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A COMPARATIVE ANTIMICROBIAL ACTIVITY OF THE *Schinus terebinthifolius* OBTAINED BY MICROPROPAGATION AND OUTDOOR GROWN

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ABSTRACT: *Schinus terebinthifolius* is widely used in popular medicine, especially as an anti- inflammatory, an antiseptic and an antimicrobial, due to that, it is commonly chosen in biological, clinical, pharmacological and chemical studies. The aim of this work was to evaluate the antimicrobial activity in of the *S. terebinthifolius* extracts obtained from outdoor-grown specimen and micropropagated ones in aseptic conditions (*in vitro*) and to study the influence of entophytic fungi in the productions of plant metabolites having antimicrobial activity by plant. Micropropagated extracts and outdoor-grown *S. terebinthifolius* plants were evaluated for their antibacterial and antifungal activities tested against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* using the agar diffusion and the microdilution methods. Phytochemical compounds were determined using HPLC. The extracts from micro propagated and outdoor-grown plants showed good antibacterial activity against the tested bacterial strains. The extracts from micropropagated plants showed the best antibacterial activity with a minimum inhibitory concentration (MIC) of 0.2 mg/mL against *S. aureus*. The results form a good basis for the use of *S. terebinthifolius* micropropagated plants as a complement to the use of outdoor-grown plants in traditional medicine, moreover this study has concluded that the endophytic fungi seem not to stimulate the plant production of phenolic compounds against the pathogenic strains used in this study

Key words: *Schinus terebinthifolius*; Endophytic fungi; Micropropagated plant.

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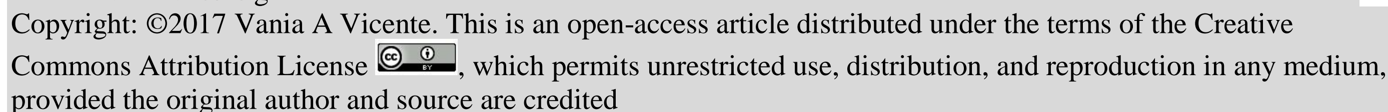

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INTRODUCTION

Schinus terebinthifolius Radii (1820) is a perennial tree of the Anacardiaceae family, native from South America, commonly known as Brazilian pepper tree, it presents a wide usage in the popular medicine and it is largely used in biological, clinical, pharmacological and chemical studies [1,2]. In addition its recognized popularity has favored the increase of its use in clinical trials and pharmacological and chemical studies [3]. The Brazilian pepper tree as the plants in general presents colonization by endophytics microorganisms [4,5] which inhabit the plant interior, for at least, one period of their life cycle without causing damage to their host [6,7]. Studies have shown that endophytic fungi are ubiquitous in plant species and they are mutualistic to their host [8]. Evidences of plant associated–microorganisms have been discovered in fossilized tissues of stems and leaves [9]. According to Damour et al. [8] the endophyte-host plant symbioses represent a broad continuum of interactions which it is possible to imagine that some of these endophytic microbes may have devised genetic systems allowing for the transfer of information between themselves and the higher plant and vice versa.

The fungi may secrete bioactive metabolites into the plant and they also may be able to transform the main plant metabolites, both of these produced metabolites may be of pharmaceutical interest [9]. When present, the endophytes fungi can influence the stability of the bioactive metabolites and change the therapeutic value of the plant material. This evidence is a neglected field of phytochemical studies [10]. Several metabolites have shown to biosynthesized and secreted products from the plant association with endophytes microorganism, these metabolites are a promising source of novel pharmaceutical molecules [11]. However, the relationship amidst endophyte fungi and the metabolites production by *S. terebinthifolius* have not yet still been elucidated through any specific study. It may be possible that the presence of endophytic microorganisms associated to plants could be related to the antibacterial and antifungal potential of the *S. terebinthifolius* [12]. In the study conducted by Gundidza and collaborators (2009) was observed chemical and antibacterial profile differences among extracts of *S. terebinthifolius* originally from Zimbabwe, South Africa and those from Brazil [13]. The *in vitro* culture represents an important tool to analyze the plant extracts activity produced and to elucidate the endophytic fungi influence in plant metabolites production with antimicrobial activity. In particular, controlled environmental conditions allow bioactive compounds to be extracted throughout the year, with no seasonal constraints [14]. Therefore, the aim of this work was to evaluate the antimicrobial activity of the extracts of *S. terebinthifolius* obtained by micro propagation in aseptic conditions (*in vitro*) and also from specimen collected in the wild.

MATERIALS AND METHODS

General experimental procedures

The analytical HPLC system consisted of a Waters liquid chromatograph model 600 equipped with a photodiode array detector (model PDA2998) and X-Terra RP-18 column (5 μm, 4.6 mm × 250 mm, Waters). For vacuum liquid chromatography, stationary phase of silica gel 60 (Merck, 40-63 m) and florisil (Merck, 60-100 Mesh) were used. The gallic acid methyl ester, gallic acid ethyl ester, gallic acid, quercetin and catechin were isolated from *S. terebinthifolius* [15] which were kindly provided for this study.

Botanical specimens

Schinus terebinthifolius leaves and seeds were collected from a sample of latitude 26.827S 25th, 49th 13.997^{O} longitude and altitude of 940 m, located in the campus of the Federal University of Parana, Brazil (UFPR). The botanical identification was performed in the Herbarium from the Botany Department at the UFPR (UPCB/UFPR) and voucher of specimen has been deposited at the Herbarium under the registration UPCB-30848. *Schinus* terebinthifolius nodal segments were collected from 6 month- old plants grown in a greenhouse at UFPR, Parana, Brazil. The segments were surface sterilized with NaOCl 2% and ethanol 70%. After sterilization 1 cm explants containing one or two buds were inoculated on a medium containing Woody Plant Medium, WPM, [16] salts supplemented with 20 g/L sucrose, 0.1 g inositol, 4.4 μM BAP, 0.1 μM NAA and 7 g/L agar. One month after inoculation, the necrotic and contaminated explants were discarded and the healthy explants were sub cultured on fresh medium containing WPM salts supplemented with 20 g/L sucrose, 0.1 g inositol, 4.4 μM BAP, 0.1 μM Ana, 0.05 g/L activated charcoal and 7 g/L agar, pH 5.8 were collected for analysis.

Associated-Plant Endophytic Fungi

The *S. terebinthifolius* leaves were collected from the wild and *in vitro* cultures plant. Isolation of the endophytic fungi was performed based on the procedures described by Petrini (1991). Stock cultures were maintained on slants of 2% malt extract agar (MEA) and Potato Dextrose Agar (PDA) at 24°C. For morphological studies, PDA slide cultures were prepared and mounted in aniline blue [7]. The cultures are permanently stored in the fungal collection of the Microbiology Laboratory (LabMicro-UFPR), Parana, Brazil.

Plant Extracts

The *S. terebinthifolius* leaves were collected from the outdoor-grown and *in vitro* samples. The extracts were obtained according to the method described by Harbone [17], the dried powdered leaves (300 g) of outdoor-grown *S. terebinthifolius* were extracted with petroleum ether (3 × 1 L), after with MeOH (3 × 1 L) in a room temperature and later they were filtered. The combined extracts were concentrated under reduced pressure. A portion of the MeOH extract (2 g) of the plant collected in the wild was subjected to vacuum liquid chomatography on silica gel and florisil mixture (1:1) eluted with petroleum ether : EtOAc 1:1, EtOAc 100%, EtOAc : MeOH 1:1, MeOH 100%, resulting fractions of 300 mL each, concentrated under reduced pressure. The leaves of *S. terebinthifolius* grown *in vitro* (100 g) were extracted with methanol (MeOH): 3x 500 mL, in a room temperature and later they were filtered and concentrated. The methanol extracts of both plants and the fractions (F-A – F-D) were analyzed by analytical HPLC with gradient of MeOH: H_2O (5:95 to 100:0). The flow rate was 0.8 mL / min at 30ºC and the injection volume was 10 µL.

Agar diffusion assay and minimum inhibitory concentration (MIC) evaluations

The antimicrobial activities of *S. terebinthifolius* were determined using agar disk diffusion method [18]. The four reference strains, *Staphylococcus aureus* (ATCC 27213), *Escherichia coli* (ATCC 35219), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231) were cultured in the dishes (106 UFC/mL) and wells were topped up with 50 µL of extracts in DMSO (Dimetil Sulfoxide). Controls included 0,01 mg/mL of chloramphenicol (CO378, ≥ 98% [TLC]; Sigma) for bacteria and 0.02 mg/mL of nystatin (N6261, ≥ 98%; Sigma) for yeast, and also 0.01 mg/mL of dimethyl sulfoxide (DMSO, Sigma). After 24 h of incubation at 37°C, the diameters of the inhibition zones were measured and compared. The presence or absence of inhibition zones was used as a criterion for the definition of active or inactive extracts. Tests were performed in triplicate. The technique described by M27-A3 CLSI manual was adapted using a solution of 2 mg/mL of the extracts that was prepared using DMSO as the solvent. One hundred microliters of MHB were added to each well of a 96-well plate. A volume of 100 µL of the test solution was added to the wells in the first row and then, a serial dilution was performed until the ninth row, resulting in concentrations that varied from 0.1 mg/mL to 1 mg/mL. The 10th, 11th, and 12th rows were used as inoculum, drug, and medium controls, respectively. Thereafter, 5 mL of the previously standardized microorganism (10^7 UFC/mL) suspension was added to all of the wells and the plate was incubated at 37°C for 18–24 h [19]. The resulting turbidity was observed, and after 24 hours the MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 595 nm with an Epoch Microplate Spectrophotometer.

Statistical analysis

The statistical analysis was performed using the ASSISTAT statistical software version 7.6 beta. Data was estimated by analysis of variance (ANOVA). For multiple comparisons, Tukey correction was performed. Quadruplicates were used in each of the essays. The level of significance set at 5% was used to perform the analysis [20].

RESULTS

Associated-Plant Endophytic Fungi

A total of 133 endophytic fungi was isolated from outdoor-grown *S. terebinthifolius* leaves. In this study, several isolates endophytics fungi were indentified from different species *Diaporthe sp* (n=23), *Phyllosticta capitalensis* (n=24), Pestalotiopsis sp (n=19), *Fusaruim* sp (n=10), *Eppicoccum nigrum* (n=8), *Xylaria* sp (n=6), Alternaria sp (n=16), *Colletotrichum* sp (n=12) e *Cryptococcus* sp (n=15). In contrast, we did not obtain endophytics isolates from the *in vitro* cultures plant leaves (Figure 1).

Figure-1: Endophytic fungi isolated from outdoor-grown and micropropagated samples of *S. terebinthifolius*.

A-outdoor-grown *S. terebinthifolius*; B- Isolation of endophytic fungi from outdoor-grown *S. terebinthifolius*; C–*in vitro S. terebinthifolius*; D- Isolation of endophytic fungi from *in vitro S. terebinthifolius*.

Plant

The *S. terebinthifolius´* methanol extracts from leaves of the outdoor-grown and *in vitro* cultures plant evaluated by diffusion method in wells showed antifungal and antibacterial activity (Table 1). These results suggested the presence of compounds with antimicrobial activity which was elucidated by Minimum inhibitory concentrations (MIC) of extracts for antibacterial and antifungal activity (Table 2). Only MIC values less than 1 mg/mL were considered sufficiently active for the crude extracts analyzed. The best antibacterial activity was shown by the methanol extracts of micropropagated plants with an MIC value of 0.2 mg/mL compared to 0.4 mg/mL from the extracts of the outdoor grown plants against *Staphylococcus aureus*, of 0.5 mg/mL to 0.8 mg/mL against Escherichia coli and an MIC value of 0.7 mg/mL compared to 0.9 mg/mL against *Pseudomonas aeruginosa*. No antifungical activity was observed against Candida albicans with MIC value used.

Table 1: Antibacterial and antifungal activity of *Schinus terebinthifolius* extracts. Mean diameters (mm) of inhibition zones.

Sample	**Extract**	**Antimicrobial Inhibition halo diameter (mm)**			
	Fraction	***Ec***	***Sa***	***Pa***	***Ca***
	Crude	22.66^{a}	30.33^{a}	15.66^{d}	21.33^{ab}
Schinus terebinthifolius	Fraction A	0.00^{c}	0.00^{d}	0.00^{f}	0.00^{d}
(Outdoor grown)	Fraction B	11.66^{b}	0.00^{d}	7.66^{e}	0.00^{d}
	Fraction C	26.33^{a}	20.33^{b}	38.33^{a}	20.66^{b}
	Fraction D	13.66^{b}	13.33^{c}	19.66^{c}	12.6^{c}
***Schinus terebinthifolius* (micropropagated)**	Crude	24.33^{a}	32.66^{a}	30.00^{b}	23.66^{a}
Chloramphenicol*		12.35	15.70	12.00	
Nystatin**					**13.24**

Ec., *Escherichia coli* (ATCC 35219); Sa, *Staphylococcus aureus* (ATCC 27213); Pa., *Pseudomonas aeruginosa* (ATCC 27853); Ca., *Candida albicans* (ATCC 10231). Inhibition values followed by the same lowercase are statistically equal considering Tukey test at 5% probability. Fraction A: petroleum ether: EtOAc 1:1; Fraction B EtOAc 100%; Fraction C EtOAc: MeOH 1:1; Fraction D: MeOH 100%. *Positive control for the antibacterial essay; **Positive control for the antifungal essay.

The leaves extracts obtained from leaves of outdoor-grown and *in vitro* plant of *S. terebinthifolius* were obtained using petroleum ether and methanol (MeOH). A portion of the MeOH extract of outdoor-grown plant was subjected to vacuum liquid chromatography and eluted under reduced pressure to give four fractions: petroleum ether : EtOAc 1:1 (Fraction A: 0,34 g), EtOAc 100% (Fraction B: 0,04 g), EtOAc : MeOH 1:1(Fraction C: 3,2 g), MeOH 100% (Fraction D: 0.84 g). Only the MeOH extract from outdoor-grown plant was fractionated due to the reduced amount of crude extracts of micropropagated plants.

Table-2: Minimum Inhibitory Concentration (MIC-mg/mL) of *Schinus terebinthifolius*.

Sample	Extract/	Antimicrobial microbial active MIC (mg/mL)			
	Fraction	***Ec***	***As***	***Pa***	***Ca***
Schinus terebinthifolius	Crude	0.8	0.4	0.9	
(Outdoor grown)	Fraction A	-	-	-	-
	Fraction B	-	-	-	-
	Fraction C	0.5	0.6	0.6	-
	Fraction D	-	0.4	-	-
Schinus Terebinthifolius **(micropropagated)**	Crude	**0.5**	**0.2**	**0.7**	-

MIC, minimum inhibitory concentration; Ec., *Escherichia coli* (ATCC 35219); Sa., *Staphylococcus aureus* (ATCC 27213); Pa., *Pseudomonas aeruginosa* (ATCC 27853); Ca., *Candida albicans* (ATCC 10231). Fraction A: petroleum ether: EtOAc 1:1; Fraction B EtOAc 100%; Fraction C EtOAc: MeOH 1:1; Fraction D: MeOH 100%.

According to the antimicrobial test of the fractions showed that -fraction D was more active (Tables 1 and 2). In addition, we provided the HPLC profile from crude extracts and fractions from outdoor-grown and micro propagated *S. terebinthifolius*. Considering as standards the five compounds (gallic acid methyl ester, gallic acid ethyl ester, gallic acid, quercetin and catechin) previoulsly isolated from *S. terebinthifolius* [15], it was possible to identify the gallic acid methyl esters as main component of the crude extract from micropropagated plant, the fraction C and the fraction D of the outdoor-grown *S. terebinthifolius* The identification this compound was done considering retention time-tR (tr =26 min) (Figure 2), and identical UV spectra with the standard (Figure 3). The others standards were not identified in the extracts and fractions A-B.

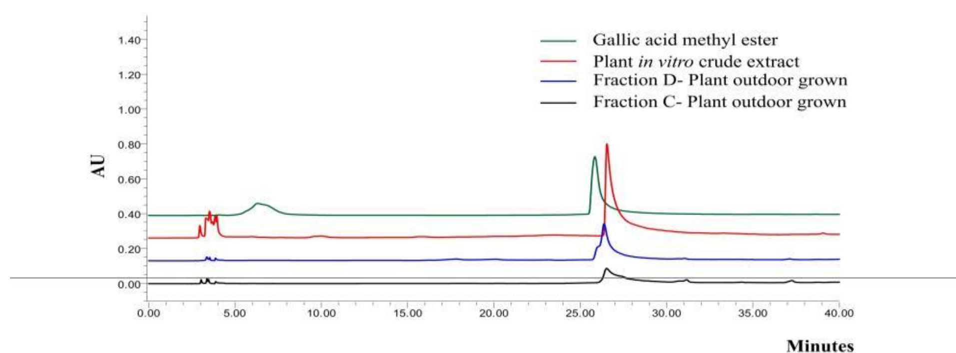

Figure-2: HPLC–DAD chromatograms of extracts from *S. terebinthifolius* plants obtained through outdoor and *in vitro* cultivation. Detection at 320 nm.

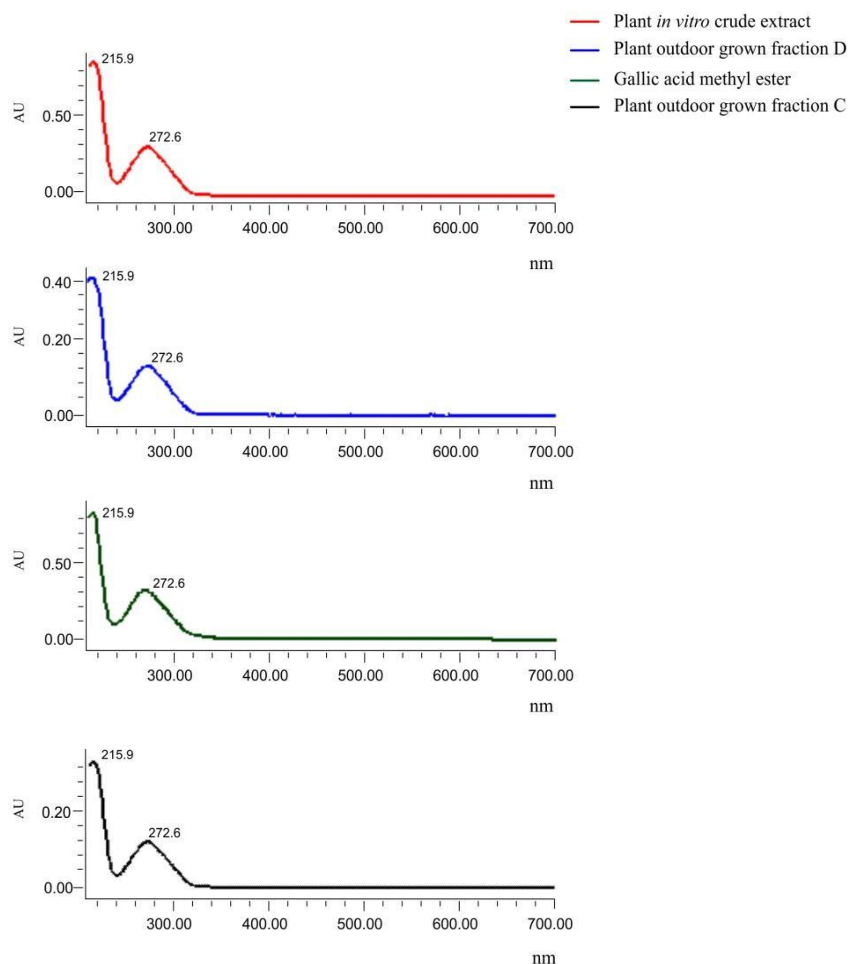

Figure 3: UV spectra at 26 min. of chromatograms in Figure 2 of extracts from S. terebinthifolius plants obtained through outdoor grown *in vitro* plants.

DISCUSSION

Biological activities from the *S. terebinthifolius* extracts, such as anti-inflammatory and antimicrobial were subjects of several studies [15,21-24]. However, this is the first report of antimicrobial activity in micropropagated plants of *S. terebinthifolius*. The potential use of micropropagated plants has been discussed regarding to the *Tulbaghia violacea* [25] and *Harpagophytum procumbens* species [26].

In our study a total of 133 endophytic fungi were isolated from leaves of *S. terebinthifolius* wild. Outdoor grown plants have several mutualistic relationships with microorganisms [27] such as endophytic fungi which have the potential to synthesize various bioactive metabolites that may directly or indirectly be used as therapeutic agents against numerous diseases [27]. According to the Plant-endophyt e Coevolution Hypothesis [28] this interaction might be possible due to the endophytes capacity of improving the plant's chemical defense by producing bioactive secondary metabolites [25]. Considering this, it is plausible that various so-called ''plant metabolites'' could be the biosynthetic products of endophytes [29].

The results of this study showed that both extracts produced by outdoor grown and micropropagated plants presented antimicrobial activity. The literature shows that antimicrobial activity of the essential oil and of the leaves extracts from various species of *Schinus* mainly *S. terebinthifolius* have demonstrated antibacterial activity *in vitro* against several clinical strains of bacteria [23, 30]. The crude extracts from micropropagated plants were more effective at lower concentrations than the ones from outdoor grown plants showing activity against *S. aureus* with a total mean of 0.2 mg/mL compared to 0.4 mg/mL from the outdoor-grown plants. According to the literature the production of secondary metabolites is influenced by environmental and physiological conditions [25,31].

In addition, Gundidza et al. observed differences in the chemical and antibacterial profile of *S. terebinthifolius* extracts [13]. Secondary metabolism could respond to oxidative stress and to production of free radicals, leading to the accumulation of different compounds in samples grown *in vitro* or outdoor. In fact, vegetative parts from outdoor samples and *in vitro* clones could present different profiles of phenolic compounds, which is not only related to genetic factors but also to environmental growth conditions [31]. The antimicrobial activity of the *S. terebinthifolius* has been related to phenolic compounds [32, 33].

According to Ncube and Ref. [25] the conditions are more favorable for phenolic compound production in micropropagated than in outdoor grown plants. In this study, the fraction D (ethyl acetate: methanol) from outdoor-grown plant and the crude extract of the micropropagated plants showed activity against pathogenic strains tested (Tables 1 and 2). However, the extracts were analyzed by HPLC and showed the presence of methyl gallate as the main compound including the crude extract from micropropagated plant. Methyl gallate is also known by its potential of growth-inhibiting activity against *Escherichia coli*, *Campylobacter jejuni*, *S. aureus* and *Shigella flexneri* [34, 35]. Moreover, it was not detected the presence of methyl gallate in the crude extracts from outdoor- grown plants, furthermore the extracts activity against evaluated microorganisms was limited. The compounds are expected to display higher antibacterial activity in the fractions than in the crude extracts, due to their higher concentration in the fractions [35]. High concentrations of the compounds in micropropagated plants may be related to the *in vitro* growing conditions that can promote the production of these compounds.

The biosynthesis of plant secondary metabolites depends on exogenous factors such as the endophytic fungi [8]. Studies have demonstrated that endophytic fungi are able to produce secondary metabolites which are also biosynthesized by their host plants. These include, for example, antineoplastic paclitaxel [36], camptothecin and its structural analogs [37], the anticancer drug lead compounds podophyllotoxin [38], deoxypodophyllotoxin [39], the antidepressant hypericin along with emodin [40], and the natural insecticides azadirachtin A and B [9]. This study aimed to investigate the role of endophytes fungi in the production of secondary metabolites with antimicrobial activity in *S. terebinthifolius*. It was observed that crude extracts of micropropagated plants showed a higher antimicrobial activity than outdoor-grown plants. The chemical characterization revealed the presence of phenolic compounds that seem to be the main compound responsible for this activity. This result indicated that the plant with endophytes microorganisms presents a different profile of the antimicrobial activity which suggested that the endophytic community acts as regulatory mechanism in the plant. Current researches are focusing in understanding the basis of biochemical and molecular production of similar compounds by endophytes, concomitant with their associated plants, although the endophytes role and their complex interactions with other plants are mostly overlooked [8].

CONCLUSION

This is a pioneer work considering the influence of endophytic microorganisms in the production of secondary metabolites by the *S. terebinthifolius*. The relationship of endophytic in compounds production by their host plant is poorly known. In this study, it was concluded that the endophytic fungi seems not to stimulate the production of phenolic compounds by plant with activity against pathogenic strains used. Furthermore, it was observed that micropropagated plants presented antimicrobial activity at lower concentrations extracts than *S. terebinthifolius* extracts collected from wild environment. Considering this study, the use of *in vitro* plants of *S. terebinthifolius* could represent an alternative to large-scale production of this secondary metabolite. However, it is necessary to investigate for better understanding the relationship between endophytic microorganisms and the activity of *S. terebinthifolius* metabolites.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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REFERENCES

[1] Farizel L, Gaglianone MC 2008. Biologia floral e fenologia reprodutiva de Schinus terebinthifolius Raddi (Anacardiaceae) em Restinga do Norte Fluminense. Acta bot. Brás, 22: 828-833.

[2] Pratti DL, Ramos AC, Scherer R, Cruz ZM, Silva AG 2015. Mechanistic basis for morphological damage induced by essential oil from Brazilian pepper tree, Schinus terebinthifolius, on larvae of Stegomyia aegypti, the dengue vector. Parasit Vectors, 8: 1-10.

[3] Mukherjee A, Williams DA, Wheeler GS, Cuda JP, Pal S, Overholt WA 2012. Brazilian (*Schinus terebinthifolius*) in Florida and South America: evidence of a possible niche shift driven by hybridization. Biol. Invasions, 14: 1415-1430.

[4] Gomes RR, Glienke C, Videira SIR, Lombard L, Groenewald JZ, Crous PW 2013. Diaporthe : a genus Of endophytic, saprobic and plant pathogenic fungi. Persoonia, 31: 1-4.

[5] Lima JS, Figueiredo JG, Gomes RG, Stringari D, Goulin EH, Adamoski D, Kava-Cordeiro V, Galli-Terasawa LV, Glienke C 2012. Genetic Diversity of *Colletotrichum* spp. an Endophytic Fungi in a MedicinalPlant, Brazilian Pepper Tree. Microbiology, 2012: 1-7.

[6] Carroll G 1988. Fungal Endophytes in Stems and Leaves : From Latent Pathogen to Mutualistic Symbiont 1988. Ecol Soc, 69: 2-9.

[7] Petrini O 1991. Fungal endophytes of tree leaves. In: ANDREWS, J. H.; HIRANO, S. S. Microbial Ecology of Leaves. Spring-Verlag, p: 179-197

[8] Damour H, Okoye FBC, Proksch P, Hakiki A, Mosaddak M, Hegazy MF, Debbab A 2015. Pretrichodermamide A and Nafuredin from *Trichoderma* sp, an endophyte of *Col nitida*. Journal of Material and Environmental Sciences. 6: 779-783.

[9] Kusari S, Hertweck C, Spiteller M 2012. Perspective Chemical Ecology of Endophytic Fungi : Origins of Secondary Metabolites. Chemistry & Biology, 19: 792-798.

[10] Gonda S, Kiss A, Emri T, Batta G, Vasas G 2013. Phytochemistry Filamentous fungi from *Plantago lanceolata* L. leaves : Contribution to the pattern and stability of bioactive metabolites. Phytochemistry, 86: 127-136.

[11] Gutierrez RMP, Gonzalez AMN, Ramirez AM 2012. Compounds Derived from Endophytes : A Review of Phytochemistry and Pharmacology. Med chem, 19: 2992-3030.

[12] Agrawal AA 2007. Macroevolution of plant defense strategies. Trends. Ecol. Evol, 22: 103-109.

[13] Gundidza M, Gweru N, Magwa ML, Mmbengwa V, Samie A 2009. The chemical composition and biological activities of essential oil from the fresh leaves of Schinus terebinthifolius from Zimbabwe. Afr J Biotechnol, 24: 7164-7169.

[14] Dias MI, Barros L, Sousa MJ, Ferreira ICFR 2011. Comparative study of lipophilic and hydrophilic antioxidants from in vivo and in vitro grown Coriandrum sativum. Plant Food Hum. Nutr 66: 181-186.

[15] Santana JS, Sartorelli P, Lago JHG 2012. Isolamento e avaliação do potencial citotóxico de derivados fenólicos de Schinus terebinthifolius Raddi (Anacardiaceae). Quim Nova, 35: 2245-2248.

[16] Lloyd G, Mccown B 1981. Commercially feasible micropropagation of montain laurel, Kalmia latifolia, by use of shoot tip culture. Com. Proc. Int. Plant Prop. Soc, 30: 421-327.

[17] Harborne JB 1998. Phytochemical methods A guide to modern techniques of plant analysis. 3rd edn. London.

[18] Ncube NS, Afolayan AJ, Okoh AI 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol, 7: 1797-1806.

[19] Manual Clinical and Laboratory Standards Institute – CLSI 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition; CLSI document M27-A3.

[20] Silva FA Z, Azevedo CAV 2009. Principal components analysis in the software assistat-statistical attendance. World congress on computers in agriculture, 7, American Society of Agricultural and Biological Engineers, Reno, NV, USA.

[21] Barbieri DS, Tonial F, Lopez PV, Sales Maia BH, Santos GD, Ribas MO, Glienke C, Vicente VA 2014. Antiadherent activity of *Schinus terebinthifolius* and *Croton urucurana* extracts on in vitro biofilm formation of Candida albicans and Streptococcus mutans. Arch Oral Biol, 59: 887-96.

[22] Johann S, Cisalpino OS, Watanabe GA, Cota BB, Siqueira EP, Pizzolati MG, Zani CL, Rezende MA 2010. Antifungal activity of extracts of some plants used in Brazilian traditional medicine against pathogenic fungus *Paracoccidioides brasiliensis*. Pharm. Biol, 48: 388-396.

[23] Lima MRF, Luna JS, Santos AF, Andrade MCC, Sant'An AEG, Genet JP, Márquez B, Neuville L, Moreau N 2006. Anti-bacterial activity of some Brazilian medicinal plants. J Ethnopharmacol, 105: 137-140.
[24] Schmourlo G, Mendonça-Filho RR, Alviano CS, Costa SS 2005. Screening of antifungal agents using ethanol precipitation and bioautography of medicinal and food plants. J Ethnopharmacol. 96: 563-568.
[25] Ncube B, Ngunge VNP, Finnie JF, Van Staden J 2011 A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. Plants. J Ethnopharmacol, 134: 775-780.
[26] Bairu MW, Novák O, Doležal K, Van Staden J 2011. Changes in endogenous cytokinin profiles in micropropagated Harpagophytum procumbens in relation to shoot-tip necrosis and cytokinin treatments. Plant Growth Regul, 63: 105-114.
[27] Kusari S, Singh S, Jáyabaskaran C 2011. Biotechnological potential of plant-associated endophytic fungi: hope versus hype. Trends in Biotechnology, 32: 297-303.
[28] Ji HF, Li XJ, Zhang HY 2009. Natural products and drug discovery: Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia?EMBO Reports, 10: 194-200.
[29] Kusari S, Lamshöft M, Zühlke S, Spiteller M 2008. An endophytic fungus from Hypericum perforatum that produces hypericin. J. Nat. Prod, 71: 159-162.
[30] Gehrke IT, Neto AT, Pedroso M, Mostardeiro CP, Da Cruz IB, Silva UF, Ilha V, Dalcol II, Morel AFJ 2013. Antimicrobial activity of *Schinuslentiscifolius* (Anacardiaceae). J Ethnopharmacol, 148: 486-91.
[31] Barros L, Duenas M, Dias MI, Sousa MJ, Santos-Buelga C, Ferreira ICFR 2012. Phenolic profiles of in vivo and in vitro grown *Coriandrum sativum* L. Food Chemistry, 132: 841-848.
[32] Ceruks M, Romoff P, Fávero AO, Lago JHG 2007. Constituintes fenólicos polares de Schinus terebentifolius Raddi (Anacardiaceae). Quim Nova, 30: 507-599.
[33] Queires LCS, Rodrigues LEA 1998. Quantificacao das substâncias fenólicas totais em órgãos da aroeira *Schinus terebinthifolius* (Raddi). Braz Arch Biol Technol, 41: 247-253.
[34] Cho JG, Kang OH, Lee YS, Oh YC, Chae HS, Jang HJ, Shin DW, Kwon DY 2009. Antibacterial activity of methyl gallate isolated from Galla Rhois or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. Molecules, 14: 1773-1780.
[35] Madikizela MA, Aderogba J, Van Staden J 2013. Isolation and characterization of antimicrobial constituents of Searsia chirindensis L. (Anacardiaceae) leaf extracts. J Ethnopharmacol, 150: 609-613
[36] Stierle A, Strobel GA, Stierle D 1993. Taxol and taxane production by *Taxomyces*. Science, 260: 214-216.
[37] Kusari S, Zühlke S, Spiteller M 2009. An endophytic fungus from *Camptotheca acuminata* that produces camptothecin and analogues. J. Nat. Prod, 72: 2-7.
[38] Puri SC, Nazir A, Chawla R, Arora R, Riyaz-Ul-Hasan S, Amna T, Ahmed B, Verma V, Singh S, Sagar R, Sharma A, Kumar R, Sharma RK, Qazi GN 2006. The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol, 122: 494-510.
[39] Kusari S, Lamsho M, Spiteller M 2009. *Aspergillus fumigates* Fresenius, an endophytic fungus from Juniperus communis L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J. Appl. Microbiol, 107: 1019-1030.
[40] Kusari S, Zühlke S, Kosuth J, Cellarova E, Spiteller M 2009. Light- independent metabolomics of endophytic *Thielavia subthermophila* provides insight into microbial hypericin biosynthesis. J. Nat. Prod,72:1825-1835.

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