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Verbascoside is not genotoxic in the ST and HB crosses of the *Drosophila* wing spot test, and its constituent, caffeic acid, decreases the spontaneous mutation rate in the ST cross

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ABSTRACT

Verbascoside (VB) is a phenylpropanoid isolated from *Buddleja* species, some of which originate in Mexico, and was first described in the sixteenth century in the codices of Mexican traditional medicine. VB is present in alcohol extracts and is widely used in the north of Mexico as a sunscreen. VB absorbs UV-A and UV-B radiation and has high antioxidant and anti-inflammatory capacities. VB and its constituent caffeic acid (CA) were screened to determine their genotoxic activity using the *Drosophila* wing spot test. Third instar larvae (72 ± 4 h) of the standard (ST) and high bioactivation (HB) crosses, with regulated and high levels of cytochrome P450s (Cyp450s), respectively, were exposed to VB or CA (0, 27, 57, 81, 135, and 173 mM). VB was not genotoxic at any of the concentrations tested in both crosses. The amount of VB residue as determined by HPLC in the adult flies that were fed with VB indicated a low metabolism of this compound, which explains the absence of genotoxicity. CA decreased the spontaneous frequencies of small and total spots and showed putative toxicity in the ST cross.

1. Introduction

1.1. Buddleja species

Various *Buddleja* species of the Buddlejaceae family have been used worldwide in folk medicine (Houghton, 1984; Arciniegas et al., 1997; Houghton et al., 2005). Some of these species originated in Mexico and are widely distributed (Table 1). The *Buddleja* species "tepozan" was described in the Aztec manuscript by De la Cruz (1991), the "Códice Badiano", as a plant used by indigenous peoples to treat the skin squamous disease "mentagra".

Iridoids (Duff et al., 1965), flavonoids (Arciniegas et al., 1997), sesquiterpenes (Yoshida et al., 1978), saponins (Emam et al., 1997), and phenylpropanoids (Houghton, 1984; Avila and Romo de Vivar, 2002) have been isolated from *Buddleja cordata*, *Buddleja perfoliata*, and *Buddleja scordioides*. Recently, special attention has been paid to natural substances isolated from plants with ethnobotanic uses. For example, the phenylpropanoids have been considered as potential sunscreens because they absorb UV-A (304–350 nm) and UV-B (352–385 nm) radiation, which can generate DNA photodamage (Toyoshima et al., 2000) and lead to immunosuppression (Gil and Kim, 2000) and the production of reactive oxygen species (ROS) (Said et al., 2007). As singlet oxygen and superoxide radicals, ROS take an active part in many pathological processes including cancer and aging (Zhao et al., 2005).

1.2. Verbascoside

B. cordata is a shrub that, in Mexico and Guatemala, grows in semi dry and temperate climates, 2050–3100 m above the sea level and in disturbed zones. In Mexico, it is known by the common name of "tepozan". Although the alcohol extract from the leaves, branches, bark and flowers has many traditional Mexican medicinal uses, such as in skin lesion treatment and wound healing (Table 1), it has only been tested as a topical antiseptic (Avila et al., 1999) and a diuretic (Houghton, 1984). Its methanol extract contains phenylpropanoid glycoside verbascoside

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Table 1
Main *Buddleja* species used in traditional Mexican medicine according to Argueta-Villamar et al. (1994) and Aguilar et al. (1994).

Specie	Common name[a]	Uses[b]	Parts of the plant used	Mexican states where it has been found	Climates that it inhabits	Meters above sea level	Origin
B. americana	Tepozan	Analgesic, antineuralgic, antiseptic, astringent, bile, cirrhosis, dermatological, diabetes, digestive disorders, diuretic, dropsy, emetocatartic, epistaxis, eupeptic, extreme thinness, fever, gastralgia, healing, hematemesis, hypnotic, inflammation, lubricant urinary tract, lung pain, menstrual derangements, paralysis of respiratory muscles and awe, postpartum baths, rheumatism, sore throat, ulcer, diuretic; wounds, injuries and burns healing; "limpias"[c], "tumores preternaturales"[c].	Leaves, root	Chiapas, DF, Hidalgo, México, Michoacán, Oaxaca, Querétaro, San Luis Potosí, Tlaxcala, Veracruz.	Warm, semi warm and template	90–1100	Austral America
B. cordata	Tepozan	Back pain, baths, bile, cancer, cleanses the body, cough, cramps, diabetes, digestive disorders, disinfect wounds, dissolves tumours and abscesses, diuretic, dropsy, fever, headaches, heal burns, diuretic, nasal bleeding, postpartum baths, rheumatism, skin lesions, snakebite, wound healing.	Branches, leaves, bark, flowers	Hidalgo, México, Puebla, Tlaxcala, Veracruz	Semi dry and template	2050–3100	Mexico and Guatemala
B. lancelolata	Saulisca,	Throat infection and itchy scalp	Undescribed	Morelos	Template	1900–3000	Unknown
B. microphylla	Salvia de bolita	Wound healing, baths.	Branches, leaves	México, Puebla	Template	2600–2900	Mexico
B. parviflora	Tepozan	Dropsy, sinusitis, postpartum baths, pain, fatigue.	Branches	Aguascalientes, Hidalgo, Puebla	Warm, semi warm and template	900–1950	Mexico
B. perfoliata	Salvia de bolita	Headache, cough and cold, heart murmurs, bile, dizziness, nervousness, polyuria, rhinitis, injuries, lower blood pressure, simple coryza, catarrh, reduces mercury in syphilitic ptyalism, antihidrotic, stomatitis by abuse with mercury, eupeptic, hypnotic, "aire"[c], "espanto"[c].	Branches, stems, leaves	D.F., Guanajuato	Tropical and subtropical	2000–2550	Mexico
B. scordioides	Escobilla	Burns, digestive disorders, eupeptic, increase heart rate.	Branches, leaves, roots	Aguascalientes, Coahuila, Durango, Guanajuato, Nuevo León, Zacatecas	Dry, semi dry and template	1800–2300	Mexico
B. sessiliflora	Lengua de vaca,	[d]Fever, inflammation, bile, astringent, digestive disorders, postpartum, contraceptive; wound, injuries and burns healing; skin lesions, sore throat and tonsils, mumps, ulcers, kidney, varicose veins, sleeping pills, "limpias"[c].	Leaves	DF, Michoacán, Morelos, Nayarit, Sonora	Warm, semi warm, semi dry and template	900–3100	USA
B. wrightii	Lengua de buey	Urinary tract.	Roots	Sonora	Warm and dry	20–700	Mexico

[a] Taken from the references as the most common name used in Mexico.
[b] Taken from the literature with ethno botany references from specialists; the knowledge and information are original source and collective creation, their owners and reenactors are the indigenous peoples of Mexico.
[c] Untranslatable word.
[d] And for stiff neck, as described in the Codice Florentino by Fray Bernardino de Sahagún.

(2-(3,4-dihydroxyphenyl)ethyl 3-*O*-(6-deoxy-α-L-mannopyranosyl)-4-*O*-[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-β-D-glucopyranoside), which possesses diverse pharmacological properties. The first property is photoprotective activity, with a Sun Protection Factor (SPF) of 24 ± 0.7 (Avila-Acevedo et al., 2005). The second property is antioxidant capabilities through a variety of mechanisms: (i) free radical scavenging and protection against lipid peroxidation as shown by the Trolox equivalent antioxidant capacity and malondialdehyde assays (Funes et al., 2009), (ii) protection against low-density lipoprotein peroxidation in microsomes (Cos et al., 2002), (iii) repair of erythrocyte membrane fluidity after oxidative stress due to muscle immobilisation as shown in rabbit (Liu et al., 2003), and (iv) protection against cell death induced by UV light through chelation as shown in cultures of HaCaT and MCF 7 cells (Kostyuk et al., 2008). The third property is anti-inflammatory effects, independent of its antioxidant capacity, via the potent inhibition of nuclear factor kappa B (NF-κB) and chemokines IL-8, IP-10, and MCP-1 as well as ERK signal and activator protein (AP-1) transactivation as shown in normal cells (Korkina et al., 2011). The fourth property is anticarcinogenic activity, related to its cytotoxic and cytostatic activities against various types of cancer cells but not primary cultures of rat hepatocytes (Saracoglu et al., 1995) and its ability to induce redifferentiation of MGc80-3 cells (Li et al., 1997). As with other phenylpropanoids, VB can induce xenobiotic responsive elements such as the cytochrome P450 CYP1 subfamily, Nrf2 and phase II enzymes by binding to the AhR transcription factor. This factor is related to growth factors, cytokines, EGFR pathway members, and MAPK pathway kinases and involved in multiple cell functions, such as wound healing and cell differentiation (Korkina et al., 2011).

The American Academy of Dermatology recommends a habitual use of sunscreens with a SPF of at least 15 for all skin types (Scherschun and Lim, 2001). Some sunscreens, however, can increase lipid and protein oxidation, producing cytotoxic effects (Armeni et al., 2004; Said et al., 2007). Santoro et al. (2008) showed