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Anti-Escherichia coli activity of extracts from Schinus terebinthifolius fruits and leaves

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SHORT COMMUNICATION

Anti-*Escherichia coli* activity of extracts from *Schinus terebinthifolius* fruits and leaves

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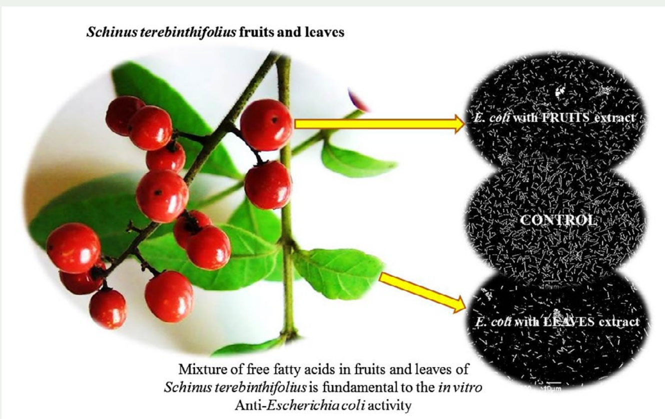
ABSTRACT
Ethanol extracts obtained from *Schinus terebinthifolius* Raddi fruits and leaves were active against *Escherichia coli* with MIC of 78 μg mL^{-1} for both extracts. Phytochemical analyses revealed a major presence of phenolic acids, tannins, fatty acids and acid triterpenes in the leaves and phenolic acids, fatty acids, acid triterpenes and biflavonoids in the fruits. Major compounds isolated from the plant, such as the acid triterpene schinol, the phenolic acid derivative ethyl gallate and the biflavonoids agathisflavone and tetrahydroamentoflavone, showed very little activity against *E. coli*. Bioautography of the ethanol extracts on silica gel plate showed inhibition zones for *E. coli*. They were removed from the plate and the compounds identified as a mixture of myristic, pentadecanoic, palmitic, heptadecanoic, stearic, nonadecanoic, eicosanoic, heneicosanoic and behenic fatty acids.



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1. Introduction

Schinus terebinthifolius Raddi has been cultivated in Espírito Santo State, Brazil, to meet the growing international market for condiments. The use of the bark for medicinal purposes was approved by the Brazilian National Health Surveillance Agency. The alcoholic extract of *S. terebinthifolius* bark is included in the National List of Essential Medicines and is indicated as an anti-inflammatory and topical antiseptic for gynaecological use (RENAME 2013). Leaves and fruits are popularly used as healing agents (Ribas et al. 2006) and to treat oral infections (Santos et al. 2009). Some papers have discussed the importance of *S. terebinthifolius* preparations as an antiadherent against the activity of bacterial formation on biofilm (Freires et al. 2010; Barbieri et al. 2014). On the other hand, foodborne illness is a major source of morbidity in developed countries. Strong evidence shows that infections caused by *Escherichia coli* originate from food-producing animals like poultry (Lazarus et al. 2015). Urinary tract infections, neonatal meningitis and sepsis are some of the human diseases associated with extra intestinal pathogenic *E. coli,* the aetiological agent of colibacillosis in chickens (Mitchell et al. 2015).

2. Results and discussions

2.1. Phytochemical analyses

Table S1 (supplementary information) lists the main substances contained in each sample. (-)-ESI-TOF-MS spectra for ethanol extracts of both fruits and leaves showed the presence of ethyl gallate ($C_9H_9O_5$, $[M–H]^-$ 197.0457), but schinol ($C_{30}H_{45}O_3$, $[M–H]^-$ 453.3372) and tetrahydroamentoflavone ($C_{30}H_{21}O_{10}$, $[M–H]^-$ 541.1145) were the major peaks of the spectrum in fruits. These compounds are commonly described to occur in *S. terebinthifolius*, along with other representatives of such classes (Carvalho et al. 2013).

Bioautography of both extracts on TLC silica gel showed an inhibition zone for the growth of *E. coli*. By comparison, fatty acids are present in both extracts. As shown in Table S2 (supplementary information), a homologous series of nine compounds, from $C_{14}H_{28}O_2$ (myristic acid) to $C_{22}H_{44}O_2$ (behenic acid) were identified.

2.2. Antimicrobial activity

Table S3 (supplementary information) shows the results of an antimicrobial screening of extracts and their fractions obtained from fruits and leaves of *S. terebinthifolius* against *E. coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Cryptococcus neoformans* and *Candida albicans*. All samples, whose minimum inhibitory concentration was determined, also showed microbicidal effect. As the values show, *E. coli* was, undoubtedly, the most sensitive microorganism to the samples of plant origin with MBC values nearly identical to those of MIC.

Ethanol extracts of both fruits and leaves were active at the same concentration (78 μg mL^{-1}). Fractionation of the fruit ethanol extract by liquid/liquid partition led to a decrease of the original antimicrobial activity, which was distributed to the more lipophilic partitions, such as hexane (156 μg mL^{-1}) and dichloromethane (156 μg mL^{-1}). Nevertheless, when the same procedure was adopted for the leaves, the antimicrobial activity increased for a more polar partition, ethyl acetate (39 μg mL^{-1}), although hexane was still very active

(78 µg mL^{-1}). This result can be attributed to chemical composition. Because of this, bioautography of both ethanol extracts was performed to identify the active molecules. For both fruits and leaves, the procedure on silica gel plates produced inhibition zones for *E. coli* growth. The compounds extracted and identified from fruits and leaves were fatty acids, highly lipophilic substances, which are often identified as the active ingredients in ethnic and herbal medicines (McGaw et al. 2002; Yff et al. 2002). Some studies carried out with the mixture of palmitic and stearic acid, or others of the same chemical nature, showed excellent antibacterial activity against *E. coli* (Hashem and Saleh 1999; Yff et al. 2002). According to Desbois and Smith (2010), FFAs are used by many organisms to defend against parasitic or pathogenic bacteria. They have the ability to kill or inhibit the growth of bacteria acting in cell membrane, where they disrupt the electron transport chain and oxidative phosphorylation, thus interfering with cellular energy production. Moreover, they may also cause the inhibition of enzymatic activity, impairment of nutrient uptake, generation of peroxidation and auto-oxidation degradation products or direct lysis of bacterial cells.

In order to observe possible morphological alterations in *E. coli* cells treated with *S. terebinthifolius* extracts, samples of ethanol extracts were selected for scanning electron microscope (SEM) observations. As shown in Figure S9 (supplementary information), both treatments resulted in a decrease in the number of cells compared to negative control; nevertheless, no morphological changes could be observed by this technique.

In relation to crude ethanol extracts, the data in this study show that the *in vitro* antimicrobial activity of *S. terebinthifolius* fruits is as efficient as that of the leaves and suggest that fruits and leaves can be excellent raw materials for products against *E. coli* infection and contamination.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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