CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITY, AND CONTROL POTENTIAL OF BOTANICAL FERMENTED PRODUCTS ON PHYTOPATHOGENIC FUNGI OF AGRICULTURAL INTEREST

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ABSTRACT

Purpose: This work aimed to evaluate the chemical composition and bioactivity of fermented extracts of the botanical species Platycladus orientalis, Cupressus sempervirens, Ricinus communis, Artemisia absinthium, and Picrasma crenata, assessed at 10 %, 20 %, and 40 % v/v on the mycelial development of phytopathogenic fungi Colletotrichum fructicola, Sclerotium rolfsii, and Fusarium oxysporum.

Theoretical framework: The search for sustainable alternatives that are less harmful to the environment regarding synthetic agrochemicals has intensified in recent years. Among the different approaches to mitigate this problem, the use of molecules of biological origin and biotransformed botanical extracts is an important research topic in the area. However, the potential for using fermented botanical extracts is still little explored, and it is necessary to study these materials to evaluate their actual biological activity systematically and, consequently, the potential for practical use, especially in areas such as agriculture.

Methods: The antioxidant activity before and after the fermentation process, as well as the levels of phenolic compounds, flavonoids, and individual phenolics, were determined by spectrophotometry and high-performance liquid chromatography. The mycelial growth of Colletotrichum fructicola was evaluated in vitro in a PDA medium containing the fermented ones at zero, 10 %, 20 %, and 40 % v/v.

Results and conclusion: The main phenolic compound identified was gallic acid in fermented A. absinthium, followed by P. crenata and R. communis. These same species showed inhibitory activity between 44.0 % and 34.6 %, at a concentration of 40 % v/v, on the mycelial development of C. fructicola. As for the antioxidant activity of the analyzed fermented products and the evaluation of phenolic compounds before and after the fermentation of plant extracts, the results before varied between 58 – 79 %, and in the post-fermentation, the activity was maintained only for the fermented products of C. sempervirens and P. orientalis, a fact also verified in the same

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species relative to the levels of phenolic compounds. No inhibitory effect was observed on the fungi, showing that these fermentates are ineffective as antifungal agents.

**Research implications:** This work showed that these botanical fermented products do not have any antifungal effect on these phytopathogens. So, further research is necessary to improve their antifungal effect.

**Originality/value:** Few studies address the biological activity of fermented extracts and their potential for agricultural application. In this sense, the present study aims to verify the antifungal activity of different botanical fermentates to evaluate their potential use as an alternative to synthetic products.

**Keywords:** Alternative Control, Flavonoids, Fermentation, Phenolic Compounds, Regenerative Agriculture.

**COMPOSIÇÃO QUÍMICA, ATIVIDADE ANTIOXIDANTE E POTENCIAL DE CONTROLE DE FERMENTADOS BOTÂNICOS SOBRE FUNGOS FITOPATOGÊNICOS DE INTERESSE AGRÍCOLA**

**RESUMO**

**Objetivo:** Este trabalho teve como objetivo avaliar a composição química e bioatividade de extratos fermentados das espécies botânicas Platycladus orientalis, Cupressus sempervirens, Ricinus communis, Artemisia absinthium e Picrasma crenata, avaliados nas concentrações de 10 %, 20 % e 40 % v/v, sobre o desenvolvimento micelial dos fungos fitopatogênicos Colletotrichum fructicola, Sclerotium rolfsii e Fusarium oxysporum.

**Referencial teórico:** A busca por alternativas sustentáveis e menos agressivas ao meio ambiente com relação à utilização de agroquímicos sintéticos vem se intensificando nos últimos anos. Dentre as diferentes abordagens a fim de mitigar este problema, o emprego de moléculas de origem biológica, bem como de extratos botânicos biotransformados, é um tópico importante de pesquisa na área. No entanto, o potencial de uso de extratos botânicos fermentados ainda é pouco explorado, sendo necessário o estudo destes materiais, a fim de avaliar de forma sistemática sua real atividade biológica e, consequentemente, o potencial de utilização prática, especialmente em áreas como a agricultura.

**Métodos:** A atividade antioxidante antes e após o processo de fermentação, bem como os teores de compostos fenólicos, flavonoides e fenólicos individuais foram determinados por espectrofotometria e cromatografia líquida de alta eficiência. O crescimento micelial de Colletotrichum fructicola foram avaliados in vitro em meio BDA contendo os fermentados a zero, 10 %, 20 % e 40 % v/v.

**Resultados e conclusão:** O principal composto fenólico identificado foi o ácido gálico no fermentado de A. absinthium, seguido por P. crenata e R. communis. Estas mesmas espécies apresentaram atividade inibitória entre 44,0 % e 34,6 %, na concentração de 40 % v/v, sobre o desenvolvimento de micelial de C. fructicola. Quanto à atividade antioxidante dos fermentados analisados e à avaliação de compostos fenólicos antes e após a fermentação dos extratos vegetais, os resultados para pré variaram entre 58 – 79 %, sendo que na pós-fermentação a atividade se manteve apenas para os fermentados de C. sempervirens e P. orientalis, fato também verificado nas mesmas espécies em relação aos teores de compostos fenólicos. Não foi observado nenhum efeito inibitório sobre os demais fungos, mostrando que estes fermentados não são eficientes como agente antifúngicos.

**Implicações da pesquisa:** Este trabalho mostrou que estes fermentados botânicos não apresentam nenhum efeito antifúngico sobre estes fitopatógenos, sendo necessária a realização de mais pesquisas para melhorar a atividade antifúngica destes produtos.

**Originalidade/valor:** Poucos estudos tratam da atividade biológica de extratos fermentados e seu potencial de aplicação agrícola. Nesse sentido, o presente estudo visa verificar a atividade antifúngica de diferentes fermentados botânicos, a fim de avaliar seu potencial uso como uma alternativa ao emprego de produtos sintéticos.

**Palavras-chave:** Agricultura Regenerativa, Controle Alternativo, Compostos Fenólicos Flavonoides, Fermentação.
1 INTRODUCTION

The prolonged and often indiscriminate use of synthetic agrochemicals to control plant diseases can harm the environment and human and animal health. In addition, the incorrect and excessive use of specific agrochemicals results, through the mechanism of natural selection, in the development of resistant phytopathogens, whose control by conventional means becomes increasingly complex and inefficient (Haq et al., 2020). In addition, studies report that the misuse of certain agrochemicals causes other problems, such as bioaccumulation and toxicity to non-target organisms (Chowdhary et al., 2018).

In general, synthetic fungicides are considered harmful and pollutant to the environment, in addition to their toxicity to consumers, when used indiscriminately. In this sense, plant extracts are safer, healthier, and more ecological alternatives when compared to synthetic molecules. These natural products can be used to manage cultivated plants due to their specific toxicity for filamentous fungi, without important toxic effects on non-target organisms (Onaran; Sağlam, 2016). Due to structural diversity, low toxicity, and lower impact on the environment, bioactive compounds from plants have become important sources in the development of products of natural origin to be used as alternatives to synthetic fungicides (Hejazi et al., 2017; Zhang et al., 2014).

In this way, the present work had the objective of carrying out the chemical characterization, evaluating the antioxidant activity and content of phenolic compounds among other compounds, and the antifungal activity of fermented botanicals of Platycladus orientalis, Cupressus sempervirens, Ricinus communis, Artemisia absinthiu, and Picrasma crenata on the mycelial development of phytopathogenic fungi of agricultural interest.

2 THEORETICAL FRAMEWORK

Natural pesticides or phytoprotective products are among the methods of alternative control of plant diseases within the so-called regenerative agriculture. These products are prepared from substances that are not harmful or have a low impact on human health and the environment, intended to control agricultural pests and diseases. The use of these products typically has little or no chemical residual potential, with less harmful effects on the environment, farmers, and consumers. Microorganisms for biological control, various liquid biofertilizers, pheromones, essential oils, and plant extracts, among others, are included in the category of natural pesticides (Silva et al., 2017).

Studies in the literature comment on the potential application of alternative control and the relevant results of this control method concerning the management of plant diseases. Thus, the use of molecules of natural origin, aiming to minimize the impacts caused by the continuous use of synthetic agrochemicals, has the additional benefits of avoiding the selection of resistant pathogens and, in some cases, reducing the production costs associated with crops (Barboza et al. al., 2013).

The use of plants or extracts from plant species dates back to the beginning of human history and has increasingly awakened research interest in interpreting the potential of bioactive plant compounds more accurately. As a result of this investigation, the use of plant species in the pharmaceutical and chemical industries has increased (Yao et al. 2015; Ishaq et al. 2019). In agriculture, the use of these bioactive compounds is also growing. The action of certain social technologies, such as the so-called botanical fermented products used mainly by family farmers linked to the field of agroecology and organic production, has been investigated. These botanical ferments have been used to control phytopathogenic insects and fungi primarily associated with organic farming practices (Sartori; Venturin, 2016; Costa et al., 2021).
The fermentation process can be an alternative method to facilitate the extraction or modification of active metabolites of certain plant species, such as phenolic compounds and terpenes. Furthermore, this process is environmentally safer and may increase effectiveness or reduce adverse environmental effects relative to unfermented extracts. Chaiyana et al. (2022) verified that, with the fermentation process, there was an increase in the biological activity of the fermented product relative to the extract that did not go through the fermentation process.

With the rapid expansion of the world's population and the growing demand for food with food security guarantees, the loss of crops due to specific plant diseases caused by fungi, viruses, and bacteria has concerned the agricultural sector. Fletcher et al. (2006) and Rajaram and Dubin (2021) report that approximately 85% of plant diseases are caused by infections caused by Sclerotinia spp., Rhizoctonia spp., Puccinia spp., Fusarium spp., Blumeria spp., Phytophthora spp., Pythium spp., and Colletotrichum spp., among others, which may cause problems about food production and, consequently, food safety.

Using natural compounds from plants can be a suppressive strategy to control several phytopathogenic fungi of agricultural interest (Gurjar et al., 2012; Javaid; Shoaib, 2013). The use of extracts from several plant species has been reported because they have antifungal potential against diseases caused by Fusarium oxysporum f. sp. lycopersici, F. oxysporum f. sp. c ceps, and S. rolfsii (Javaid; Rauf, 2015; Sana, 2016).

Colletotrichum is another fungal genus of great agricultural importance, whose belonging species cause anthracnose disease, affecting different plant species. These phytopathogens attack dozens of crops worldwide, causing significant economic and production losses (Huang et al., 2021). For example, olive anthracnose, caused by different species of Colletotrichum spp., can be considered the most harmful disease in the olive crop worldwide. Punica granatum bark extract is effective in in vitro disease control against Colletotrichum acutatum (Pangallo et al., 2017).

Some species of phytopathogenic fungi, such as athenelia (Sclerotium rolfsii), are not controlled by a single method, requiring an association between techniques, such as the association between cultural and chemical control methods, such as the use of fungicides and/or resistant plants (Sousa; Blum, 2013). Therefore, integrated plant management (IPM) can be used as an alternative control strategy. Its advantages are that it employs methods with less environmental impact, which is efficient and economically viable, causing less pollution than chemical control alone. In addition, the use of IPM and alternative control can be carried out in systems that follow the principles of organic agriculture (Cavalcanti et al., 2018).

It is important to emphasize that fungal plant diseases compromise global food security. Some primary crops with high economic and agronomic value, such as corn, potatoes, soybeans, and beans, are threatened by several fungal diseases that can lead to important yield losses. This can cause crop failures, with deleterious effects on supply and price variations in the medium and long term (Pennisi, 2010; Savary et al., 2019).

On the other hand, changes in the metabolism of phenolic compounds and antioxidant capacity in plants can be observed. They can be altered by biotic and abiotic stress factors, pathogen attacks, different fertilizations, nutritional deficiency, low temperature, and mechanical damage as a mechanism for protection against such damage. (Dixon; Paiva, 1995). Evaluating these compounds, in addition to the intrinsic characteristics of each species, helps us to improve the understanding of the action of these bioactive compounds and their possible biological activity. Exploring plants' physiological and metabolic state in response to stress and defensive stimuli has also been the subject of research worldwide (Isah, 2019).
3 MATERIALS AND METHODS

3.1 Fungal isolate and plant material

The experiment was carried out from July to October 2022. The fungi Colletotrichum fruticola, Sclerotium rolfsii (060/19), and Fusarium oxysporum (A35/14) used came from the mycotheque of the Phytopathology Laboratory of the University of Caxias do Sul (Caxias do Sul, RS, Brazil).

The plant species Platycladus orientalis L. Br Medianeira (HUCS: 51956), Cupressus sempervirens L. (HUCS 51957) Cupressaceae, Ricinus communis L. (HUCS 54065) Euphorbiaceae, and Artemisia absinthium L. (HUCS 54061), were collected in the municipality of Caxias do Sul (29°10'4'' S, 51° 10'46'' W) and Picrasma crenata (Vell.) Engl. (HUCS 52730) Simaroubaceae was collected in the central area of the municipality of Nova Roma do Sul (28°59'25'' S, 51°24'29'' W).

3.2 Preparation of fermented botanicals and determination of total phenolic compounds and flavonoids

The fermented products of different plant species were produced by mixing 1.5 L of untreated water and 500 g of fresh plant (smaller branches and leaves), liquefied until completely crushed, in a proportion of one part of plant material to three parts of water (1:3). Fermentation occurred spontaneously and aerobically, kept in a dark environment, at room temperature (10 – 25 °C) for 15 days.

For chemical analysis, the botanical fermented samples were homogenized. A 10 mL aliquot was transferred to 15 mL centrifuge tubes. The mixture was centrifuged for 15 min at 3000 g, and the clear supernatant was used in the assays. The content of phenolic compounds was determined spectrophotometrically by the Folin-Ciocalteu method, according to the procedure described by Pereira et al. (2018), and the results were expressed in milligrams of gallic acid equivalent per 100 mL of sample. The quantification of the total flavonoid content followed the spectrophotometric method of aluminum chloride, according to the procedures of Matic et al. (2017), and the results were expressed in milligrams of quercetin equivalent per 100 mL of sample.

3.3 Determination of individual contents of phenolic compounds

The individual levels of phenolic compounds in the fermented products were analyzed by High-Performance Liquid Chromatography (HPLC) according to the procedure and specifications described by Agostini et al. (2017). Initially, the samples were pre-treated by dilution in Milli-Q water (5.0 g·L⁻¹) and filtered through a nylon membrane with a pore diameter of 0.45 µm. An HP model 1100 liquid chromatograph was used, coupled to a Lichrospher RP 18 column (5 µm) and a UV detector at a wavelength of 210 nm. The reverse phase analysis comprised solvent A (Milli-Q water with 1.0 % v/v phosphoric acid) and solvent B (acetonitrile). The mobile phase pumping system was of the gradient type, with 90 % solvent A from zero to 5 min, 60 % A from 5 min to 40 min, and 90 % A from 45 min to 50 min. The mobile phase flow rate was maintained at 0.5 mL·min⁻¹, as proposed by Morelli (2010).

The phenolic compounds were identified according to their elution order and by comparing their retention time with their pure standards, previously injected and following the same chromatographic conditions. Quantification was performed using the external standardization method through the correlation of the peak area of the compound to the curve
of each evaluated standard. The phenolic compounds gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin, apigenin, and kaempferol were evaluated. Results were expressed in milligrams of each compound per 100 mL of sample.

**3.4 Determination of the before and post-fermentation antioxidant activity of plant extracts**

The antioxidant activity of fermented products was evaluated at two moments on the day of assembly of plant extracts in fermenters (extract without fermentation) and plant extracts after 15 days of fermentation. Antioxidant activity was determined by inhibition of the DPPH• radical according to the method described by Yamaguchi et al. (1998), where Tris-HCl buffer (100 mM, pH 7.0) containing 250 µM of the DPPH• radical dissolved in ethanol was added to the pre- and post-fermented extracts. The tubes were stored in the dark for 20 min, and the absorbance was determined at a wavelength of 517 nm. Results were expressed as a percentage of DPPH• radical inhibition.

**3.5 Antifungal activity of fermented botanicals on the mycelial growth of phytopathogenic fungi**

To verify the antifungal activity of the fermented botanicals, PDA (Potato-Dextrose-Agar) culture media were prepared to which, while still melting, the fermented botanicals were added at concentrations of zero (control), 10 %, 20 %, and 40 % v/ v. The media were autoclaved at 121 °C for 15 min to sterilize them. These were poured into Petri dishes in five replicates. After solidification of the medium, a 5 mm mycelial disc, with seven days of development, of the fungi *C. fructicola*, *S. rolfsii*, and *F. oxysporum* was deposited in the center of the plate. The plates were sealed with plastic film and incubated in a BOD chamber at 25±2 °C and 12 h light/dark photoperiod.

Mycelial growth diameters were measured on the 14th day after inoculation. With the data obtained, the percentage of inhibition of growth of the treatments relative to the control was determined according to the method described by Pansera et al. (2023).

**3.6 Experimental design and statistical analysis**

The fermented products were produced in batches, and chemical characterization and antioxidant activity tests were performed in triplicates. Antifungal activity assays were performed in quintuplicate. Antifungal activity assays followed a completely randomized design.

The results obtained were submitted to the Levene test (homoscedasticity) and the Shapiro-Wilk test (normality of the residues), followed by an Analysis of Variance (ANOVA). Subsequently, the means were compared using Tukey’s test at a 5 % probability of error using the AgroEstat® software (Brazil).

**4 RESULTS AND DISCUSSION**

The results of the total phenolic compounds and flavonoid content of the fermented products produced are compiled in Table 1.
Table 1. Contents of total phenolic compounds and flavonoids of the different botanical fermentates produced.

<table>
<thead>
<tr>
<th>Botanical fermentates</th>
<th>Phenolic compounds$^1$ (mg∙100 mL$^{-1}$)</th>
<th>Flavonoids$^2$ (mg∙100 mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia absinthium</td>
<td>57.0±3.3 e</td>
<td>12.5±1.0 e</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>911.8±10.2 b</td>
<td>532.3±11.7 a</td>
</tr>
<tr>
<td>Picrasma crenata</td>
<td>313.5±2.9 d</td>
<td>150.8±3.1 c</td>
</tr>
<tr>
<td>Platycladus orientalis</td>
<td>348.3±8.8 e</td>
<td>227.3±9.1 b</td>
</tr>
<tr>
<td>Cupressus sempervirens</td>
<td>1094.9±12.5 a</td>
<td>83.5±2.3 d</td>
</tr>
</tbody>
</table>

$^1$– Expressed as gallic acid equivalent. $^2$– Expressed as quercetin equivalent. Means in columns followed by the same lowercase letter do not differ statistically by Tukey’s test at a 5 % error probability.

Source: Authors (2023).

The highest levels of phenolic compounds were observed in fermented *Cupressus sempervirens* and *Ricinus communis*, followed by *Platycladus orientalis* and *Picrasma crenata*. The highest levels of flavonoids were identified in fermented *Ricinus communis*, followed by *Platycladus orientalis* and *Picrasma crenata*.

No specific reports of phenolic compounds are identified from fermented botanicals in the literature. According to Al-Snafi (2016), in the phytochemical analysis of *Cupressus sempervirens* essential oil, mainly tannins, flavonoids, alkaloids, saponins, phenols, and other biologically active compounds were identified. According to Romani et al. (2002), the main chemotaxonomic chemical components identified in the genus *Cupressus* are biflavonoids and flavonoid-flavonoid dimers, with a variety of chemical structures and different types of biological activity, such as antimicrobial and antioxidant, among others. According to Omafuvbe et al. (2009), the antimicrobial and antifungal potential of the extract of fermented plants is due to bioactive compounds of different classes, such as tannins, saponins, phenolic compounds, and flavonoids in general.

The species *Ricinus communis* L., commonly known as castor bean, has been extensively investigated from a phytochemical point of view, whose literature reports the presence of alkaloids, flavonoids, tannins, alkaloids, sterols, and terpenes in their tissues (Darmanin et al., 2009; Jena; Gupta, 2012). Species of the genus *Artemisia* are also recognized for having different phenolic compounds, flavonoids, and terpenes as secondary metabolites (Silva-Alves et al., 2013).

Several compounds with bioactive properties have already been identified concerning the genus *Picrasma*, including phenolic compounds and flavonoids, although a more detailed characterization has not been presented (Liu et al., 2019).

The individual contents of phenolic compounds, analyzed by high-performance liquid chromatography (HPLC), are shown in Table 2.

Table 2. Individual contents of phenolic compounds identified in the botanical fermentates analyzed by high-performance liquid chromatography (HPLC).

<table>
<thead>
<tr>
<th>Botanical fermentate</th>
<th>Gallic acid (mg∙100 mL$^{-1}$)</th>
<th>Epicatechin (mg∙100 mL$^{-1}$)</th>
<th>Rutin (mg∙100 mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia absinthium</td>
<td>758.7</td>
<td>17.7</td>
<td>-</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>352.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Picrasma crenata</td>
<td>330.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Platycladus orientalis</td>
<td>1.1</td>
<td>32.6</td>
<td>-</td>
</tr>
<tr>
<td>Cupressus sempervirens</td>
<td>32.9</td>
<td>37</td>
<td>53.8</td>
</tr>
</tbody>
</table>

The phenolic compounds epigallocatechin, catechin, epigallocatechin gallate, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin, apigenin, and kaempferol were not detected in the analyzed fermented products. 

Source: Authors (2023).
The phenolic compound identified in greater quantity in the fermented botanicals analyzed was gallic acid. This compound was detected mainly in the fermentate of Artemisia absinthium, followed by the Ricinus communis and Picrasma crenata fermentates.

Gallic acid, a natural polyphenol, is found in several plant species and is reported in the literature to have antifungal and antibacterial properties (Li et al., 2017).

Several phenolic compounds (gallic acid, coumaric acid, acid, syringic acid, and salicylic and chlorogenic acids) and flavonoids (quercetin and rutin) are commonly found in essential oils and extracts of A. absinthium (Jahid et al., 2016; Ali; Abbasi, 2013; Craciunescu et al., 2012; Dehghani; Bidgoli, 2021).

The antioxidant activity of the pre-fermentation extracts and after 15 days of fermentation were evaluated using the method of percentage of inhibition of the DPPH radical (Yamaguchi et al., 1998) and total phenolic compounds by the Folin-Ciocalteau method (Pereira et al., 2018) and the results are shown in Table 3.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pre-fermentation DPPH (%)</th>
<th>Total Phenolics</th>
<th>After 15 days of fermentation</th>
<th>Total Phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemisia absinthium</td>
<td>76.5±0.8 b</td>
<td>41.9±1.8 d</td>
<td>26.1±0.9 d</td>
<td>30.3±0.2 d</td>
</tr>
<tr>
<td>Ricinus communis</td>
<td>72.8±0.6 c</td>
<td>238.6±26.2 b</td>
<td>51.7±2.9 c</td>
<td>142.9±11.3 bc</td>
</tr>
<tr>
<td>Picrasma crenata</td>
<td>78.8±0.4 a</td>
<td>426.6±51.3 a</td>
<td>49.9±1.3 c</td>
<td>216.5±12.9 a</td>
</tr>
<tr>
<td>Platycladus orientalis</td>
<td>58.3±0.2 d</td>
<td>166.8±10.5 c</td>
<td>66.0±0.5 b</td>
<td>156.3±10.7 b</td>
</tr>
<tr>
<td>Cupressus sempervirens</td>
<td>78.1±0.3 a</td>
<td>119.0±2.1 c</td>
<td>80.0±0.8 a</td>
<td>122.4±6.5 c</td>
</tr>
</tbody>
</table>

Total phenolics are expressed as milligrams of gallic acid equivalents in 100 mL of fermentate (mgGA/100 mL⁻¹). Means in column followed by the same lowercase letter do not differ statistically by Tukey's test at a 5% error probability.

Source: Authors (2023).

The results showed that the highest antioxidant activities occurred for extracts of P. crenata and C. sempervirens in pre-fermentation and for C. sempervirens after 15 days of fermentation. A significant reduction in antioxidant activity was also observed from pre- to post-fermentation in most plants tested, except for P. orientalis and C. sempervirens.

The content of total phenolic compounds evaluated for pre- and post-fermentation extracts showed higher rates for P. crenata pre- and post-fermentation. As observed for antioxidant activity, there was a reduction of the activity between pre- and post-fermentation, except for P. orientalis and C. sempervirens.

Polyphenols are studied mainly due to their antioxidant properties (Shin; Lee, 2021) and antimicrobial activity against some phytopathogenic microorganisms, including those of food origin (Aguilar-Veloz et al., 2020).

Antioxidant activity has already been reported for A. absinthium extracts (Ali et al., 2013), ethanolic extracts of P. orientalis leaves (Ren et al., 2019), methanolic and chloroform extracts, and essential oil of C. sempervirens (Toroglu, 2007; Asgary et al., 2013).

According to Naczk and Shahidi (2006), plants may contain several bioactive compounds, including phenolic compounds, carotenoids, and anthocyanins. These molecules can be used in fermentation bioprocesses, being transformed by the metabolic activity of the microorganisms involved in the fermentation process. This fermentation activity can decompose or convert substrates into compatible components under the action of microbial enzymes, improving substrate properties through production and increasing the extraction of bioactive compounds (Parvez et al., 2006). According to Zhang et al. (2012), an extensive alteration of the extract chemistry can occur during fermentation, leading to important changes.
in its properties, such as developing or losing some biological activity. Therefore, compounds with bioactive properties can be altered or even formed from fermentation.

Regarding the antifungal activity of the fermented botanicals, none of the fermented products evaluated in this work effectively controlled the fungi tested. Only the phytopathogenic fungus *C. fruticola* suffered partial inhibition against some of the evaluated fermented products.

*In vitro* assays of the antifungal activity of fermented products against the phytopathogenic fungus *Colletotrichum fruticola* are compiled in Table 4.

**Table 4.** Percentage inhibition of mycelial growth of *Colletotrichum fruticola* from exposure to different concentrations of botanical fermentates.

<table>
<thead>
<tr>
<th>Botanical fermentate</th>
<th>Control</th>
<th>10%</th>
<th>20%</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Artemisia absinthium</em></td>
<td>0.0 Ca</td>
<td>15.6 Aa</td>
<td>20.7 Aab</td>
<td>4.2 Bb</td>
</tr>
<tr>
<td><em>Ricinus communis</em></td>
<td>0.0 Ba</td>
<td>7.7 Bab</td>
<td>31.6 Aa</td>
<td>34.6 Aa</td>
</tr>
<tr>
<td><em>Picrasma crenata</em></td>
<td>0.0 Ba</td>
<td>2.0 Bb</td>
<td>3.2 BC</td>
<td>42.9 Aa</td>
</tr>
<tr>
<td><em>Cupressus sempervirens</em></td>
<td>0.0 Ba</td>
<td>0.0 Bb</td>
<td>11.1 ABC</td>
<td>7.6 Ab</td>
</tr>
<tr>
<td><em>Platycladus orientalis</em></td>
<td>0.0 Aa</td>
<td>0.0 AB</td>
<td>0.0 AC</td>
<td>5.8 AB</td>
</tr>
</tbody>
</table>

Means followed by the same letter, uppercase in line (concentration) and lowercase in column (botanical fermentate), do not differ by Tukey’s test at a 5% probability of error.

Source: Authors (2023).

The botanical fermentate that had the greatest effect on the mycelial development of *C. fruticola* was the fermented *Artemisia absinthium*, with 44.2% inhibition, followed by *Picrasma crenata*, with 42.9% inhibition, and *Ricinus communis*, with 34.6% inhibition, respectively (Table 4).

Plant extracts are used as one of the alternatives for controlling pathogens that cause diseases in cultivated plants, as they have chemical compounds with bioactive activity, which can be present in different parts of plants, such as stems, leaves, branches, bark, roots, among others (Hao et al., 2013). The extracts can be prepared green or dry, and their bioactive properties can be extracted from the plant tissue using solvents such as water, ethanol, and ethyl acetate (Cos et al., 2006). However, considering agricultural use and food production, water-based or hydroalcoholic mixtures are the most suitable solvents (Boeing et al., 2014).

The species of the genus *Artemisia* are described as producing different types of metabolites with antimicrobial and antioxidant activity. Elevated alkaloids, flavonoids, phenol, quinines, and terpenoid contents have been reported in *Artemisia extracts* (Juteau et al., 2002; Ahameethunisa; Hopper, 2010). Recently, research has shown that this species has antioxidant, hepatoprotective, analgesic, estrogenic, cytotoxic, antibacterial, and antifungal activity, among others (Bora; Sharma, 2011; Ahameethunisa; Hopper, 2012).

*Artemisia gmelinii* (wormwood) extracts have shown potent antibacterial and antifungal activity (Mamatova et al., 2019). Different parts of the *Artemisia santolinifolia* species, such as root, leaves, and stem, have metabolites such as terpenoids, tannins, phenolic compounds, and flavonoids. Some chemical compounds were characterized in aqueous extracts (alkaloids, flavonoids, saponins, tannins, and steroids) and showed antifungal activity (Salhi et al., 2017). Phenolic compounds are present in different species of *Artemisia* (Silva-Alves et al., 2013). Tannins and other compounds were also isolated, which showed antimicrobial and phytotoxic activity in controlling fungi (Salhi et al., 2017; Nowsheen et al., 2021). Chung et al. (2009) verified inhibitory activity on *Mucor rouxii* and *Penicillium citrinum* from the extract of Artemisia species, identifying terpenoids, such as the compounds carvone and piperitone.

The promising potential of the application of extracts and derivatives of plants of the genus *Picrasma*, with cytotoxic potential, was reported by Zhao et al. (2014). According to
Khan et al. (2001) and Xu et al. (2021), the secondary metabolites of the genus *Picrosma* have shown biological activity in *in vitro* studies, such as antibacterial, antifungal, and antiviral effects. These reports may explain the partial control (42.9 %) on developing the phytopathogenic fungus *C. fruticola* when exposed to fermented diluted 40 % v/v of *Picrosma crenata*.

Chen et al. (2021) evaluated the bioactivity of several compounds isolated from *Picrosma javanica* leaves. The biological activity of eleven of the most abundant isolates was assessed against five phytopathogenic fungi *in vitro*, and several of these showed moderate inhibitory effects compared to chemical controls, such as the synthetic fungicide carbendazim.

Table 4 shows that the fermented product of *R. communis*, at a concentration of 40 % v/v, inhibited the development of the phytopathogen *C. fruticola* by 34.6 %. There are reports in the literature on the antifungal and antimicrobial activity of extracts from the leaves of *R. communis* obtained by the infusion process (Panghal et al., 2011). Several flavonoids, such as quercetin and rutin, have been reported as the main phytoconstituents of this plant (Kumar, 2017). The antifungal, antimicrobial, larvicidal, and antioxidant activity of different *R. communis* extracts was also verified (Taur et al., 2011; Naz; Bano, 2012; Suurbaar et al., 2017; Hussain et al., 2021; Singh et al., 2009; Soni; Dhiman, 2017).

The antimicrobial activity of *R. communis* extracts is reported in the literature. Its plant extracts have been reported as having antimicrobial activity against different microorganisms. However, the compounds responsible for this activity were not identified (Ribeiro et al., 2016). Ricinin, an alkaloid extant in different parts of the *R. communis* plant, is a simple and moderately toxic α-pyridone alkaloid with antimicrobial activity (Swarupa et al., 2017).

A variety of phytochemicals such as gallic acid, quercetin, triterpenoids, ingenol, kaempferol, catechin, epicatechin, gentisic acid, ricinoleic acid, linoleic acid, camphor, α-thujone, and β-thujone have been reported in infusions obtained from different parts of *R. communis* (Kumar, 2017). In addition, several studies have reported antimicrobial, fertility-reducing, antidiabetic, antiarthritic, hepatoprotective, anticancer, and laxative properties of *R. communis* leaf extracts (Shokeen et al., 2008). Carolina et al. (2019) observed that *R. communis* leaf extracts showed antifungal activity against *Aspergillus niger*.

Aromatic and medicinal plants are particularly valuable for bioprocesses as they contain many bioactive compounds, including phenolic compounds, terpenes, carotenoids, anthocyanins, and tocopherols (Mousavi et al., 2013). Furthermore, the content of these compounds can be increased by the metabolic activity of the microorganisms involved in the fermentation process, enhancing their biological activity against the unfermented extract (Katina et al., 2007; Ng et al., 2011).

**5 CONCLUSION**

The fermented extracts of *A. absinthium*, *P. crenata*, and *R. communis*, at a concentration of 40 % v/v, showed partial inhibitory activity only on the development of the fungus *Colletotrichum fruticola*. The main phenolic compound identified via HPLC of these fermented extracts was gallic acid. Plant extracts showed variable results in their antioxidant activity and content of phenolic compounds pre- and post-fermentation, whose behavior varied for each plant species.

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