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Article

Cytotoxic activity of extracts from *Tecoma* species and isolated lignans

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A phytochemical study of *Tecoma* genus (Bignoniaceae) was accomplished by antitumor activity of ethanolic extracts. Species of this genus are composed of small shrubs often used as ornamental plants. The *Tecoma stans* species is used in folk medicine for different purposes. Recent work shows *in vitro* anticancer activity against human breast cancer. The ethanolic extracts from leaves and trunks of *Tecoma casneifolia*, *T. garrocha*, *T. stans* var. *angustata* and *T. stans* var. *stans* were tested *in vitro.* The assays used were against line tumor cells by the MTT method and the most active extracts were further studied. In this way, the ethanolic extract from *T. stans* var. *stans* trunks presented the higher cytotoxicity against the tumor cell lines studied (CC_{50} 0.02 to 0.55 µg/ml) when compared to the other extracts tested (CC_{50} 0.08 to 200.0 µg/ml). Accordingly, this extract was selected for chromatographic fractionation from which five known lignans were isolated. Further, paulownin, paulownin acetate, sesamin, olivil and cycloolivil were identified using ^{13}C and ^{1}H NMR, IR, UV and spectroscopy and spectrometric MS techniques. These isolated compounds were tested and exhibited CC_{50} ranging from 13.01 to100.0 µg/ml which is superior to the ethanolic extract of trunk of *T. stans*.

Keywords: *Tecoma castaneifolia. Tecoma garrocha. Tecoma stans.* Lignan.

INTRODUCTION

Cancer accounts for about 13% of all causes of death in the world and more than 7 million people die each year from the disease (Who, 2018). The World Health Organization estimated that by the year 2030, 27 million cases of cancer can be expected. The greatest effect of this increase will be on low-and-middle income countries (Who, 2018).

Some prophylactic actions to prevent cancer include health education at all levels of society, early diagnosis prevention and support for research that includes new forms of treatment (drugs, vaccines) (Who, 2018). Hence, research of new bioactive molecules against cancer is an important area of research that can diminish fatality by this disease.

Within this context, the importance of plants as sources of new drugs is universally recognized. Already, many active compounds and their synthetic derivatives are used in the treatment of this illness (Newman, Cragg, 2016). Further studies involving new isolated molecules and/or modified molecules with antineoplastic potential are a promising field (Newman, Cragg, 2016).

Different classes of natural products with cytotoxic activity are currently in clinical use, among alkaloids (vincristine, vinblastine and camptothecin derivatives), diterpenes (taxanes), lignans (podophyllotoxin derivatives) and others (Patrick, 2017). According to Newman and

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Cragg (2016), from 136 antitumor drugs approved and marketed in United States, between 1981 and 2014, about 83% are of natural origin or synthetic products developed from a natural model.

Bignoniaceae Juss. species are known to be sources of cytotoxic compounds. In this context, naphthoquinones have some active compounds including β-lapachone (Oliveira *et al*., 1990). Species of this botanical family produce many different compounds, from which they have already isolated terpenoids, flavonoids, xanthones, quinones and lignans (Brandão *et al.,* 2017; Oliveira *et al*., 1993). Several reported species contain active compounds against major diseases. Cancer (bark of *Tabebuia*), hepatitis, rabies, diabetes, malaria, leishmaniasis and syphilis (Fischer, Theisen, Lohmann, 2004) are just part of this extended list that can be treated with bignoniaceae obtained compounds.

The genus *Tecoma* Juss. is composed mostly of shrubs or small trees and rarely subscandent plants. There are about fourteen species occurring in tropical regions of Africa and America, especially in the Andes and Arizona (Gentry, 1992). In Brazil, several species are used for ornamental purpose. Species of this genus have terminal inflorescences racemes or thryrses, yellow, orange-red corolla widely cultivated due to its natural beauty (Gentry, 1992; Anburaj *et al*., 2016). In addition, some natural occurrence is described in urban areas.

The most widespread species, *T. stans,* is found in Mexico with at least three varieties. Aerial parts are known to feed animals. Leaves are used in the popular medicine to treat diabetes (Kampati *et al*., 2018). Description from Mexican Pharmacopoeia of this plant is attributed to digestive properties. Thus, it can be prescribed for gastrointestinal disorders including gastritis from alcoholic derivation. A different traditional use for *T. stans* is designated by tribal people as anthelmintic (Kampati *et al*., 2018). Recent extract studies from *T. stans* demonstrate some anti-cancer activity against human breast cancer (Anburaj *et al*., 2016).

The present work contain studies from ethanolic extracts of *Tecoma* sp and their isolated lignans. Both have their cytotoxic activity evaluated against some different tumor cell lines.

MATERIAL AND METHODS

Collection, Taxonomical Determination and Processing of Plant Materials

T. castaneifolia (D. Don) Melchand and *T. garrocha* Hieron were collected in Santana de Pirapama, MG, Brazil, with the geographic coordinates 19º 00' 22" S and 44º 02' 35" W. *T. stans* var. *angustata* Rehder and *T. stans* var. *stans* (L.) Juss. ex Kunth were collected in Belo Horizonte, MG, Brazil, with the geographic coordinates 19º 55' 15" S and 43º 56' 16" W. The plants were taxonomically identified by Dr. JR Stehman, Botany Department of the Institute of Biological Sciences, UFMG, Belo Horizonte, Brazil. Voucher specimens were deposited at the BHCB/UFMG, Belo Horizonte, Minas Gerais, Brazil.

Preparation of Extracts

Plant material was dried in a circulating air oven at 40°C during 72 h. Aerial parts such as leaves and trunks were ground and extracted by percolation with 96% EtOH at room temperature. The solvent was removed in rotary evaporator under reduced pressure at 50ºC, leaving dark residues which were kept in a vacuum desiccator until constant weight.

Isolation of Chemical Components from trunk Extract

A portion of ethanolic extract of trunk of *T. stans* (EETTS, 100.0 g) was dissolved in methanol-H_2O (6:4) and this solution was submitted to successive extractions with immiscible solvents using firstly, dichloromethane and then ethyl acetate. There were three isolated parts produced: Organic layer corresponding *T. stans* dichloromethane trunk extract (TSDT, 63.4 g); *T. stans* ethyl acetate trunk extract (TSET, 6.2 g) and final *T. stans* aqueous fraction trunk extract (TSAT, 26.7 g).

The fractioning of TSDT occurred to the formation of a white precipitate (10.7 g) that was separated by decantation. A portion of this precipitate (8.5 g) was chromatographed on a silica gel column employing n-hexane, n-hexane/CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2/EtOAc

(1:1), EtOAc, EtOAc/MeOH (1:1), and MeOH as eluents. The fractions were collected in glass flasks of 20.0 ml obtaining a total of 60 fractions. The fractions were analyzed by a thin layer chromatography (TLC) and the ones that presented similar profiles were combined. In the TLC analyzes, n-hexane/EtOAc (1:1) was used as mobile phase, sulfuric anisaldehyde developer. Subsequently, the soluble part of the dichloromethane fraction (19.0 g) was fractionated using the same conditions utilized previously in the precipitated fractionation. This dichloromethane fraction gave a total of 182 fractions. They were assembled according to their similarity between analogous profiles from TLC analysis using the same conditions employed in the fractionation of the insoluble part. Final fractionations resulted in 19 combined fractions of precipitate and 24 combined fractions from the soluble portion.

Combined fractions 4 (115.5 mg) and 5 (583.5 mg), from insoluble portion of CH_2Cl_2 fraction, were submitted to silica gel chromatography on preparative TLC (silica gel 60 F_{254}-Merck®; 20x20 cm, layer thickness 1.0 mm) using CH_2Cl_2/EtOAc (8:2) as mobile phase. From the fractionation of Fr 4, a white solid called TST-3 (32.0 mg) was obtained while the fractionation of Fr 5 led to the isolation of a solid called TST-2 (55.0 mg).

The combined fractions 8 (478.5 mg), 9 (358.6 mg) and 10 (679.5 mg) from insoluble portion of CH_2Cl_2 fraction, were subjected to successive recrystallization using absolute ethanol leading to the isolation of a solid called TST-1 (1217.0 mg). Further quantities of TST-1 (292.0 mg) solid were obtained from the recrystallization of the pooled fractions 13 (776.3 mg), 14 (1079.4 mg), 15 (1098.7 mg) and 16 (1045.4 mg) from fractionation of the soluble part of the CH_2Cl_2 fraction.

The EtOAc fraction obtained from the liquid-liquid partition of the ethanolic extract was also fractionated on a silica gel column using the same conditions used in the fractionation of CH_2Cl_2 fraction. A total number of 41 fractions were obtained, which were combined according to their TLC chromatographic profiles, obtaining 15 combined fractions. The combined fractions 7 (141.6 mg) and 8 (153.5 mg) were rechromatographed on preparative TLC (silica gel 60 F_{254}-Merck®; 20x20 cm, layer thickness 1.0 mm) using CH_2Cl_2/EtOAc (8:2) as mobile phase, yielding small amounts of called TST-4 (14.0 mg) and TST-5 (11.0 mg).

Structural Determination

Isolated compounds were identified based on spectral analyses and literature comparison. NMR ^{1}H and ^{13}C-NMR including ^{1}D and ^{2}D spectra such as COSY, HSQC, and HMBC were obtained on a Bruker Avance DRX400 instrument in DMSO-*d6* with TMS as internal standard. Spectra of isolated compounds are shown in the supplemental material. Chemical shifts are given as δ (ppm) (Brandão *et al*., 2013) and coupling constants (*J*) are given in hertz. Melting points (mps) were measured with a Reichert melting point apparatus and are uncorrected. Infrared spectra were recorded on FT-IR Spectrometer, Varian 640-IR, Varian with system ATR and are reported in wave number (cm^{-1}). Samples were diluted with methanol-formic acid 0.1 % solution and ESI mass and UV spectra were recorded on a Waters ACQUITY TQD Tandem Quadrupole UPLC-DAD-MS System with direct injection.

Spectroscopic Data for Isolated Lignans

Paulownin (TST-1): White solid (MeOH); m.p. 107.0-108.5 °C; Lit. 105-106 °C (Ragasa *et al*., 2015); $[\alpha]_D$ = +30.8; UV (MeOH) l_{max} 234, 285 nm; IR n_{max} 3487, 2935, 2875, 1608, 1592, 1493, 1457, 1405, 1358, 1254, 1231, 1081, 1023, 1007, 959, 938, 910, 881, 805, 761, 715 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.93 (2H, dd, *J* = 1.2, sl Hz, H-2, H-2'), 6.78–6.88 (4H, m, H-5, H-3', H-6, H-6'), 4.83 (1H, d, *J* = 5.2 Hz, H-7), 3.04 (1H, m, H-8), 3.83 (1H, dd, *J* = 6.0, 2.8 Hz, H-9a), 4.50 (1H, dd, *J* = 8.4, 8.8 Hz, H-9b), 4.81 (1H, s, H-7'), 3.91 (1H, d, *J* = 9.6 Hz, H-9'a), 4.04 (1H, d, *J* = 9.2 Hz, H-9'b), 5.97 (2H, s, –OCH_2O–), 5.95 (2H, s, –OCH_2O–); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 129.24 (C-1), 119.81 (C-2), 108.23 (C-3), 147.32 (C-4), 148.05 (C-5), 106.91 (C-6), 85.83 (C-7), 60.47 (C-8), 71.63 (C-9), 134.66 (C-1'), 120.13 (C-2'), 108.60 (C-3'), 147.95 (C-4'), 148.19 (C-5'), 107.43 (C-6'), 87.49 (C-7'), 91.69 (C-8'), 74.87 (C-9'), 101.27 and 101.13 (2x –OCH_2O-); ESI-MS m/z 371.27 $[M+H]^+$, m/z 393.22 [M+Na] (calcd for $C_{20}H_{19}O_7$, 371.1130).

Paulownin acetate (TST-2): white solid (MeOH); m.p. 144.5-145.5 °C; Lit. 143-144 °C (Takahashi, Hayashi, Takani, 1970); UV (MeOH) l_{max} 237, 286 nm; IR n_{max} 3017, 2987, 2875, 1745, 1602, 1504, 1481, 1455, 1389, 1250, 1200, 1171, 1054, 932, 805, 780, 745 cm^{-1}; ^{1}H NMR

($CDCl_3$, 400 MHz): δ 6.92 (2H, dd, *J* = sl, sl Hz, H-2, H-2'), 6.75–6.88 (4H, m, H-5, H-6, H-3', H-6'), 4.73 (1H, d, *J* = 4.0 Hz, H-7), 3.27 (1H, m, H-8), 4.24 (1H, m, H-9a), 4.40 (1H, m, H-9b), 5.03 (1H, s, H-7'), 3.76 (1H, d, *J* = 4.0 Hz, H-9'a), 3.78 (1H, d, *J* = 4.0 Hz, H-9'b), 5.97 (2H, s, $–OCH_2O–$), 5.94 (2H, s, $–OCH_2O–$), 1.74 (3H, s, $–CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 130.26 (C-1), 119.84 (C-2), 108.22 (C-3), 147.41 (C-4), 147.58 (C-5), 106.81 (C-6), 85.78 (C-7), 59.01 (C-8), 69.93 (C-9), 134.08 (C-1'), 122.35 (C-2'), 108.87 (C-3'), 147.43 (C-4'), 148.10 (C-5'), 107.99 (C-6'), 86.84 (C-7'), 97.20 (C-8'), 75.18 (C-9'), 101.17 and 101.10 (2x $–OCH_2O–$), 169.45 (–C=O), 20.93 ($–CH_3$); ESI-MS m/z 413.48 $[M+H]^+$ (calcd for $C_{22}H_{21}O_8$, 413.1158).

Sesamin (TST-3): white powder (MeOH); m.p. 123.0-125.5 °C; Lit. 123-124 °C (Ragasa *et al*., 2015); UV (MeOH) l_{max} 235, 286 nm; IR n_{max} 3065, 2998, 2875, 1508, 1452, 1385, 1254, 1200, 1100, 1079, 1045, 986, 931, 815, 765, 708 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): 1H NMR (400 MHz, $CDCl_3$): δ 6.87 (2H, d, *J* = SL, H-2, H-2'), 6.79–6.82 (4H, m, H-5, H-5', H-6, H-6'), 4.75 (2H, d, *J* = 4.0 Hz, H-7, H-7'), 3.07 (2H, m, H-8, H-8'), 3.90 (2H, dd, *J* = 3.6, 5.6 Hz, H-9, H-9'), 4.26 (2H, dd, *J* = 6.8, 2.0 Hz, H-9, H-9'), 5.97 (2x $–OCH_2O–$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.12 (C-1 e C-1'), 106.50 (C-2 e C-2'), 147.99 (C-3 e C-3'), 147.13 (C-4 e C-4'), 108.18 (C-5 e C-5'), 119.34 (C-6 e C-6'), 85.80 (C-7 e C-7'), 54.36 (C-8 e C-8'), 71.73 (C-9 e C-9'), 101.27 and 101.06 (2× $–OCH_2O–$); ESI-MS m/z 355.26 $[M+H]^+$ (calcd for $C_{20}H_{19}O_6$, 355.1181).

Olivil (TST-4): white powder (MeOH); m.p. decomposes at 269.0-279.0 °C; Lit. 135 °C (Ghogomu-Tih *et al*., 1985), UV (MeOH) l_{max} 222, 257, 275 (sh), 320 (sh), 366 nm; IR n_{max} 3583, 2920, 1598, 1512, 1444, 1361, 1260, 1171, 1066, 1047, 1022, 883, 833 cm^{-1}; ^1H NMR (DMSO-*d6*, 400 MHz): δ 6.91 (2H, dd, *J* = 1.8, 16.8 Hz), 6.77–6.86 (m, 4H), 5.97 (s, 2H, $-OCH_2O-$), 5.94 (s, 2H, $-OCH_2O-$), 4.80 (1H, s, H-1), 4.03 (1H, d, *J* = 9.0 Hz, H-3), 3.92 (1H,d, *J* = 9.6 Hz, H-3), 4.82 (1H, d, *J* = 4.8 Hz, H-4), 3.03 (1H, m H-5), 3.82 (1H, dd, *J* = 6.0, 9.0 Hz, H-6), 4.50 (1H, dd, *J* = 8.4, 9.0 Hz, H-6); ^{13}C NMR (DMSO-*d6*, 100 MHz δ 129.63 (C-1), 115.17 (C-2), 147.75 (C-3), 146.08 (C-4), 115.34 (C-5), 122.96 (C-6), 83.76 (C-7), 61.06 (C-8), 59.41 (C-9), 134.91 (C-1'), 111.53 (C-2'), 147.34 (C-3'), 145.17 (C-4'), 115.26 (C-5'), 119.65 (C-6'), 39.63 (C-7'), 80.99 (C-8'), 76.67 (C-9'), 56.05 and 56.04 (2× $–OCH_3–$); ESI-MS m/z 375.53 $[M-H]^-$ (calcd for $C_{20}H_{23}O_7$, 375.1122).

Ciclolivil (TST-5): white solid (MeOH); m.p. 285.6-287.9 °C; Lit. 289-291 °C (Ghogomu-Tih *et al*., 1985); UV (MeOH) l_{max} 267, 313 (sh) nm; IR n_{max} 3452, 2980, 1608, 1516, 1447, 1352, 1259, 1168, 1049, 1032, 1023, 873, 821 cm^{-1}; ^1H NMR (DMSO-*d6*, 400 MHz): δ 7.24 (1H, d, *J* = 2.0 Hz, H-2), 6.62 (1H, d, *J* = SL, H-5), 6.62 (1H, dd, *J* = 2.0 e 6.4 Hz, H-6), 4.50 (1H, d, *J* = 12.0 Hz, H-7), 2.50 (1H, m, H-8), 4.21 (1H, m, H-9a), 4.01 (1H, m, H-9b), 7.06 (1H, s, H-2'), 7.22 (1H, s, H-5'), 3.67 (1H, d, *J* = 16,40 Hz, H-7'a), 3.02 (1H, d, *J* = 14.0 Hz, H-7'b), 4.29 (1H, m, H-9'a), 4.01 (1H, m, H-9'b), 4.24 and 4.23 (6H, s, 2× $–OCH_3$); ^{13}C NMR (DMSO-*d6*, 100 MHz): δ 143.21 (C-1), 123.56 (C-2), 158.00 (C-3), 155.65 (C-4), 126.46 (C-5), 132.87 (C-6), 54.27 (C-7), 57.17 (C-8), 79.26 (C-9), 147.96 (C-1'), 122.35 (C-2'), 156.35 (C-3'), 154.84 (C-4'), 125.29 (C-5'), 136.18 (C-6'), 49.89 (C-7'), 83.69 (C-8'), 70.40 (C-9'), 65.92 and 66.03 (2× OCH_3); ESI-MS m/z 375.51 $[M-H]^-$ (calcd for $C_{20}H_{23}O_7$, 375.1122).

Cell lines

A panel of human cancer cell lines were used for the cytotoxicity studies. ATCC® cell lines including hepatocellular carcinoma Hep G2 (ATCC® HB-8065™), ovarian cell carcinoma TOV-21G (ATCC® CRL-11730™), urinary bladder transitional cell carcinoma T24 (ATCC® HTB-4™), cervix cell carcinoma HeLa (ATCC® CCL-2™) and breast cell carcinoma MDA-MB-231 (ATCC® HTB-26™), as well as normal human lung fibroblast cell MRC-5 (ATCC® CCL-117™) were used in the assays. The cells were cultivated in complete cell medium consisting of Dulbecco's modified Eagle medium (DMEM, Cultilab, Campinas, SP, Brazil), supplemented with 5% fetal bovine serum, 50 µg/mL gentamicin, 100 U/mL penicillin and 5 µg/mL amphotericin B (Brandão *et al*., 2013). The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 and harvested in log-phase for experimental use.

Cytotoxicity Assay

Cell lines were exposed to different concentrations of extracts/fractions/compounds for 72 h [6]. After incubation,

cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Merck) assay at a concentration of 2 mg/mL in PBS (Brandão *et al*., 2013; Twentyman, Luscombe, 1987). Each sample was assayed in three replicates for concentrations ranging from 200.0 to 3.125 µg/mL. The cytotoxicity of each sample was expressed as CC_{50}, i.e. the concentration of the sample that inhibited cell growth by 50% (Brandão *et al*., 2013).

RESULTS AND DISCUSSION

Identification of compounds isolated from trunk ethanolic extract of *T. stans* var. *stans*

The ethanolic extract from the trunk of *T. stans* var. *stans* showed the most cytotoxic results for the five tumor cell lines. Therefore, it was selected for a chromatographic fractionation. This fractionation resulted in isolation of five known lignans (Figure 1). The lignans were identified using a ^{1}H and ^{13}C NMR spectroscopy. Later, structural elucidation was confirmed by comparison of bibliography data. Spectra of ^{1}D and ^{2}D were used such as COSY, HSQC and HMBC (Brandão *et al*., 2013). Literature data of ^{1}D and ^{2}D was also used during the identification (Takahashi, Hayashi, Takani, 1970; Ghogomu-Tih *et al*., 1985; Ragasa *et al*., 2015), Spectra were attached in supplementary file. The presence of lignans in the species of Bignoniaceae is widely reported (Cipriani *et al*., 2008).

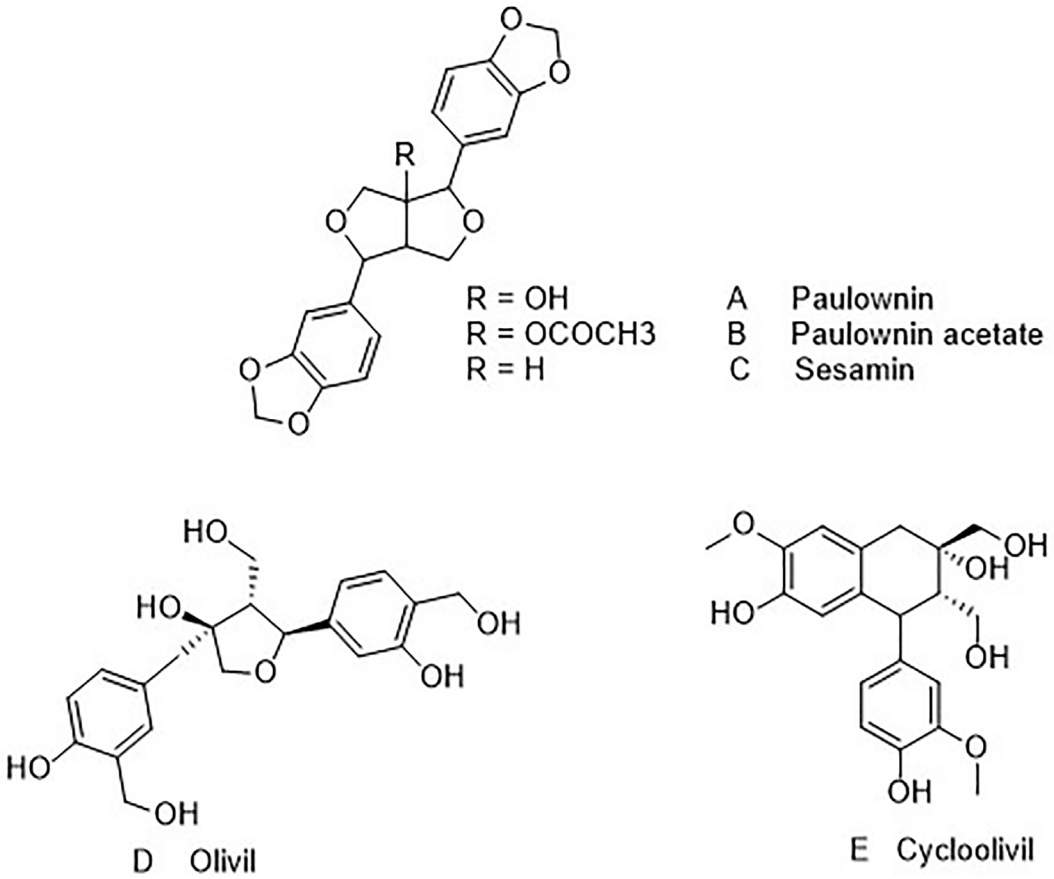

FIGURE 1 - Chemical structures of paulownin (A), paulownin acetate (B), sesamin (C), olivil (D) and cycloolivil (E).

Paulownin **A** (TST-1) was isolated in large quantities from the dichloromethane fraction after chromatographic separation. About 1.5 g of this amorphous white solid was obtained. The ^{1}H NMR spectrum of TST-1 showed six signals of aromatic hydrogen at δ 6-94 to 6.78 ppm corresponding to two trisubstituted aromatic rings. The two singlets at δ 5.97 and δ 5.95 ppm, referring to two hydrogens, characterizing two groups of methylenedioxy coupled to aromatic ring in its structure. Furthermore, two oxymethine hydrogens were observed as a duplet at δ 4.84 ppm referring to the H-7 and as a singlet at δ 4.81 ppm corresponding to the H-7', the couplings of this signals were confirmed at the COSY contour map. The ^{13}C NMR spectrum presents the signals at δ 101.27 ppm and δ 101.13 ppm, the confirmed groups of methylenedioxy switched on to the aromatic system. The signals observed at δ 129.24 ppm and δ 134.66 ppm, referring to the carbons C-1 and C-1', with these values they were characteristic of the furofuran basic structure with an aril group type 3,4-methylenedioxyphelinic at equatorial position. Moreover, the difference at 5.5 ppm between C-1 and C-1' observed indicates the presence of a hydroxyl group at the carbon C-8' (Agrawal, Thakur, 1985). The MS of TST-1 showed an $[M + Na]^{+}$ ion at *m/z* 393.22 and $[M + H]^{+}$ ion at *m/z* 371 peaks. The molecular formula of TST-1 was determined as $C_{20}H_{20}O_7$ (371.1130). The data allowed identification of TST-1 as a natural paulownin product (1,4-bis(3,4-methylenedioxyphenil)-tetrahydro-*1H,3H*-furo[3,4-c]furan-3-ol).

The ^{1}H and ^{13}C NMR spectra of paulownin acetate **B** (TST-2) (55.0 mg) showed similar signals observed by TST-1. Likewise, this different molecule is also a furofuran lignan containing an aril group type 3,4-methylenedioxyphelinic at equatorial position. Furthermore, the ^{13}C NMR spectrum presents the signals at δ 169.45 ppm referring to the carbonile coupled to the C-8' and the methyl group at δ 20.93 ppm. The MS spectrum of TST-2 showed an $[M]^{+}$ ion at *m/z* 413.4777 as a base peak. The molecular formula of TST-2 was determined as $C_{22}H_{21}O_8$ (413.12). Obtained data allowed for the identification of TST-2 as paulownin acetate (1,4-bis(3,4-methylenedioxyphenil)-tetrahydro-*1H,3H*-furo[3,4-c] furan-3-il).

A different furofuran lignan isolated from dichloromethane fraction was sesamin **C** (TST-3) (32.0 mg), where the ^{1}H and ^{13}C NMR spectra showed six signals of aromatic hydrogen at δ 6-87 to 6.79 ppm corresponding to two trisubstituted aromatic rings. Signals characteristic of furofuran lignans were observed as double-doublet at δ 4.26 and δ 3.90 ppm, attributed to the oxymethylenic hydrogens H-9 and H-9' at equatorial and axial position, respectively. The ^{13}C NMR spectrum showed ten signals that suggest a symmetric molecule, like the methylenedioxy signal at δ 101.06 ppm coupled to the aromatic systems. The MS of TST-3 showed an $[M]^{+}$ ion at *m/z* 355.2642 as a base peak. The molecular formula of TST-3 was determined as $C_{20}H_{19}O_6$ (355.1181). These data allowed the identification of TST-3 as sesamin (1,4-bis(methylenedioxy)-tetrahydro-*1H,3H*-furo[3,4-c] furan-3-o).

The fractionation of the ethyl acetate portion isolated small amounts of olivil **D** (TST-4) (14.0 mg). NMR spectrum of ^{1}H showed aromatic hydrogens due to the signals at δ 7.03 ppm, δ 6.65 ppm and δ 6.78 ppm and the multiplet from δ 6.85 to 678 ppm. Analysis of ^{13}C NMR data and HSQC contour map suggest the presence of two aromatic rings. These spectra showed the presence of carbon signals at δ 115.17 ppm (C-2), δ 111.53 ppm (C-2'), δ 115.34 ppm (C-5), δ 115.26 ppm (C-5'), δ 122.96 ppm (C-6) and δ 119.65 (C-6'), which correspond to the aromatic region. These followed correlations of spectra allowed for the conclusion that aromatic rings are 1,3,4-trisubstituted, identified as a tetrahydrofuran lignan skeleton. The MS of TST-4 showed an $[M]^{-}$ ion at *m/z* 375.3220 as a base peak. The molecular formula of TST-4 was determined as $C_{20}H_{23}O_7$ (375.1152). These data allowed the identification of TST-4 as a natural olivil product (3-(4-hidroxy-3-methoxybenzil)-5-(4-hidroxy-3-methoxyphenyl)-4-(hidroxymethyl) tetrahydrofuran-3-ol).

NMR ^{1}H spectrum of cycloolivil **E** (TST-5) (11.0 mg), isolated from the ethyl acetate fraction, showed five aromatic hydrogens. The coupling constants from these signals corresponded to two different rings. The signals at δ 7.12 ppm (1H, J = 2.0 and 6.40 Hz) couple with the signals of doublet-doublet at δ 7.24 ppm (1H, d, J = 2.0 Hz) and δ 6.62 ppm (1H, J = 6.40), and were attributed to the hydrogens of the 1,3,4-trisubstituted ring. The second set of signals at 7.06 (1H, s) and 7.22 (1H, s) suggests the presence of a 1,3,4,6-tetrasubstituted ring. These described signals characterize a lignan

skeleton tetrahydronaphthalene type. The MS of TST-5 showed an $[M]^-$ ion at *m/z* 375.3220 as a base peak. The molecular formula of TST-5 was determined as $C_{20}H_{23}O_7$ (375.1152). These data allowed for the identification of TST-5 as cycloolivil (4-(4-hidroxy-3-methoxyphenyl)-2,3-bis(hidroxymethyl)-7-methoxy-1,2,3,4-tetrahydronaphthalene-2,6-diol).

Cytotoxic essay of *Tecoma* species extract and constituents

Ethanolic extracts of species from genus *Tecoma*, *T. stans* var. *stans*, *T. stans* var. *angustata*, *T. castaneifolia* and *T. garrocha* were evaluated for *in vitro* cytotoxic activity. Biological essay was evaluated against five tumor cell lines including: Hep G2, T24, TOV-21G, HeLa and MDA-MB-231 and normal cell line, MRC-5, in concentrations ranging from 200.0 to 3.125 µg/mL. The *T. stans* var. *stans* trunk extract presented CC_{50} between 0.0156 and 0.5533 µg/mL against tumor cell lines while leaf extract presented CC_{50} from 39.89 to 200.0 µg/mL. The *T. stans* var *angustata* trunk extract also were very cytotoxic presenting CC_{50} from 0.084 to 56.03 µg/mL whereas leaves extract presented a moderate CC_{50} cytotoxicity between 24.22 and 200.0 µg/mL. The extract of *T. castaneifolia* stems presented CC_{50} ranging from 15.90 to 110.80 µg/mL and the extract of leaves from this species had values between 18.31 to 200.0 µg/mL. Cytotoxicity of *T. garrocha* extracts also showed similar results previously ranging from 12.96 to 200.0 µg/mL for the trunk extract and from 27.93 to 200.0 µg/mL for the leaves extract.

Trunks extracts from all species of the genus *Tecoma* were more cytotoxic against tumor cell lines when compared to leaves extracts. Thus, the most active *T. stans* var. *stans* ethanolic extract was selected for fractionation by liquid-liquid partition. Five lignans were isolated: paulownin, paulownin acetate, sesamin, olivil and cycloolivil. These compounds were also evaluated for cytotoxic activity against the five tumor cell lines at concentrations ranging from 100 to 1.5625 µg/mL.

Paulownin presented mean cytotoxicity values between 29.35 and 100.0 µg/mL, whereas paulownin acetate showed CC_{50} from 28.15 to 100.0 µg/mL, the CC_{50} values of sesamin ranged from 13.01 to 100.0 µg/mL, while the olivil was not cytotoxic at the highest concentration tested (100.0 µg/mL) to any of the cell lines tested. Finally, the cycloolivil presented CC_{50} between 45.98 and 100.0 µg/mL. Extracts and isolated compounds showed CC_{50} values for normal cell line, while MRC-5 was higher than those found for tumor cell lines. The results of mean cytotoxic concentrations (CC_{50}) for each extract and isolated compound are described in Table I.

TABLE I - Cytotoxic activity (CC_{50}) of *Tecoma* species extract and lignans

Extract/ Compound		CC_{50} µg mL^{-1} (µM)										
MRC-5		HeLa	IS	Hep G2	IS	T24	IS	TOV-21G	IS	MDA-MB-231	IS	
Tecoma castaneifolia	Trunk	131.30 ± 1.39	110.80 ± 1.12	1.19	25.56 ± 1.37	5.14	15.90 ± 1.61	8.26	17.51 ± 1.66	7.50	23.24 ± 1.49	5.65
	Leaves	171.60 ± 1.18	58.86 ± 1.16	2.92	48.03 ± 1.40	3.57	18.31 ± 1.32	9.37	83.40 ±1.42	2.06	> 200.00	< 0.86
Tecoma garrocha	Trunk	> 200.00	> 200.00	> 1.00	92.53 ± 1.32	> 2.16	12.96 ± 1.98	> 15.43	16.76 ± 1.47	> 11.93	53.07 ± 1.34	> 3.77
	Leaves	> 200.00	> 200.00	1.00	119.10 ± 1.27	> 1.68	27.93 ± 1.31	> 7.16	88.94 ± 1.45	> 2.25	> 200.00	1.00

TABLE I - Cytotoxic activity (CC_{50}) of *Tecoma* species extract and lignans

Extract/ Compound		CC_{50} µg mL^{-1} (µM)										
MRC-5		HeLa	IS	Hep G2	IS	T24	IS	TOV-21G	IS	MDA-MB-231	IS	
Tecoma stans var. *angustata*	Trunk	80.25 ± 1.18	56.03 ± 1.17	1.43	0.1963 ± 1.40	408.81	0.0841 ± 1.51	954.22	0.1697 ± 1.69	472.89	19.59 ± 1.49	4.10
	Leaves	144.10 ± 1.33	185.80 ± 1.50	0.78	64.41 ± 1.68	2.24	24.22 ± 5.95	4.49	140.30 ± 1.47	1.03	> 200.00	< 0.72
Tecoma stans var. *stans*	Trunk	0.4258 ± 1.30	0.5533 ± 5,69	0.77	0.1198 ± 1.96	3.55	0.0156 ± 1.96	27.30	0.1043 ± 1.56	4.08	0.2680 ± 1.15	1.59
	Leaves	> 200.00	> 200.00	1.00	62.48 ± 1.68	> 3.20	39.89 ± 1.12	> 5.01	69.28 ± 1.23	> 2.89	> 200.00	1.00
Paulownin (TST–1)		> 100.00	> 100.00	1.00	31.88 ± 1.71 (86.08 ± 1.71)	> 3.14	29.35± 1.21 (79.25 ± 1.21)	> 3.40	29.51± 1.49 (79.68 ± 1.49)	> 3.39	> 100.00	1.00
Paulownin acetate (TST–2)		> 100.00	> 100.00	1.00	34.13 ± 2.07 (82.76 ± 2.07)	> 2.93	31.31 ± 1.35 (75.92 ± 1.35)	> 3.19	28.15 ± 1.55 (68.26 ± 1.55)	> 3.55	> 100.00	1.00
Sesamin (TST–3)		> 100.00	> 100.00	1.00	73.34 ± 1.76 (206.97 ± 1.76)	> 1.36	13.01 ± 1.44 (36.72 ± 1.44)	> 7.69	94.73 ± 1.39 (267.33 ± 1.39)	> 1.06	> 100.00	1.00
Olivil (TST–4)		> 100.00	> 100.00	1.00	> 100.00	> 1.00	> 100.00	> 1.00	> 100.00	> 1.00	> 100.00	1.00
Cycloolivil (TST–5)		> 100.00	> 100.00	1.00	78.57 ± 1.65 (208.74 ± 1.65)	> 1.27	45.98 ± 118 (122.16 ± 1.18)	> 2.17	65.62 ± 2.14 (174.34 ± 2.14)	> 1.52	> 100.00	1.00
Podofilotoxin		0.1339 ± 1,49 (0.3231 ± 1.49)	0.1293 ± 1.48 (0.3120 ± 1.48)	1.04	0.0043 ± 1.47 (0.0104 ± 1.47)	31.14	0.0025 ± 1.43 (0.0062 ± 1.43)	53.56	0.0048 ± 1.75 (0.0117 ± 1.75)	27.90	0.0986 ± 1.85 (0.2379 ± 1.85)	1.36

CC_{50}: Mean cytotoxic concentration; MRC-5: normal human lung fibroblast cell; HeLa: cervix cell carcinoma; Hep G2: hepatocellular carcinoma, T24: urinary bladder cell carcinoma; TOV-21G: ovarian cell carcinoma; MDA-MB-231: breast cell carcinoma; SI (Selectivity Index): CC_{50} MRC-5/ CC_{50} Tumor cell line

The cytotoxic activity of some lignans are widely known, including podophyllotoxin, which interferes with the mitotic spindle interacting with tubulin (Gordaliza *et al*., 2004). Semisynthetic derivatives from podophyllotoxin etoposide and teniposide are potent anticancer agents with different mechanisms of action. These in relation to modified lignans stabilize the covalent intermediate formed between DNA and topoisomerase II, and are also thought to produce strand breakage by free radical production (Gordaliza *et al*., 2004; Nobili *et al*., 2009).

The presence of paulownin in extracts of Bignoniaceae species was previously reported at *Kigelia africana* (Sidjui *et al*., 2015) and *Markhamia lutea* (Ali *et al*., 2015). Paulownin (TST-1) had cytotoxic concentrations ranging from 79.25 to 86.08 µM. A study by Huang *et al*. (2013) demonstrated paulownin with antitumor activity against human chronic myelogenous leukemia (K-562) cell lines with CC_{50} of 70.6 µM and lung cancer cells (A549) with CC_{50} of 22.6 µM.

The evaluation of the paulownin acetate cytotoxicity resulted in cytotoxic concentrations between 68.29 and 82.76 µM, demonstrating that paulownin acetate has antitumor activity similar to paulownin. This lignan was found in *Gmelia arborea* (Verbenaceae) as one of the major chemical constituents (Acharya *et al*., 2015). However, in the Bignoniaceae family, the only report in scientific literature about the isolation of paulownin acetate was found in the work performed by Caetano (1983), who also isolated it from the trunk part of *T. stans* species. It has been the first report of cytotoxic activity of this compound in cell culture.

In the Bignoniaceae family, sesamin was isolated from the species *Kigelia africana* (Sidjui *et al*., 2015) and *Markhamia lutea* (Ali *et al*., 2015). In the present study, this compound had a cytotoxic concentration ranging from 36.72 to 267.33 µM. Akl *et al*. (2013) evaluated antitumor activity from sesamin against breast cancer cell lines, MCF-7 and MDA-MB-231, obtaining CC_{50} of 98.0 µM and CC_{50} of 43.9 µM, respectively. In another study, Hirano *et al*. (1994) considered sesamin to have low potency against leukemia tumor cells (HL-60 and MOLT-4), since it had CC_{50}> 0.0028 µM. Using this same parameter, sesamin can be considered with low potency evaluated against cell lines that was used in this work.

The olivil is a lignan with wide distribution between botanic families. Occurrence reports in Bignoniaceae family involving this compound can be found in *Stereospermum* species such as *S. cylindricum* (Kanchanapoom *et al*., 2006) and *S. kunthianum* (Ghogomu-Tih *et al*., 1985). Olivil biological data did not demonstrate any cytotoxic effect at the concentrations tested (CC_{50}> 100.0 µg/mL) against any tested cell lines in this present study. Previous research has also failed to detect the cytotoxic effect of these compounds against breast cancer cell line (MCF-7), lung cancer cells (A-549) and normal lung fibroblast cell line (WI-38) at concentrations below 100.0 µg/mL (Wangteeraprasert *et al*., 2012).

The fifth lignan isolated from *T. stans* var. *stans* trunk extract was identified as cycloolivil. The cycloolivil is a lignan commonly found in the family Bignoniaceae, as in species of the genus *Tabebuia*, such as *T. heptaphylla* (Schmeda-Hirschmanna, Papastergioub, 2003), and species of the genus *Stereospermum*: *S. kunthianum* (Ghogomu-Tih *et al*., 1985) and *S. cylindricum* (Kanchanapoom *et al*., 2006). Wangteeraprasert *et al*. (2012) evaluated the cytotoxic effect of cycloolivil against cell lines of breast cancer (MCF-7), lung cancer (A-549) and normal cell line of lung fibroblasts (WI-38) and an antiproliferative activity at the highest tested concentration of 100 µg/mL was not observed. In the present study, the cycloolivil showed CC_{50} of 78.57, 45.98 and 65.62 µg/mL against Hep G2, T24 and TOV-21G cell lines, respectively. Finally, cycloolivil did not demonstrate any cytotoxic effect against HeLa and MDA-MB-231 even at the highest tested concentration (100 µg/mL).

Our results reveal that the trunk of the *T. stans* var *stans* is rich in lignans and may be a source for obtaining paulownin. We can also conclude by the cytotoxicity tests results using tumor cell lines evaluating ethanolic extracts of *T. stans* var. *stans* and *T. stans* var *angustata* as potential sources of cytotoxic compounds. However, some lignans such as podophyllotoxin and its derivatives etoposide and teniposide show marked cytotoxic activity (Patrick, 2017). The lignans isolated from the ethanolic extract of *T. stans* trunks (paulownin, paulownin acetate, sesamin, olivil and cycloolivil) have only moderate cytotoxic activity when compared to the activity of ethanolic extract of origin. These data may suggest the presence of other bioactive compounds, not yet isolated from the extracts and with more cytotoxic activity. Another hypothesis can be the synergic effect promoted by a set of substances.

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