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Hexane Extract from *Tradescantia pallida* (Rose) D.R. Hunt (Commelinaceae): Its Volatile Constituents and *in vitro* Antifungal and Cytotoxic Activities

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HIGHLIGHTS

- *Tradescantia pallida* has shown promising antibacterial, antioxidant and anticancer activities.
- Botanical extract products are environmentally friendly, inexpensive and may reduce losses by discouraging pathogen growth.
- HE-TP was highly active in *in vitro* assays against *P. digitatum, S. sclerotiorum* and *R. stolonifer*.
- HE-TP demonstrated cytotoxic activity against different human tumor cell lines.
- The major compound identified in hexane extract from *T. pallida* was (*E*)-4-methoxycynnamic acid (50.2%).

Abstract: *Tradescantia pallida* (Commelinaceae) has shown promising antibacterial, antioxidant and anticancer activities. This study aimed at extracting hexane from *T. pallida* (HE-TP) aerial parts to identify and quantify its volatile compounds by GC-MS and GC-FID and at evaluating its antifungal and antiproliferative activities. (*E*)-4-Methoxycynnamic acid (50.2%), 2,5-di-tert-butyl-1,4-benzoquinone (13.7%) and epijuvabione (10.4%) were the major components identified in HE-TP. HE-TP was incorporated into PDA

medium, poured into Petri dishes and transferred to mycelial discs of pathogens. Percentages of inhibition of fungal growth were determined. HE-TP showed remarkable antifungal potential at the dose of 400 µL since it inhibited 100% of *Penicillium digitatum* and *Sclerotinia sclerotiorum* growth and 92.6% of *Rhizopus stolonifer* growth. Besides, HE-TP demonstrated cytotoxic activity against different human tumor cell lines with IC_{50} values between 231.43 and 428.76 µg/mL. Therefore, results showed that HE-TP has potential against fungi of agronomic interest and tumor cells.

Keywords: *Penicillium digitatum*; *Sclerotinia sclerotiorum*; *Rhizopus stolonifer*; (*E*)-4-Methoxycynnamic acid; alternative control; cytotoxic activity.

INTRODUCTION

Tradescantia Ruppius ex L., as currently circumscribed, is the second largest genus of the Commelinaceae family, which has 70 species. They are plants found in remarkable ecological niche worldwide, mainly in tropical and temperate regions [1-2]. Some species are *T. fluminensis* Vell*, T. sillamontana* Matuda*, T. navicularis* (Ortgies) D.R.Hunt*, T. albiflora* Nanouk*, T. zebrina* hort. Ex. Bosse. and *T. pallida* [3-4]. *T. pallida* (Rose) D.R. Hunt is well-known in Brazil (called "*taboquinha roxa"* and "*tetrapoeraba roxa*" in Brazilian Portuguese) due to its sumptuous purple leaves [1-2].

The genus *Tradescantia* has been much used as an ornament in all Brazilian states not only because it grows and propagates easily but also because it is highly resistant to climatic conditions and environmental factors [1]. Regarding its pharmacological benefits, *T. pallida* leaves have been used as dyes and have acted as anodes against rheumatism and joint pain [1]. The literature has often shown that this species is an excellent bioindicator of air pollution levels [1-2].

In addition, Menegazzo and collaborators (2020) have recently stated that *T. pallida* exhibits other biological activities, such as antimicrobial, antioxidant and anticancer ones [5]. This report deepens the issue that has been investigated by researchers worldwide, i. e., the fact that plant extracts and essential oils from different plant species exhibit antifungal activity against fungi of agronomic interest [6-7].

Considering that there is a single report of antifungal activity of aqueous extract from *T. pallida* leaves against *Fusarium solani*, *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides* in the literature [8], this study aimed at determining volatile constituents of hexane extract from *T. pallida* (HE-TP) aerial parts by gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detection (GC-FID) and its *in vitro* antifungal activity against the following three fungi of agronomic interest: *Penicillium digitatum, Sclerotinia sclerotiorum* and *Rhizopus stolonifer.* In addition, the cytotoxic activity of HE-TP against tumor cell lines was also evaluated.

MATERIAL AND METHODS

Plant material

Tradescantia pallida (Figure 1) aerial parts were collected on January 21st, 2021, at 1 pm in the *Cerrado* region, Rio Verde, Goiás (GO) state, Brazil. They were stored in paper bags, identified and preserved. Plant material was identified by the botanist Erika Amaral and deposited in the herbarium that belongs to the Instituto Federal Goiano, Campus Rio Verde, GO, exsiccate no. 231-TP. Access to the botanical material was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN) under the code AEACDCA.

Figure 1. *Tradescantia pallida (purple secretia - taboquinha roxa* and *tetrapoeraba roxa*, in Brazilian Portuguese) and its sumptuous purple leaves.

Preparation of hexane extract (HE-TP)

Aerial parts (100 g) were air-dried and milled by a Wiley mill. Subsequently, they were exhaustively cold-extracted with hexane. Each resulting extract was filtered and concentrated under reduced pressure. Then, 5.3 g crude hexane extract was obtained.

Chemical identification of HE-TP constituents

HE-TP was dissolved in ethyl ether and analyzed by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC–MS) with the use of Shimadzu QP5000 Plus and GCMS2010 Plus (Shimadzu Corporation, Kyoto, Japan) systems. The temperature of the column in GC-FID was programmed to rise from 60 to 240°C at 3°C/min and was held at 240°C for 5 min; the carrier gas was H_2 at a flow rate of 1.0 mL/min. The equipment was set to operate in the injection mode; the injection volume was 0.1 µL (split ratio of 1:10); and injector and detector temperatures were 240 and 280°C, respectively. Relative concentrations of components were obtained by normalizing peak areas (%). Relative areas consisted of the average of triplicate GC-FID analyses. GC-MS conditions and the identification have been previously reported [9]. Identification of volatile components of hexane extract from *T. pallida* (Table 1) was based on their retention indices on an Rtx-5MS (30 m X 0.25 mm; 0.250 µm) capillary column under the same operating conditions used for GC relative to a homologous series of *n*-alkanes (C_8-C_{20}). Structures were computer-matched with Wiley 7, NIST 08 and FFNSC 1.2 and their fragmentation patterns were compared with literature data [10].

Antifungal activity of HE-TP

Isolates of *Penicillium digitatum*, *Sclerotinia sclerotiorum* and *Rhizopus stolonifer* were provided by Eugenio Miranda Sperandio, Ph. D., in the Phytopathology Laboratory at the Instituto Federal Goiano - *Campus* Rio Verde. Assays were carried out in the agricultural microbiology laboratory at IF Goiano - *Campus* Rio Verde and the antifungal activity of HE-TP was evaluated in agreement with the disc-diffusion method described by Valadares and coauthors (2018) [11], with modifications. Doses of hexane extract were 20, 50, 100, 200, 300 and 400 µL for HE-TP diluted in isopropyl palmitate (100%). Negative controls and the solvent used for HE-TP dilution, isopropyl palmitate (100%), were dishes with no addition of HE-TP (control) whereas the positive control was the fungicide Frowncide 500 SC (dose of 5 µL), at 10 µg/mL of the active ingredient. Petri dishes were sterilized and prepared with PDA culture medium. After medium solidification, HE-TP, at previously mentioned doses, were added and smeared on the surface of dishes with the help of a Drigalski spatula. Afterwards, 5 mm diameter PDA medium discs with 10-day-old mycelium were placed in the center of the dishes. Then, they were incubated at 28 ± 2°C and mycelial growth was measured daily, up to full growth of the fungus on control dishes. The treatment was carried out in quadruplicate and the experimental design was thoroughly randomized. Data were submitted to the analysis of variance (ANOVA) and means of

treatments were evaluated by the Scott-Knott test at 5% significance level by the ASSISTAT software. The percentage of inhibition of mycelial growth (IMG) was calculated by the following formula [18]:

$$\boldsymbol{IMG}\ (\%) = \frac{(\boldsymbol{control\ growth - treatment\ growth})}{\boldsymbol{control\ growth}}\boldsymbol{x100} \quad (1)$$

Results of this study were analyzed by the analysis of variance and means were compared by the Tukey's test at 5% probability, with the use of the statistical software BioEstat version 5.0.

Cytotoxicity assessment

In this study, different human cell lines were used for evaluating cytotoxic activity of samples: non-tumoral fibroblasts (GM07492A), cervical adenocarcinoma (HeLa), breast adenocarcinoma (MCF-7) and glioblastoma (U-251MG). Cells were cultured in Ham's Nutrient Mixture F10 (HAM-F10) plus Dulbecco's Modified Eagle's medium (1:1; Sigma-Aldrich) supplemented with 10% fetal bovine serum (Cultilab), antibiotics (0.01-mg/mL streptomycin and 0.005-mg/mL penicillin; Sigma-Aldrich) and 2.38-mg/mL Hepes (Sigma-Aldrich), at 37°C with 5% CO2. All in vitro assays were performed on three different days to ensure reproducibility.

Evaluation of cytotoxicity was carried out by the colorimetric assay of toxicology in vitro - Kit XTT (Roche Diagnostics), according to the manufacturer's guidelines. To carry out the experiments, 1x104 cells were seeded in 96-well microplates. Samples were first dissolved in 1% dimethylsulfoxide (DMSO; Sigma-Aldrich) and then diluted in complete medium. Since concentrations under investigation were limited due to the sample solubility, they ranged from 31.25 to 1000 µg/mL. Wells for negative (untreated) and positive (cisplatin; Sigma-Aldrich) controls were included. Treatment and analysis procedures were conducted as described by Silva and coauthors (2017) [19]. A non-linear regression analysis was performed by the GraphPad Prism program in order to calculate the sample concentration that inhibits 50% of cell viability (IC50, half maximal inhibitory concentration).

RESULTS AND DISCUSSION

Volatile constituents of HE-TP aerial parts were identified by GC-FID and GC–MS. Major compounds found in HE-TP were (E)-4-Methoxycynnamic acid (50.2%), 2,5-di-tert-butyl-1,4-benzoquinone (13.7%) and epijuvabione (10.4%) (Table 1).

Table 1. Volatile constituents of hexane extract from *T. pallida* aerial parts.

RT (min)	Compound	RI_{exp}	RI_{lit}	%RA
4.71	2-Methyl- tetrahydro-2H-pyran	837	838	0.1
4.85	(*E*)-2-Hexen-1-ol	862	862	3.3
5.04	*n*-Nonane	898	899	2.2
5.21	2-Hydroxy-butanoic acid methyl ester	906	907	0.3
5.63	3,5-Dimethyl-octane	922	924	3.2
6.61	Cyclohexancarbaldehyde	961	963	1.0
6.95	2-Propenoic acid pentyl ester	975	976	0.1
7.66	*n*-Decane	999	999	0.2
10.19	5-Methyltetrahydrofuran-2-methanol	1073	1075	0.1
11.05	*n*-Undecane	1098	1099	2.4
14.59	*n*-Dodecane	1197	1199	3.5
18.89	*n*-Tridecane	1299	1299	5.3
25.19	2,5-Di-tert-butyl-1,4-benzoquinone	1465	1466	**13.7**
26.44	Pentadecane	1498	1500	0.9
37.00	(*E*)-4-Methoxycynnamic acid	1733	1733	**50.2**
37.75	Epijuvabione	2015	2017	**10.4**
	Total			96.9

RT = Retention time; **RI_{exp}** = Retention index relative to *n*-alkanes (C_8–C_{20}) on the Rtx-5MS column; **RI_{lit}** = Kovats retention index (values from the literature - [10]). **%RA** = Relative abundance

Few studies of their chemical composition can be found in the literature. A recent publication has only introduced the chemical composition of essential oil from *T. pallida* leaves; its authors identified the major constituents caryophyllene oxide (18.84%), β-caryophyllene (13.65%) and α-copaene (6.08%) [5] while two major anthocyanins were reported by Shi and coauthors (1992) [12].

Concerning biological properties, the leaf extract exerted antimicrobial activity on Gram-positive and Gram-negative bacteria and antioxidant one, as reported by Tan and coauthors (2014) [13] and Silva and coauthors (2015) [1], besides anticancer activity against cervical cancer cell lines, whose positive results were found by assays carried out with ethanolic and methanolic extracts [14]. Kamiya and coauthors (2019) showed that the aqueous leaf extract may also inhibit *Pseudomonas aeruginosa* growth [15].

'Studies and reassure the biotechnological potential of the species *T. pallida*. Figures 2-4 show the high antifungal activity of HE-TP against *Penicillium digitatum, Sclerotinia sclerotiorum* and *Rhizopus stolonifer.*

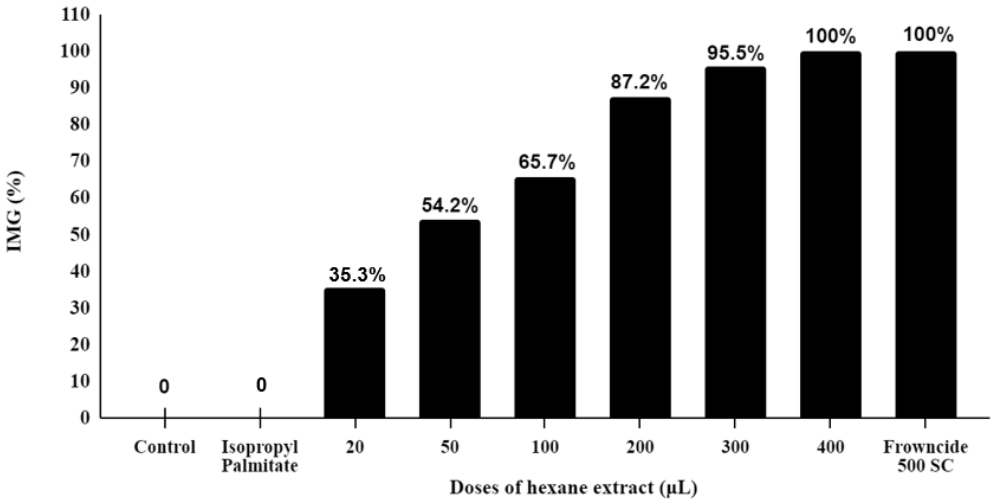

Figure 2. Percentages of inhibition of mycelial growth of *Penicillium digitatum* at different HE-TP doses.

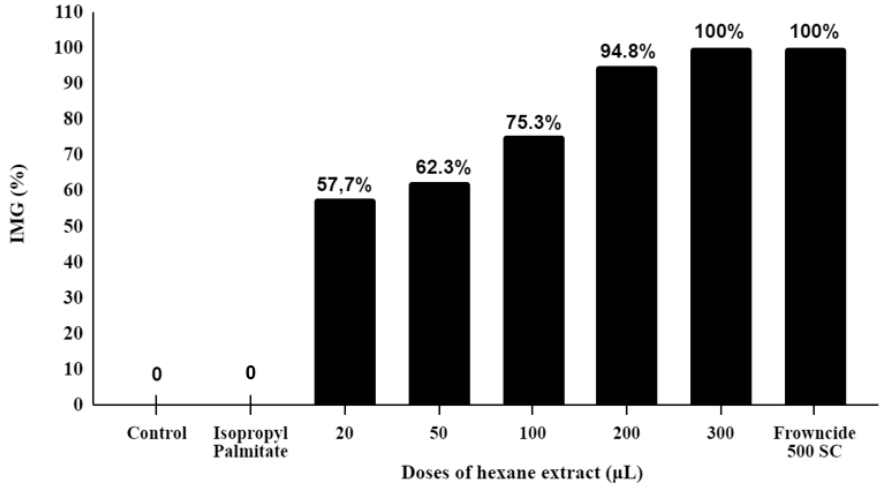

Figure 3. Percentages of inhibition of mycelial growth of *Sclerotinia sclerotiorum* at different HE-TP doses.

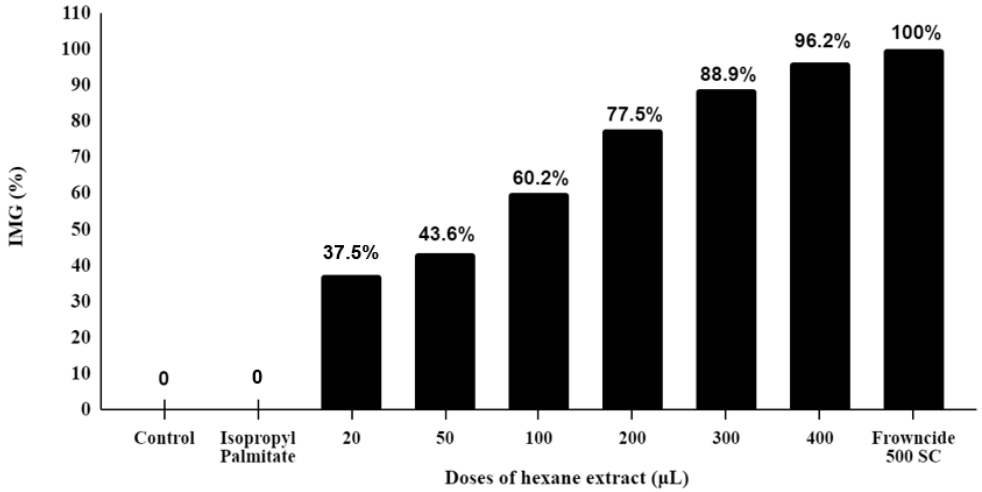

Figure 4. Percentages of inhibition of mycelial growth of *Rhizopus stolonifer* at different HE-TP doses

The dose of 400 µL was enough to inhibit 100% and 92.6% of *P. digitatum* and *R. stolonifer* growth, respectively. It should be highlighted that the dose of 300 µL inhibited 100% of *S. sclerotiorum* mycelial growth. It is also worth mentioning that the solvent isopropyl palmitate, which was used for solubilizing HE-TP, did not exhibit any antifungal activity when it was evaluated separately.

The literature has confirmed that isopropyl palmitate, which has been widely used in pharmaceutical formulations, is not toxic [16]. In short, satisfactory results found by this study – and described by this paper – add new and relevant information to the literature. They corroborate data which was previously published by de Lima and coauthors (2019) [8], who discovered that aqueous extract from *T. pallida* fresh leaves has promising potential against three phytopathogenic fungi.

In addition, the authors of this study believe that the good antifungal activity exhibited by HE-TP may be related to (*E*)-4-methoxycynnamic acid (50.2%; Table 1; Figure 5), a derivative of cinnamic acid and the major constituent found in HE-TP. This acid and its derivatives have been known in the literature due to their antifungal activity [17].

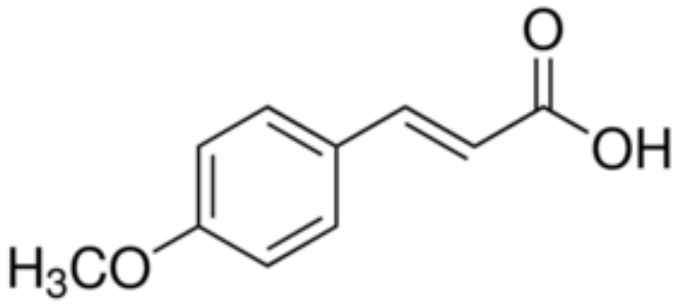

Figure 5. Chemical structure of (*E*)-4-methoxycynnamic acid.

HE-TP was also evaluated regarding its cytotoxic activity against different human tumor cell lines. Results shown in Table 2 indicate that HE-TP reduced cell viability of all cell lines under evaluation after 24 h of treatment but without any selectivity. IC_{50} values were 244.83, 231.43, 428.76 and 207.46 µg/mL for HeLa, MCF-7, U-251MG and GM07492A cell lines, respectively.

Cytotoxic activity of HE-TP may be due to the high content of (*E*)-4-methoxycynnamic acid, one of its major constituents (50.2%). In recent years, (*E*)-4-methoxycynnamic acid have demonstrated high potential as cytotoxic and anticancer agents [20-22]. Results found by Gunasekaran and coauthors (2015) suggest that cytotoxicity induced by (*E*)-4-methoxycinnamic acid in human colon adenocarcinoma cell line (HTC-116) can be attributed to the intrinsic mitochondrial apoptosis pathway mediated through DNA damage and high levels of reactive oxygen species [20].

Table 2. IC_{50} values (µg/mL) found against different human cell lines after 24 h of treatment at different HE-TP concentrations (31.25 to 1000 µg/mL)

Treatments	Cell lines			
	GM07492A	**HeLa**	**MCF-7**	**U-251MG**
HE-TP	207.46 ± 15.56	244.83 ± 16.65	231.43 ± 7.57	428.76 ± 0.05
Positive control	41.99 ± 1.33	69.26 ± 1.94	54.16 ± 0.42	102.03 ± 8.05

IC_{50} - sample concentration that inhibits 50% of cell viability; **GM07492A** - non-tumoral fibroblasts; **HeLa** - cervical adenocarcinoma; **MCF-7** - breast adenocarcinoma; **U-251MG** – glioblastoma; **HE-TP** - hexane from *T. pallida* aerial parts; **Positive control** – cisplatin.

CONCLUSION

This study showed the biotechnological potential of HE-TP against three fungi which cause losses to agriculture and, consequently, negative impact on economy. HE-TP was highly active in *in vitro* assays against *P. digitatum, S. sclerotiorum* and *R. stolonifer*. At the dose of 400 µL, it inhibited 100% of *P. digitatum* and *S. sclerotiorum* mycelial growth, besides 96.2% of *R. stolonifer* mycelial growth. On the other hand, it should be highlighted that, at low concentrations, such as 100 µL, inhibition of all fungi under evaluation was above 50%. In addition, HE-TP was able to exert cytotoxic activity against human tumor cell lines. The volatile composition of HE-TP, determined by GC-MS and GC-FID, and its antifungal activity were determined for the first time. Botanical extract products are environmentally friendly, inexpensive and may reduce losses by discouraging pathogen growth. It is notable that plant extracts contain active compounds that inhibit growth of plant pathogens. In short, isolation of bioactive constituents of HE-TP, chemical studies of extracts with high polarity and evaluation of other *in vitro* and *in vivo* biological activities are needed.

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Conflicts of Interest: The authors declare no conflict of interest. Funders had no role in the design of the study; in the collection, analyses and interpretation of data; in the writing of the manuscript, nor in the decision to publish the results.

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