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RESEARCH ARTICLE

Antifungal Activity of two Plant Extracts against Aspergillus Niger

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ABSTRACT

Although there have been recent improvements in the development of antifungal treatments, fungi still pose a significant risk to human and animal health. Plant-based antimicrobials are an abundant and underutilized resource that has great promise. In this study, we demonstrate the antifungal potency properties of Salvadora persica and Juglans regiaextracts against significant pathogens, Aspergillus niger, which is known to cause harm to humans, animals, and plants. The plant extracts were produced using six distinct organic solvents: chloroform, petroleum ether, acetone, ethyl acetate, ethanol, and methanol. The antifungal activity of the plant extracts was assessed using the agar-well diffusion technique at various concentrations: 200, 100, 50, 25, and 15 mg/ml. The results indicated that extracts from Juglans regia exhibited high antifungal activity against A.nigerand appeared more effective than extracts from Salvadora persica. The ethanolic extract of J. regia showed the highest efficacy among all extracts from the two plants investigated (mean diameter of inhibition zone = 21 mm) at 200 and 100 mg/ml. Our study suggests that the crude extract of regia's bark exhibits significant antifungal activity, suggesting potential antimicrobial agents could be derived from this plant.

INTRODUCTION

Aspergillus fungi, belonging to the filamentous fungal group, are found everywhere and can decompose organic matter. Due to this, they can lead to a variety of clinical symptoms. Aspergillus conidia are commonly found in the environment. When people come into touch with and retain these conidia, they may have a range of clinically essential consequences, from asymptomatic colonization to invasive infection (Allizond et al., 2023). Aspergilli can cause hypersensitivity reactions, and superficial and cutaneous mycoses (Pérez-Cantero et al., 2020; Merad et al., 2021).

Fungal pathogens can infect a diverse array of hosts, and it is not uncommon for an individual species to be responsible for both human illnesses and crop damage. An example of such specie is Aspergillus niger (Setzer et al., 2014). Aspergillus niger is a filamentous fungus found in litter, soil, compost, and decaying plant material; in addition, it is a human pathogen that opportunistically causes aspergillosis and is frequently responsible for otomycosis. Invasive aspergillosis is the most problematic disease, with high morbidity and mortality rates, especially in patients with compromised immunity. (Surapuram et al., 2014; Pérez-Cantero et al., 2020; Gizaw et al., 2022). Aspergillosis treatment using polyenes, azoles, and echinocandins is challenging due to toxicity, poor solubility, resistance, low efficacy, and potential allergic reactions, while most antimycotic drugs are fungistatic (Gupta and Venkataraman, 2022; Pawar and Thaker; 2006).

The global issue of antimicrobial resistance to commercially available drugs has emerged in recent years. Similarly, numerous species are acquiring resistance to the antifungal treatments that are

presently accessible (Gizaw et al., 2022). Natural antifungal medications offer potential alternative remedies with reduced or nonexistent negative effects. Therefore, it is crucial to explore natural antifungal compounds as alternate approaches to counteract fungal diseases (Da et al., 2019). Plant extracts derived from medicinal and aromatic plants are a natural resource that can contain a range of biologically active compounds. These chemicals can directly impact microbial diseases (Benhaoued et al., 2024)

Salvadora persica, a member of the Salvadoraceae family, is a highly significant plant among the 182 species used as chewing sticks. It has been widely used (Noumi et al., 2010). This plant has potent antifungal effects against different microorganisms (Naseem et al., 2014). Juglans regia, belonging to the family Juglandaceae, is the most widely distributed tree nut worldwide and possesses considerable economic importance. The plant is a well-known botanical species used in traditional medicine (Ellafi et al., 2023; Vieira et al., 2019). Essentially, every component of the plant has a traditional medicinal use. The bark extract of Juglans regia exhibits a wide range of antibacterial properties against several pathogens (Zakavi et al., 2013).

Our primary objective in this study was to assess the effectiveness of certain extracts derived from medicinal and aromatic plants from Algeria against Aspergillus niger. We focused on evaluating the antifungal properties of these extracts, a crucial step in understanding their potential as antifungal agents.

MATERIEL AND METHODS

The stems of Salvadora persica and the bark of Juglans regia were obtained from local markets in Khenchela City, Algeria, in April 2023. The plant materials were identified by a taxonomist at the Department of Agronomy, University Abbes Laghrour Khenchela, Algeria. Voucher specimens were deposited under PS-1-2 and PR-1-2 at the academic laboratory, University of Khenchela, Algeria. The plant material was dehydrated in the laboratory using natural air circulation at ambient temperature. The two plants were dried at room temperature for ten days. The dried samples of the plant were cut into circular shapes and pulverized into fine powder using a laboratory grinder.

The production of the different extracts involved combining 20 g of powdered Salvadora persica and Juglans regia with 200 mL of the specified solvents, namely 95% methanol, ethanol, chloroform, petroleum ether, acetate extract, and acetone, obtained from Sigma-Aldrich in St. Louis, USA. The solution was agitated for 30 minutes and allowed to rest for 24 hours. The solution was filtered using Whatman number 1 filter paper, and the resulting solution was subsequently evaporated in a vacuum evaporator at a temperature of 40 °C. The specimens were stored in sterile containers and kept at a temperature of 4 °C until they were needed for future purposes (Mau et al., 2001). The yield (in %) was determined using the following formula Rd % = (m_1 X 100) / m_0; Where: - Rd: Yield - (m_1): Mass of the dry extract (in g) - (m_0): Mass of the dry plant material (in g) (Majhenic, 2007).Dried extracts of S. persica and J. regia were used to create various quantities of each extract: 200, 100, 50, 25, and 15 mg/ml. They used dimethyl sulfoxide (DMSO) as the solvent (Abhary & Al-Hazmi, 2015).

Antifungal activity of plant extracts

The antifungal activity was evaluated using the approach Yazdani et al. (2012) described, following the steps outlined below. A standard saline solution created a colloidal suspension with an optical density (OD) ranging from 0.15 to 0.17 at a wavelength of 530 nm. Inoculating Petri dishes containing PDA medium was performed using a swab. 6 mm diameter sterile discs were saturated with 20 µl of various extract concentrations. These discs were then placed on the surface of Mueller Hinton and PDA agar. The Petri dishes were incubated at 37 °C for 48 to 72 hours. The activity is assessed by quantifying the inhibition areas surrounding the discs; the experiment was reproduced thrice. Aseptic DMSO was employed as a negative control. The mean diameter of the overall growth inhibition was measured and reported in millimeters for the inhibition zones (Lino & Deogracious, 2006).

Statistical analysis. Three measurements were collected for each new data point. The data were presented utilizing the statistical metrics of average and variability. The obtained data were analyzed

using the GraphPad Prism® 7.01 software program by Software Inc., San Diego, CA, USA. The data were analyzed using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post-hoc test for conducting multiple comparisons. P values less than 0.05 were considered significant.

RESULTS

Yield of plant extracts

The percentage yield of each crude extract was determined and is shown in Table 1. Comparatively, the extraction yields ranged from 9.45% for the Juglens regia -methanolic extract to 0.40 % for the Salvadora persica petroleum ether extract (Table 1), J. regia with methanol produced a higher yield while S. persica Ether of petroleum had a lower yield.

Table 1: Dry weight of the extracts of the two studied plants

Plant Extract	Juglans regia	Salvadora persica
Ether of petroleum	1.46%	0.4%
Chloroform	0.45%	2.3%
Methanol	9.45%	4%
Ethanol	7.07%	0.86%
ethyl acetate	3.15%	3.12%
Acetone	5.23%	4.23%

Antifungal activities of plant extracts

In this investigation, we evaluated the antifungal activities of various extracts from Salvadora persica and the bark of Juglans regia. The extracts were tested against A. niger at concentrations of 200, 100, 50, 25, 15 mg/ml.

The particular findings are presented in Table 2. The observations made on the effect of the different extracts on the growth of the fungus strain demonstrate that all the extracts of Juglans regia have antifungal activity against the tested strain. Overall, all the extracts of J. regia were more potent than the extracts of JS. Persica.

The extracts of J. regia were active against A. niger, of which the diameter of the inhibition zone was between 12 and 21 mm with ethanol extract, between 14 and 19 mm with methanol extract, between 12 and 19 mm with Acetate extract, between 09 and 16 mm with Acetone extract, between 10 and 12 mm with petroleum ether extract, and between 09 and 10 mm with chloroform extract (Table 2). At a concentration of 200 and 100 mg/ml, the Ethanol extract of Juglans regia exhibited more potent antifungal effects against A. niger with inhibition zones measuring 21 mm.

Table 2: Inhibition diameter (mm) of the studied extracts against A. niger

Methanolic extract (E1), Ethanolic extract (E2), Chloroform extract (E3), petroleum ether extract (E4), Acetate extract (E5), Acetone extract (E6). *Values are presented as the mean of triplicates ± standard deviation of 3 independent experiments. a, b,c, d in the lines indicated significant differences between treatments according to Tukey test ($p<0.05$).

	Salvadora persica					**Juglens regia**				
Extract	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml
E1	11± 0.0 [a]	10± 0.0 [a]	11± 0.0 [a]	10± 0.0 [a]	12± 0.0 [a]	15± 0.0 [a]	19± 0.5 [a]	15± 0.3 [d]	14± 0.8 [d]	18± 0.8 [a]
E2	11± 0.0 [a]	09± 0.0 [a]	10± 0.0 [a]	11± 0.5 [a]	11± 0.0 [a]	21± 0.0 [a]	21± 0.0 [b]	16± 0.0 [a]	16± 0.0 [a]	12± 0.0 [a]
E3	00	00	00	12± 0.0 [a]	11± 0.0[b]	00	00	00	09± 0.0 [a]	10± 0.0 [a]
E4	00	00	00	10± 0.0 [a]	00± 0.0 [a]	00	00	00	10± 0.0 [b]	12± 0.0 [a]
E5	00	11± 0.0 [b]	13± 0.0 [a]	12± 0.0 [a]	12± 0.0[b]	00	14± 0.0 [a]	12± 0.9[c,d]	19± 0.0 [a]	12± 0.5 [a]
E6	00	11± 0.0 [a]	10± 0.0 [a]	9± 0.0 [a]	08± 0.0 [a]	00	16± 0.8[d]	12± 0.0 [a]	09± 0.0 [b]	09± 0.0 [a]

The methanol and acetone extracts of Salvadora persica showed an activity of 11 mm. Likewise, the petroleum ether presented an inhibition zone of 10 mm.

For the antifungal activity of Salvadora persica, the extracts were active against the A. niger strain, of which the diameter of the inhibition zone was between 08 and 13mm. Hence, the acetate extract exhibited the most vigorous activity, of which the inhibition zone was 13 mm at 50 mg/ml. In contrast, chloroform and Ethanolic extracts had an inhibitory effect of 12 mm at 25 and 15 mg/ml, respectively, while other concentrations had less activity.

DISCUSSION

Abkhoo and Jahani (2017) indicated that the yields of plant extracts vary depending on the extraction solvent and the plant extract used. It is highly recommended to use solvents with varying polarities to extract a wide range of phytophenolic chemicals with high precision. The variations in values can be attributed to the genetic variety of these plants, which impacts the production of bioactive metabolites (Ellafi et al., 2023). The yields of extracts obtained from the same organ and the same species can be influenced by several factors, such as the extraction method and the conditions applied, such as the drying time of the plant material, the quantity of the plant to be extracted, the time, stirring speed, temperature, and polarity of the solvent (Koné et al., 2017).

A study conducted by Fujita et al. (1995) revealed that acetone and chloroform extracts inhibited the growth of A. niger. Moreover, the methanol extract demonstrated significant activity against Aspergillus niger compared to the two other extracts (Fujita et al., 1995). Several substances commonly found in plants, such as polyphenols, flavonoids, tannins, and alkaloids, are responsible

for their antimicrobial properties (Nguyen, 1983). In the antifungal activity, the ethanol extract of J. Regia was tested on different fungi, such as Aspergillus niger; the extract inhibited the growth (7 to 15 mm) (Sumbul et al., 2021). Previous studies analyzing various extracts of Juglans regia have consistently shown that juglone is the main component responsible for the most potent antifungal activity in walnut green husk extracts, regardless of the type of extract evaluated. However, the activity differed based on the quantity of other essential extract components and the type of fungal infection being treated (Bhat et al., 2023).

For the antifungal activity of Salvadora persica, the extracts were active against the A. niger strain. Pirzada et al. (2009) showed that chloroform extracts of S. persica have a median antifungal effect against Aspergillus niger, which was in concordance with our results. These findings disagree with a previous study by Paliwal et al. (2007); they showed that the ethanol extract of S. persica leaf extract was found to be significantly active against Aspergillus niger with an inhibition zone of 16 to 25 mm.

The methanol, acetone and petroleum ether extracts of Salvadora persica showed a median activity. Likewise, the presented an inhibition zone of 10 mm. This observation was consistent with earlier studies that have revealed less than optimal antifungal activity of S. persica (Al Sadhan & Almas, 1999);

Furthermore, it was noticed that this antimicrobial activity differs from one extract to another due to the selective action of the solvent extractor on a certain number of microorganisms. The selective antimicrobial power of J. regia and S. persica depends on the antimicrobial substances of plant origin present in each extract. Indeed, according to their polarity, the compounds extracted are not the same, so the change in extraction protocol and the solvents used would make it possible to detect the antimicrobial activities of J. regia and S. persica (Nguyen, 1983). The effectiveness of an extract depends on its concentration, the plant from which it is derived, and the strain tested (Klervi, 2005).

To summarize, this work demonstrated the antifungal properties of several extracts derived from J. regia and S. persica leaves against the A. niger strain. The strain exhibited varying levels of susceptibility to the extracts. The results obtained serve as a foundation for future investigations aimed at identifying the highly bioactive components in two plant extracts that demonstrate the potential to inhibit the growth of fungi.

CONCLUSION

The need for new antifungal chemicals is an often discussed subject in academic literature and continues to be a primary focus of ongoing scientific investigation. This is due to the growing number of microbial strains resistant to antifungals. This study examined the antifungal efficacy of several extracts derived from S. persica and J. regia, commonly used in our country's traditional medicine. The findings demonstrated that extracts derived from Juglans regia showed significant antifungal activity against A.niger and seemed to be more potent than extracts from Salvadora persica. The ethanolic extract of J. regia was more effective than all other extracts from the two plants. Thus, these findings are still preliminary. More studies will be necessary to determine the specific chemicals responsible for the antifungal effects of Salvadora persica and Juglans regia.

Authors' contributions

BS conceived the idea, Methodology, conducted the study and wrote the manuscript. MN participated in the design of the study and helped in writing the manuscript. All authors read and approved the final manuscript.

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