. The mixture was keep at 23 ± 1 °C in the dark for 16 h afterwards, the ABTS solution was diluted with ethanol until an absorbance of 0.7 at 734 nm was achieved in a UV–Vis spectrophotometer. 10 μl of sample was added in the reaction cuvette, immediately after 1 mL of ABTS solution was added. After 10 min, the percentage inhibition of absorbance at 734 nm was calculated for each concentration, relative to the blank absorbance (ethanol). The DPPH radical is characterized by an unpaired electron, which is a free radical stabilized by resonance. A solution of DPPH radical at a concentration of 60 mM by diluting with methanol was prepared. Was added 100 μL of extract of tarbush in test tubes covered with foil after was added 2.9 mL of DPPH solution and allowed to stand for 30 min. The absorbance was recorded at a wavelength of 517 nm.