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Title:

Evaluation of antifungal activity of *Laurus nobilis* leaves extracts against food spoilage fungi.

Presnted by:

DJEDI Ahlem & DRICI Amani

Defended on 10 / 06/ 2025, before the jury:

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Dedication

With sincere gratitude and appreciation, I dedicate this work to all those who have helped me and guided me until I made it to this point.

To my beloved parents, who instilled in me noble values and provided me with comfort, love, and unlimited support. You have always been my role model, my source of inspiration. Without your countless sacrifices, I would not have made it. I give you all my love, gratitude, and deep respect.

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Ahlem

Dedication

To Amani,

That ambitious little girl, who paved her way with confidence and hope despite all the exhaustion, and walked steadily towards her dream until she became the woman, I'm proud to be today. A woman who doesn't wait for applause, but applauds herself every time she accomplishes something, believes in her dreams, and knows that falling is not the end, but a new beginning and another chance to rise again. She has always whispered to herself firmly, "I know who I am."

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I ask God that this graduation be the beginning of a journey filled with goodness and success.

Amani

Abstract

This work aims to study the antifungal activity of *Laurus nobilis* against food spoilage fungi. The laurel leaves extracts were obtained using simple conventional methods (maceration, decoction, and infusion). The fungi strains used in this study were isolated from various spoiled foods (tomato, yogurt, potato…). The antifungal activity was realized with the well diffusion method on agar media, then the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) for the three extracts obtained. The total phenolic and flavonoid content of laurel leaves was also determined using maceration extract (hydroethanolic). The isolated fungi were *Rhizopus* sp*., Penicillium* sp*., Fusarium* sp. and *Aspergillus niger*. The results of antifungal activity of the previous extracts on these fungal strains were very interesting, an inhibition zone diameter of 20mm against *Fusarium* sp., with maceration extract and 25mm against *Rhizopus sp.* with the infusion extract. MIC and MFC test results conducted to minimum inhibitory and fungicidal concentration of 3.125mg/ml against *Penicillium sp*. (DDTC2.2) with the maceration extract. The results of phenols and flavonoids dosage showed that the Laurel plant we tested contains a considerable and satisfactory amount of total phenolics (55.58 µgEQ/mgE) and flavonoids (18,04 µgEQ/mgE) compared to previous research. The results obtained showed interesting antifungal activity with strongest antifungal effect of the maceration extract compared to the other extracts.

Résumé

Le présent travail porte sur l'étude de l'activité antifongique de la plante *Laurus nobilis* vis-à-vis de champignons de détérioration d'aliments. Les extraits des feuilles du laurier ont été obtenus par les méthodes conventionnelles d'extraction (macération, infusion et décoction). Les souches fongiques utilisées dans cette étude ont été isolées à partir d'aliments altérés (tomates, yaourt, pomme de terre…). L'activité antifongique a été réalisée avec la méthode des puits sur gélose, ainsi que la détermination de la Concentration Minimale Inhibitrice (CMI) et de la Concentration Minimale Fongicide (CMF) pour les trois extraits obtenus. Le contenu en polyphénols et flavonoïdes a été dosé pour l'extrait de macération (hydro éthanolique). Les champignons isolés étaient *Rhizopus sp., Penicillium sp., Fusarium sp*. et *Aspergillus niger*. Les résultats de l'activité antifongique des extraits précédents sur ces souches fongiques étaient très intéressants, avec un diamètre de zone d'inhibition de 20mm contre *Fusarium* sp. avec l'extrait de macération et 25 mm vis-à-vis de *Rhizopus* sp. avec l'extrait d'infusion. Les tests de la CMI et de la CMF ont abouti à la concentration minimale inhibitrice et fongicide de jusqu'à 3,125 mg/ml vis à vis *Penicillium sp*. (DDTC2.2). avec l'extrait de macération. Les résultats du dosage des composes phénoliques et flavonoïdes ont montré que la plante du laurier contient une quantité considérable de polyphénols totaux de (55.58 µgEQ/mgE) et de flavonoïdes totaux de (18,04 µgEQ/mgE). Les résultats obtenus ont montré une activité antifongique intéressante avec un effet antifongique plus intéressant de l'extrait de macération (hydro éthanolique) par rapport aux autres extraits.

ملخص

يهدف هذا العمل إلى دراسة النشاط المضاد للفطريات لنبات الغار (*Laurus nobilis*) ضد فطريات فساد الطعام.

تم الحصول على مستخلصات أوراق الغار باستخدام طرق تقليدية بسيطة (النقع، الغلي، والنقع في الماء). عُزِلَت سلالات الفطريات المستخدمة في هذه الدراسة من أطعمة فاسدة متنوعة (طماطم، زبادي، بطاطس...). تم تحقيق النشاط المضاد للفطريات باستخدام طريقة الانتشار في الأجار، ثم تم تحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MFC) للمستخلصات الثلاثة. تم أيضًا تحديد المحتوى الكلي للفينولات والفلافونويدات في أوراق الغار باستخدام مستخلص النقع (الهيدروإيثانولي). الفطريات المعزولة كانت*Rhizopus sp.* ، *Penicillium sp.*، *Fusarium sp.* و*Aspergillus niger*.كانت نتائج النشاط المضاد للفطريات للمستخلصات السابقة على هذه السلالات الفطرية مثيرة جدًا للاهتمام، على سبيل المثال، منطقة التثبيط لمستخلص النقع بقطر 20 مم ضدFusarium sp ، ومناطق التثبيط لمستخلص النقع بقطر 25 مم ضد *Rhizopus sp.* تم إجراء اختبارات MIC و MFC لتحديد الحد الأدنى من التركيز المثبط والقاتل للفطريات، حيث أظهر مستخلص النقع أكبر تأثير بتركيز 3.125 ملغ/مل ضد *Penicillium sp.* (DDTC2.2). كان المنقوع أقل فعالية، حيث لم يكن له أي تأثير على *Fusarium sp.* و *Aspergillus niger* حتى عند أعلى تركيز 50 ملغ/مل. أظهرت نتائج جرعة الفينولات والفلافونويدات أن نبات الغار الذي اختبرناه يحتوي على كمية كبيرة ومرضية من الفينولات الكلية (55.58 ميكروغرام مكافئ/ملغ من الاستخلاص) والفلافونويدات (18.04 ميكروغرام مكافئ/ملغ من الاستخلاص) مقارنةً بالأبحاث السابقة. أظهرت النتائج التي تم الحصول عليها أن مستخلص النقع كان له أقوى تأثير مضاد للفطريات مقارنةً بالمستخلصات الأخرى.

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MIC: Minimum Inhibitory Concentration

MFC: Minimum Fungicidal Concentration

PDA: Potato Dextrose Agar

SDA: Sabouraud Dextrose Agar

Introduction

Among the global problems people face is food spoilage **(Sevindik & Uysal., 2021)**, and the microbial factor remains the largest contributor **(Snyder & *al.,* 2024).** This spoilage can be the cause of food poisoning or foodborne illness when the spoilage microorganisms are pathogenic or produce toxins. A report by the WHO in 2016 stated that approximately 600 million people worldwide fall ill due to contaminated food, with an estimated 420,000 deaths each year.

Microbial food spoilage is defined as a complex ecological phenomenon resulting from the biochemical activity of microbial chemical processes, where changes occur in the food that make it unsafe for consumption. These microbes include: bacteria (*Enterobacter, Erwinia, Clostridium*), molds (*Aspergillus, Fusarium, Rhizopus, Penicillium*) and yeasts (*Saccharomyces*, *Candida*). **(Nychas & Panagou, 2011).**

Several methods have been used to protect food from disease-causing microbial spoilage, such as storing it at low temperatures, and other alternative methods like high pressure and pulsed electric, etc. However, none of these methods has eliminated undesirable microorganisms. This has led them to rely on extracts from medicinal and aromatic plants to extend its shelf life, as these extracts are considered a crucial component of food safety and quality due to the essential oils produced by secondary metabolism **(Holley & Patel, 2005; Boniface & *al*., 2012; Tuğba, 2023).**

The laurel is considered one of the aromatic medicinal plants used as a food preservative due to its antimicrobial and insecticidal properties. The laurel is an aromatic medicinal herb of significance with potential therapeutic effects thanks to its chemical composition. Its essential oil and some of its parts possess properties that allow its use in various fields, such as medicine, food, pharmaceuticals, and cosmetics **(Kilic & *al*.,2004; Sırıken & *al*., 2018).**

This study aims to evaluate, examine, and investigate the antifungal properties of the noble laurel "*Laurus nobilis*" plant and its effect on microorganisms causing food spoilage. This is due to its known antimicrobial activity and the significant interest this topic currently receives from researchers, to eliminate chemical preservatives and replace them with natural preservatives that are safer for consumer health.

Our research seeks to:

- ✓ Bibliographic part, with general information on *Laurus nobilis* and spoilage microorganisms, most known in the deterioration of foodstuffs.

- ✓ Practical part, with the isolation and purification of some fungi from spoiled food products. The extraction of bioactive molecules and the evaluation of the antifungal activity of noble laurel. Then the study of the phytochemical properties of laurel.

Chapter I: Bibliographic Part

I.1 *Laurus nobilis*

I.1-1 History

Laurus nobilis is the genuine shrub of Greek and Roman mythology **(Paparella et *al.*, 2022)**. The use of bay leaves was not limited to being a spice; they were also considered a medicine and were widely used by Greek doctors. Bay bark helps alleviate liver diseases and dissolve kidney stones, as proven by the study conducted by the Greek physician Dioscorides. Thibault Lespleigney also regarded bay as a proper healing medicine **(Iserin *et al.*, 2001; Gérard et François, 2009).**

I.1-2 Description of *Laurus nobilis*

It is a small aromatic tree that may reach a stature of 20 meters with leaves that are almost 5-8 cm long and 3-4 cm wide. They are dark green, smooth, leathery, glossy green above, and lighter underneath. As for its blooms, they are either elongated or lanceolate with wavy edges. Its flowers are small, yellow, male, and female. When ripe, the fruit is ovoid, black, and 10-15 mm in size **(Iserin *et al.*, 2001; Afifi *et al.*, 1997; Demir *et al.*, 2004; Patrakar *et al.*, 2012)**.

Figure 01: *Laurus nobilis* (Beloud, 2005)

I.1-3 Botanical classification

Laurus nobilis, also called Apollo's laurel or bay laurel, belongs to the Lauraceae family, which has between 2500 and 3500 species **(Dobroslavić *et al.*, 2022)** organized into about 50 genera **(Alejo-Armijo *et al.*, 2017)**.*Laurus azorica* (*Laurus canariensis*), *Laurus nobilis*, and *Laurus novocanariensis* are the three main species that make up the genus Laurus **(Khodja *et al.*, 2023)**.

I.1-4 Chemical Composition of Leaves

Leaves of *Laurus nobilis* are rich in bioactive compounds, of which the principal compounds are phenolics, terpenoids, fixed oils, and essential oils. Phenolic compounds comprise flavonoids like kaempferol and apigenin glycosides, phenolic acids like gallic,

caffeic, rosmarinic acids, flavonols like catechin, epigallocatechin, and phenolic glycosides, the compound that is responsible for its antioxidant activity **(Awada *et al*., 2023).**

I.1-5 Origin and distribution of *Laurus nobilis*

Laurus nobilis is originally from Asia Minor, it is now widespread in Mediterranean regions, the former Yugoslavia, India, and the Maghreb. It grows both wild and cultivated in southern and western Europe. It thrives in humid forests and ravines, such as those in Algiers and Constantine **(Miliani *et al*., 2017)**. Beyond the Mediterranean countries (Algeria, Turkey, Spain, Morocco, Italy, Greece, and Portugal), it also grows in tropical and subtropical Asia, Australia, the Pacific, and South Asia **(Paparella *et al*., 2022).**

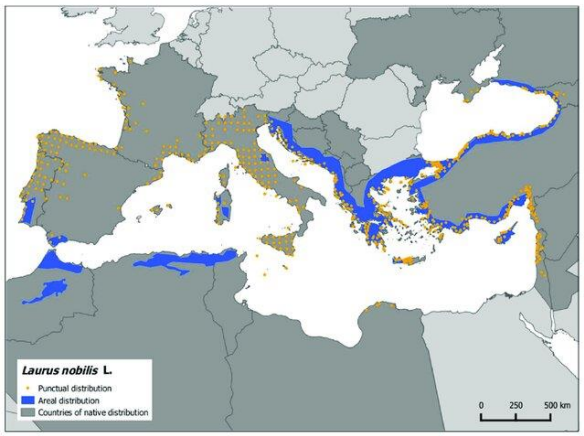


Figure 02: Distribution of *Laurus nobilis* (Source: FAO)

I.1-6 Uses of *Laurus nobilis*

The uses of *Laurus nobilis* vary from one place to another. It has been historically valued in traditional medicine for treating gastrointestinal issues like bloating and flatulence. In Iran, its leaves have been used to alleviate symptoms of epilepsy, neuralgia, and Parkinsonism and treat dermatitis. Turkish folk medicine employs its aqueous extract as an anti-hemorrhoidal, anti-rheumatic, diuretic, antidote for snakebites, and stomachache relief. Moreover, recent studies highlight its potential in diabetes treatment and migraine prevention **(Awada *et al*., 2023).** Industrially, *Laurus nobilis* is important for its dried leaves and essential oils (EOs); they are widely used as a spice and flavoring agent in meat products, soups, and fish, as well as for food preservation due to their antimicrobial and insecticidal properties. Its volatile oil is also utilized in hair lotions and the treatment of psoriasis, thanks to its strong antioxidant and antifungal properties. Given the proven effects of *Laurus nobilis* volatile oil and previous studies, its use in cosmetics can contribute to product functionality and add commercial value. It is valued in both the cosmetic and medicine fields for its anti-inflammatory, analgesic, antioxidant, and antibacterial properties **(Awada *et al*., 2023)**.

I-2. Spoilage Organisms

Spoilage organisms are a group of microbes that cause sensory changes in food, making it inedible (unacceptable for human consumption) due to their growth and metabolism. This spoilage can be either chemical or physical, or it can involve alterations in taste and the appearance of unpleasant odors **(Gram *et al*., 2002)**. These organisms include bacteria (*Clostridium, Xanthomonas, Erwinia, Salmonella, and Escherichia*) and fungi (*Alternaria, Aspergillus, Botrytis, Fusarium,* and *Rhizopus*) **(Kusstatschera *et al.*, 2020).** When consumed, toxins produced by a variety of spoilage microorganisms can result in food poisoning**.**

I-2. 1. Spoilage Fungi

Fungi are prolific food-altering organisms. Most species are saprophytic, expressing extracellular enzymes responsible for the digestion of polymers during vegetative decomposition **(Snyder *et al*., 2019).** Spoilage fungi are either toxigenic, as some types of mold produce toxins called mycotoxins, or pathogenic, causing infections or allergies **(Akinyele et Akinkunmi, 2012).**

Species associated with citrus spoilage are *Penicillium digitatum, Penicillium italicum, Aspergillus niger,* and *Alternaria alternata* **(Cai *et al.*, 2023)**.

Dairy products provide suitable conditions for the growth of fungi. The low pH of cheese supports the growth of yeasts and molds such *as Geotrichum candidum, Candida spp., Penicillium commune, P. nalgivoense,* and *Aspergillus versicolor*. As for yogurt, many microorganisms cause its spoilage, such as *Candida, Pichia, Kluyveromyces, Rhodotorula, Debaryomyces, and Torulopsis* **(Lu et Wang, 2017).**

The high water content in tomatoes makes them more susceptible to microbial damage **(Ghosh, 2009)**. Among the fungi that can damage tomatoes are *Alternaria tenuis, Colletotrichum coccodes, Rhizopus stolonifer andother, Rhizopus spp.,* and *Mucor piriformis.*

There are several genera of fungi that cause potato damage, including *Fusarium sp*., which causes dry rot, and *Pythium ultimum*, which causes leak, in addition to *Alternaria solani*, *Aspergillus*, *Cladosporium*, *Spongospora subterranea f. sp. subterranea*, *Verticillium alboatrum,* and *Verticillium dahliae* **(Idriss *et al.*, 2024).**

Among the most dangerous types of spoilage that bread and bakery products are exposed to is microbial spoilage. Where several types of fungi grow, including *Eurotium, Aspergillus, Penicillium, Cladosporium, Mucor, Rhizopus, Monilia, and Pichia butonii* **(Saranraj et Sivasakthivelan, 2016)**.

I-2. 2. Factors affecting the growth of spoilage microorganisms in food

There are two types of factors that cause fungal spoilage: intrinsic factors and extrinsic factors.

Intrinsic factors:

These are factors that relate to the food itself. These factors are:

- **Nutrient content of the food:** Food contains nutrients (carbohydrates, proteins, and lipids) that support the growth of microbes, as the latter use these nutrients as sources of energy, carbon, nitrogen, minerals, and vitamins.
- **pH value:** Low-pH foods are more susceptible to spoilage by yeasts and molds compared to bacteria.
- **Water activity:** Foods with high water content and a water activity above 0.90 are more prone to bacterial spoilage (**Hamad, 2012),** while yeast and mold proliferate at an aw > 0.62, which includes moderately moist foods **(Zhao *et al*., 2020).**
- **Antimicrobial substances:** The antimicrobial substances present in foods, added to them, or formed during processing work to prevent or delay the growth of spoilage microorganisms Among these antimicrobial agents are allicin (in garlic), eugenol (in spices), preservatives, bacteriocins, and enzymes **(Hamad, 2012)**.

Extrinsic factors:

These are factors that relate to the environment in which the food is stored.

- **Temperature:** Temperature is a major factor in food spoilage. The temperature range in which microbes grow varies from below zero degrees Celsius to over 100 degrees Celsius (**Zhao *et al*., 2020).**
- **Humidity:** Humidity can cause food spoilage and affect its condition by altering its water activity, as high moisture supports the growth and development of microorganisms in food **(Zhao *et al*., 2020).**

I-2. 3. The colonization process

There are many mechanisms that fungi use to spoil food, including enzymatic degradation, metabolic activity, and the production of secondary metabolites **(Pouris *et al*., 2024).** The infection process follows the development of the appressorium. Fungi adhere to the surface of foods and produce enzymes **(Akinro *et al.*,2015**). These lytic enzymes degrade the polymers (proteins and lipids) present in food, leading to the release of water and other components. Fungi use these as nutrients for their growth **(Barth *et al*., 2009).** These byproducts cause undesirable bad flavors and odors **(Pouris *et al*., 2024).**

I-3. Antifungal activity of Laurus nobilis

The growing issue of microbial resistance is a major concern worldwide due to the limited number of antifungal drug classes and the increasing number of patients requiring antifungal treatment**. (Dammak *et al*., 2019).** Fungal infections are responsible for the morbidity and mortality of thousands annually. In Africa for example, cryptococcosis causes 624,700 deaths annually, while another 250,000 deaths occur annually due to aflatoxin ingestion **(Belasli et al., 2020; Dammak *et al*., 2019).**

Researchers have directed their studies nowadays toward the discovery of natural substances with greater efficacy and lower toxicity **(Dammak *et al*., 2019)**, some of these studies applied on *Laurus nobilis* proved that it contains important bioactive compounds with antimicrobial, antitumor, and anti-inflammatory properties. **(Bayar et *al.*, 2018; Rizwana et al., 2019)**. Several researches have proved the effectiveness of *Laurus nobilis* against a wide range of fungi, including *Alternaria spp., Aspergillus spp., Bipolaris sp., Candida spp., Cryptococcus spp., Fusarium spp.,* and *Sclerotinia spp.* **(Bayar et *al.*, 2018; Belasli et *al.*, 2020; Dammak et *al.*, 2019; Rizwana et *al*., 2019)**.

Chapter II: Materials and Methods

II.1. Materials

II.1.1. Plant

The laurel leaves were harvested from Bejaia (Algeria) in late January 2025.

Figure 03: *Laurus nobilis* sample (original)

II.1.2. Fungal strains

The samples (tomato, potato, yogurt, bread, and canned tomato) were gathered and stored at room temperature with moderate humidity, observed daily until they showed spoilage signs. Once they were spoiled, they were transferred to the laboratory for fungi isolation.

Figure 04: Samples from which spoilage fungi were isolated (Original)

Table I: Food material used and spoilage conditions

Food used	Conditions favoring mold growth	References
Bread	-Room temperature - High humidity - Slices or cut pieces - Stored in a sealed plastic container while it is still warm (freshly baked) - Left for 5 days	(Legan, 1993) (Nuhu et Yusuf., 2023) (Pateras, 1998) (Thilanka, 2019)
Yogurt (brand Faida, produced by Hodna factory)	- Room temperature - Limited ventilation - Left for 5 days	(Buehler *et al*., 2018) (Shi, 2022) (Zubairi *et al*., 2021)

Potato	- Stored in a warm place - High humidity - Wounds on the surface of the potato - Limited ventilation - Stored in a semi-closed plastic bag - Left for 20 days	(Doan et Davidson, 2000) (Idriss *et al*., 2024) (Rupp et Jacobsen, 2017) (Muhammad *et al*., 2023)
Full fresh tomato and half a fresh tomato	- High humidity - Room temperature - Exposure to sunlight and air - Must be ripe, soft, and juicy - Left for 7 days	(Ghosh, 2009) (Kalyoncu et Oskay, 2014) (Parnell *et al*., 2004) (Van Laanen et Scott, n.d.)
Canned tomato (brand Izdihar)	- Room temperature - Exposure to air (opened can) - Left for 10 days	(Mariutti et Soares, 2009) (Morka *et al*., 2025) (Ogofure, 2015)

II.1.3. The reactants and equipment used

Table II: The reactants and equipment used

The reactants	**Equipment used**
• Ethanol • Methanol • PDA (Agar-agar, glucose) • SDA • Distilled water • Physiological water	• Petri dishes • Autoclave • Incubator • Rotary evaporator • Filter paper

II.2. Methods

II.2.1. Isolation of spoilage fungi

II.2.1.1. The preparation of culture medium PDA

In 300 ml of distilled water, 20 g of agar-agar was dissolved and stirred well. 200 g of potatoes were weighed, peeled, and chopped, mixed with 300 ml of distilled water, and boiled for 20 to 25 minutes at 100 °C. Then the two preparations were mixed. Distilled water was added to the previous mixture until the total volume reached 1000 ml. The mixture was sterilized. The resulting solution was transferred to Petri dishes **(Kinshasa, 2009).**

Petri dishes containing Potato Dextrose Agar (PDA) were inoculated with spoilage fungus samples (from potato, tomato, bread, and yogurt, canned tomato) using flame-sterilized forceps and then incubated at 28°C for 7 days **(Nareen et *al.*, 2016)**. After 7 days, the fungal colonies were purified by subculturing with an inoculation needle into the sterile medium.

II.2.2. Extraction of crude extract

The laurel leaves were dried using the air-drying method. They were placed in boxes on the table at 28°C (room temperature), away from sunlight, for 20 days. Afterward, they were ground and stored in glass vials **(Silva et *al.*, 2020).**

II.2.2.1. Maceration

Hydroethanolic extract was prepared using 10g of laurel powder mixed with a solvent mixture of 70 mL ethanol and 30 mL water. The homogenates were filtered using 3mm filter paper. Using a rotary evaporator set at 40°C, the filtrates obtained in the previous step were condensed under a vacuum. The resulting dark powder is the hydroethanolic extract **(Mathieu et *al.*, 2014).**

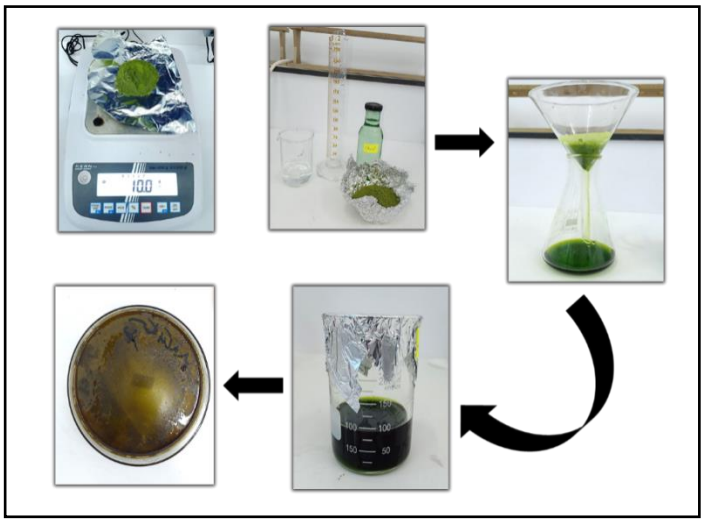


Figure 05: Extraction protocol of hydroethanolic extract (original)

II.2.2.2. Decoction

A flask was filled with 100 ml of distilled water, and 10 g of laurel leaves powder was added. Then this mixture was boiled for 30 minutes. The mixture was left to cool, then filtered and concentrated using a rotary evaporator under vacuum at 50 °C. Then the extract was placed for 24 hours at 37°C in the oven **(Bohui et *al.*, 2018).**

II.2.2.3. Infusion

100 ml of distilled water was boiled, then 10 g of laurel powder was added, then the mixture was left for 30 min to cool, then filtered and concentrated using a rotary evaporator under a vacuum set at 50°C. In the oven, the extract was placed for a whole day at 37°C **(Bohui et *al.*, 2018).**

Crude extracts were dissolved in methanol and then stored in tubes at 4°C until use.

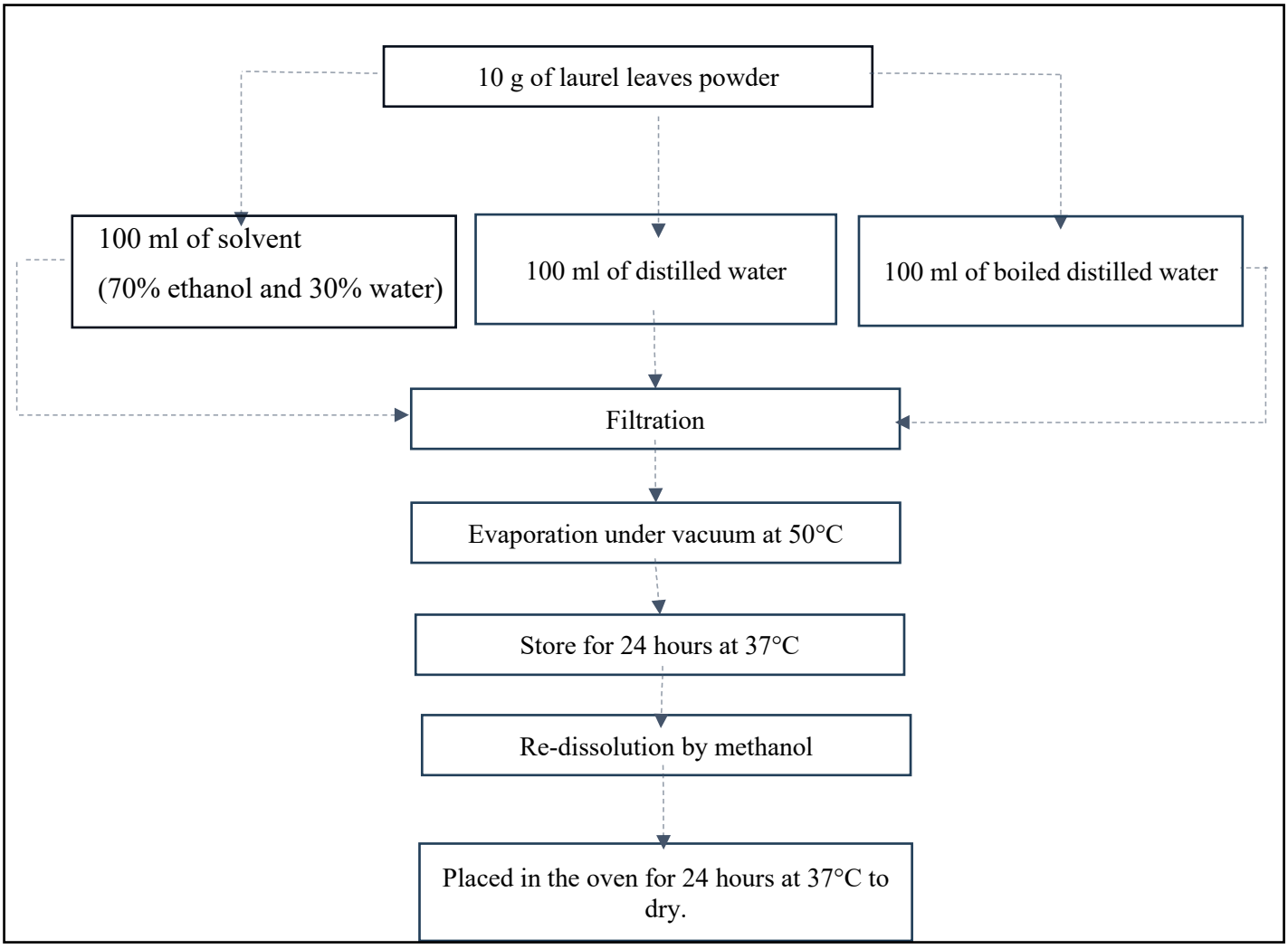


Figure 06: Extraction Protocol Diagram

II.2.3. Determination of extraction yield

The dry extract weight was determined by evaporating the solvent and measuring the remaining solid mass.

The dry extract weight was calculated using the following equation:

Wr = Wb – Wa

Wr: Residue weight

Wa: Empty container weight

Wb: Container weight with the residue

While the yield of crude extract is calculated using the equation:

Y% = (WME \ WPM) x 100

Y% : Yield

WME: Weight of the methanolic crude extract (g)

WPM: Weight of plant material (g)

II.2.4. Determination of Antifungal Activity

II.2.4.1. Agar Well Diffusion Method

The surface of the agar plate was inoculated by spreading fungal inoculum (0.5 McFarland) over the entire agar surface. Then, the agar was punctured with a 6 mm diameter using the open end of a sterile Pasteur pipette, making 4 wells in each plate. These wells were filled with 50µl of plant extracts (maceration, decoction, and infusion) and methanol (control). Subsequently, the agar plates were incubated at 28°C for 24 to 72 hours. The plates were examined at 24, 48, and 72h, during which the plant extracts and methanol spread in the agar medium and inhibited fungal growth **(Balouiri et *al.*, 2016).**

II.2.5. Determination of the Minimum Inhibitory Concentration (MIC)

Each test tube was filled with 2 mL of sterile PDA broth. then, 1 mL of each extract was added to the first tube, and a serial ten-fold dilution was performed up to reach the following concentrations in descending order: 50, 25, 12.5, 6.25, and 3.125 mg/ml. After that, 20 µL of fungal suspension was added to all tubes **(Benkova et *al.*, 2020; Schumacher et *al.*, 2018)**. Positive control tubes were prepared by adding 20 µL of fungal suspension to 2 mL of PDA broth. while negative control tubes contained the PDA broth and 1 ml of extract only **(Radetsky et *al.*, 1986).** All tubes were incubated at 28C°, and observations were made after 24, 48, and 72 hours. The presence of turbidity indicated fungal growth, while clear tubes indicated inhibition or death of the fungi. All tubes were carefully compared with both positive and

negative control tubes to verify fungal growth by assessing the level of turbidity or clarity in each sample **(Benkova et *al*., 2020).**

II.2.6. Determination of Minimum Fungicidal Concentration (MFC)

Following 72 hours of incubation, small volumes from the clear tubes were carefully streaked in straight lines onto Sabouraud agar plates using sterile cotton swabs. The plates were then incubated at 28C° for 4 days.

A complete absence of fungal growth along the streaked lines indicated a fungicidal effect. In contrast, the appearance of fungal growth indicated that the concentration used was fungistatic and insufficient to eliminate the fungus (**Espinel-Ingroff et *al*., 2002; Maurya et *al*., 2019).**

II.2.7. Dosages of Secondary metabolite

II.2.7.1. Determination of Total Phenolic Content

The total phenolic content of *Laurus nobilis* leaves was determined according to the protocol of **Singleton and Rossi (1965)** with some modifications. By dissolving 2 mg of maceration extract in 1 ml of ethanol, then adding 1 ml of diluted Folin-Ciocalteu reagent (1 ml in 9 ml of water) and mixing the solution thoroughly. After 4 minutes, 800 µl of sodium carbonate (600 mg in 8 ml of water) was added to the mixture, which was then shaken and incubated for 1 hour at room temperature, protected from light. The absorbance was then measured at 765 nm. The results are obtained from the gallic acid calibration curve, prepared according to the same procedure; the same standard working solutions were therefore used in a range of concentrations.

II.2.7.2. Determination of Total Flavonoid Content

The total flavonoid content was determined according to **Lamaison and Carnat (1990)** with some modifications, using the aluminum chloride colorimetric method. A 4 ml ethanolic extract containing 4 mg was prepared and dissolved in 4 ml of ethanol. Then, 1 ml of a 2% aluminum chloride solution was added, mixed, and incubated for 40 minutes at room temperature away from light. The absorbance was measured at 415 nm. The results were obtained from the calibration curve of Quercetin, which was performed according to the same operating procedure with a range of concentrations **(Djeridane et *al.*, 2006).**

Chapter III: Results and discussion

III.1 Results

III.1.1 Extraction results

III.1.1.1 Properties of the obtained powder

The powder obtained from grinding dried laurel leaves is characterized by a dark green color, a soft and uniform texture, and a robust aroma, as laurel is known to be an aromatic herb. The extract was obtained using 3 different methods, each yielding distinct properties. As shown in the table:

Table III: Laurel extracts characteristics

Method / Properties	Aspect	Odor	Texture
Maceration	Dark green liquid	Very aromatic and intense	Viscous
Decoction	Greenish-brown	less fragrant than maceration	Watery
Infusion	Greenish-orange	Characteristic of bay laurel	Watery

III.1.1.2 Determination of Extraction Yield

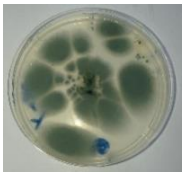
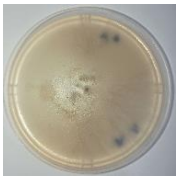
The yield obtained from the ethanolic extract was 11,8%, while that of the infusion extract was 2,62%, and the decoction yield was 12%.

III.1.2 Results of fungi isolation and identification

III.1.2.1 Macroscopic identification

The results of the macroscopic aspects of the fungi strains isolated are presented in the table IV bellow:

Table IV: Macroscopic identification of isolated fungus

	Macroscopic aspect	**Description of the aspect**	**Isolation source**
DDT2		Fast-growing, with extensive and rapidly spreading colonies, capable of covering an entire Petri dish quickly. Very cottony texture. Initially, it's white, but turns gray over time due to spore production.	Tomato
DDTC2.2		Circular colonies appear as a white fluffy mass that later turns blue-green with a white border.	Canned tomato
DDTC3.2		This fungus rapidly spreads on the surface of PDA medium and is characterized by a powdery appearance and an olive-green color resulting from the formation of dense spores.	Canned tomato
DDY1		The colonies of this fungus appear almost circular, with a powdery texture and a green color, while the peripheral areas are translucent/ whitish. These colonies are numerous and spread throughout the culture.	yogurt
DDPOT1		Presents a white to pink mycelium with colonies showing white hyphae radiating outward to give a spider web appearance.	potato
DDPI1.1		In the PDA, these fungi grew within a short period at 25 °C. Their color turned black during formation (black spores).	Bread

III.1.2.2 Microscopic identification

III.1.2.2.1 DDT2

This fungus was isolated from altered tomatoes. Microscopic observation showed the presence of long, thick, unsegmented fungal filaments **(Figure 06)**. The end of these filaments are large, dark spheres (sporangia) containing spores that are released when the walls of the sporangia rupture. By examining the macroscopic and microscopic appearance and comparing it with reference images **(Figure 06c, d)** and similar fungi causing tomato damage, we identified these fungi as *Rhizopus* sp.

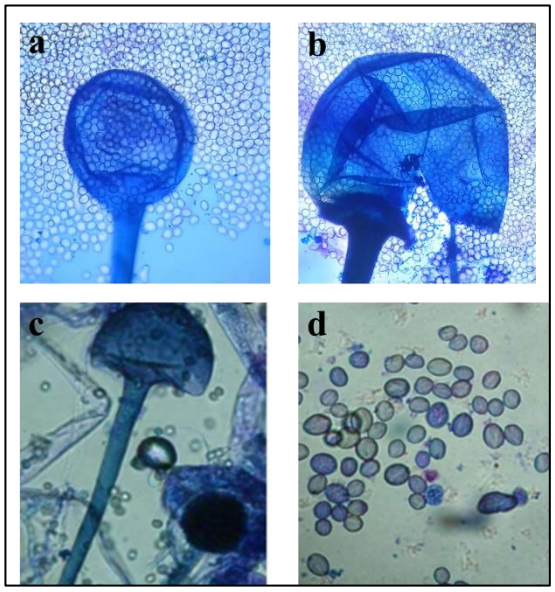


Figure 07: Microscopic observation of *Rhizopus* sp. (DDT2) (a, b) (x 40) (Original). *Rhizopus oryzae* under microscope (c,d) **(Ibatsam et *al*., 2019).**

III.1.2.2.2 DDTC2.2

A fine and dense mycelium with brush-like structures called “Phialides” and small round spores (Conidia) **(Figure 07)**. Compared to the reference image and the information previously collected, according to the observed characteristics, this fungus belongs to the *Penicillium* genus, and can be one of the *Penicillium* species.

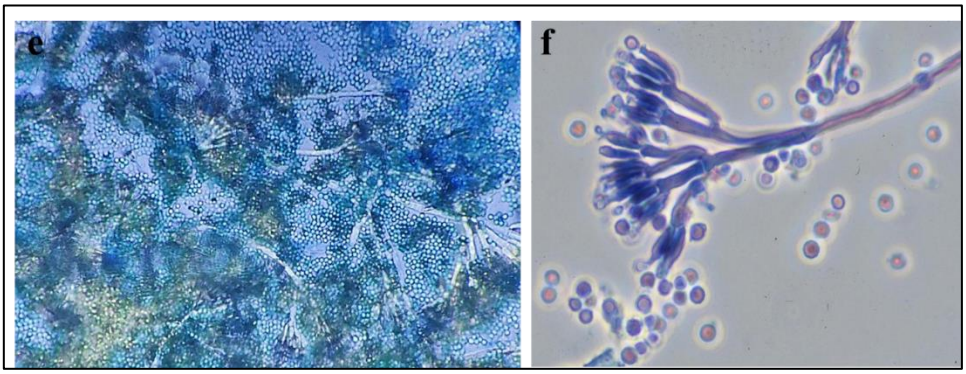


Figure 08: Microscopic observation of *Penicillium* sp. (DDTC2.2) (e) (x 40) **(Original)**. *P. chrysogenum* under the microscope (f) **(Ogórek et al., 2020).**

III.1.2.2.3 DDTC3.2

This fungus was isolated from canned tomato and is characterized by short, segmented fungal filaments that end in branched heads resembling brushes. They are surrounded by numerous spores that spread widely and abundantly **(Figure 08)**. According to these characteristics, this fungus can be classified as *Penicillium* sp.

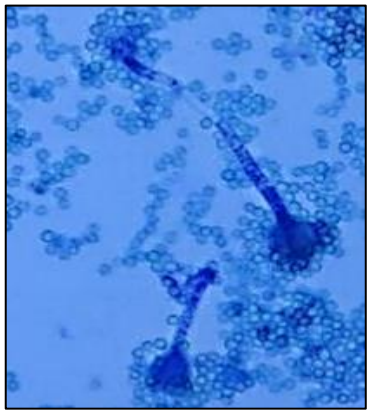


Figure 09: Microscopic observation of *Penicillium* sp. (DDTC3.2) under microscope (x 40) **(Original).**

III.1.2.2.4 DDY1

The fungus was isolated from spoiled yogurt and was found to contain densely branched mycelium, consisting of tubular hyphae, and conidiophores terminating in brush-like structures. Furthermore, it was observed to contain countless, widely distributed spores (conidia) **(Figure 09)**. Based on all the above characteristics, this fungus can be identified *as Penicillium* sp.

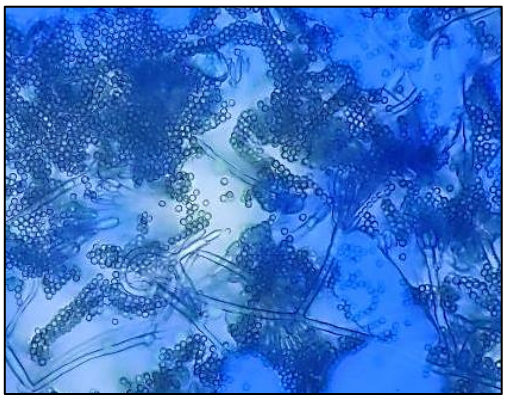


Figure 010: Microscopic view of *Penicillium* sp. (DDY1) (x 40) **(Original).**

III.1.2.2.5 DDPOT1

This fungus was isolated from damaged potatoes. The microscopic examination showed that the spores of this fungus are different and distinct from the spores previously observed, as their shape resembles a sickle with divisions (2 to 3 septa). There are also oval-shaped spores

and round cells with thick walls **(Figure 10g).** Compared to the reference image **(Figure 10h).** of the *Fusarium* genus, it can be said that the sickle-shaped spores are (Macroconidia), while the oval spores are (Microconidia), and the round cells are (Chlamydospores). This fungus is identified as *Fusarium* sp.

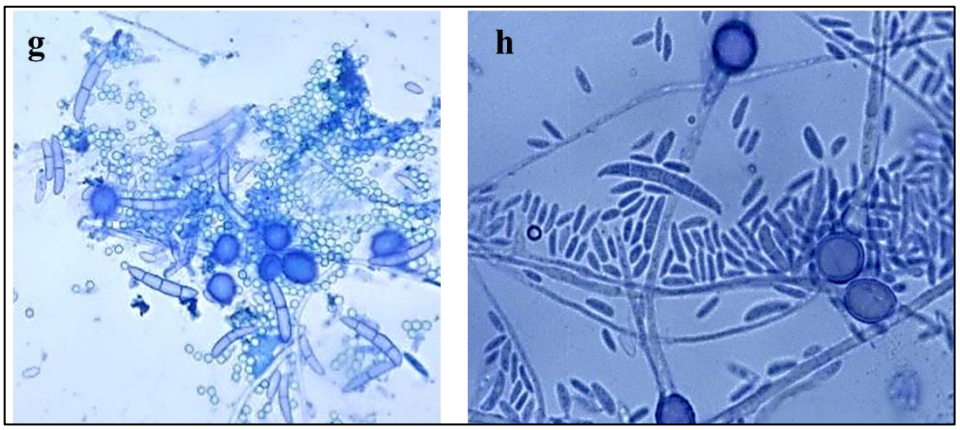


Figure 11: Microscopic observation of *Fusarium* sp. (DDPOT1) (g) (x 40) **(Original).** Microscopic observation of *Fusarium oxysporum* f.sp. melonis (h) **(Snyder & Hansen.,1940).**

III.1.2.2.6 DDPI1.1

This fungus was isolated from moldy bread. After adding the methylene blue dye and placing the fungus under the microscope, a dark, semi-circular, central mass was observed at the tip of a thick vertical filament, with small granules visible on it. In the background, thin and branched filaments appear **(Figure 11)**. Compared to the structure of *Aspergillus niger*, it can be said that the semi-circular mass is a Conidial Head and that the thin threads covered with spores are hyphae. As for the small granules, they are represented by dense chains of spores (Conidia), and the thick filament is the Conidiophore. So, this fungus is *Aspergillus niger*.

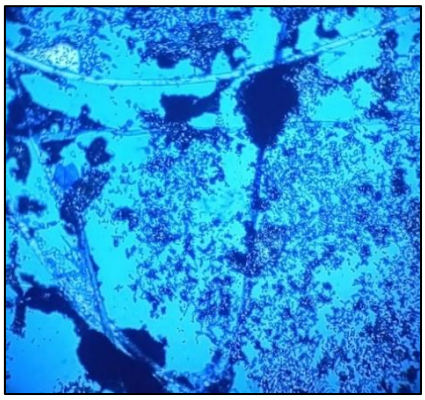


Figure 12: Microscopic observation of *Aspergillus niger* (DDPI1.1) (x 40) **(Original).**

III.1.3 Evaluation of the antifungal activity

The antifungal activity of *Laurus nobilis* leaf extracts was evaluated using the agar-well diffusion method on previously isolated fungi in SDA medium.

To reveal the antifungal activity of laurel leaf extracts, the presence or absence of fungal growth around the wells was observed, along with measuring the diameters of the inhibition zones. The obtained results were converted into the bar chart shown below **(Figure 12)**.

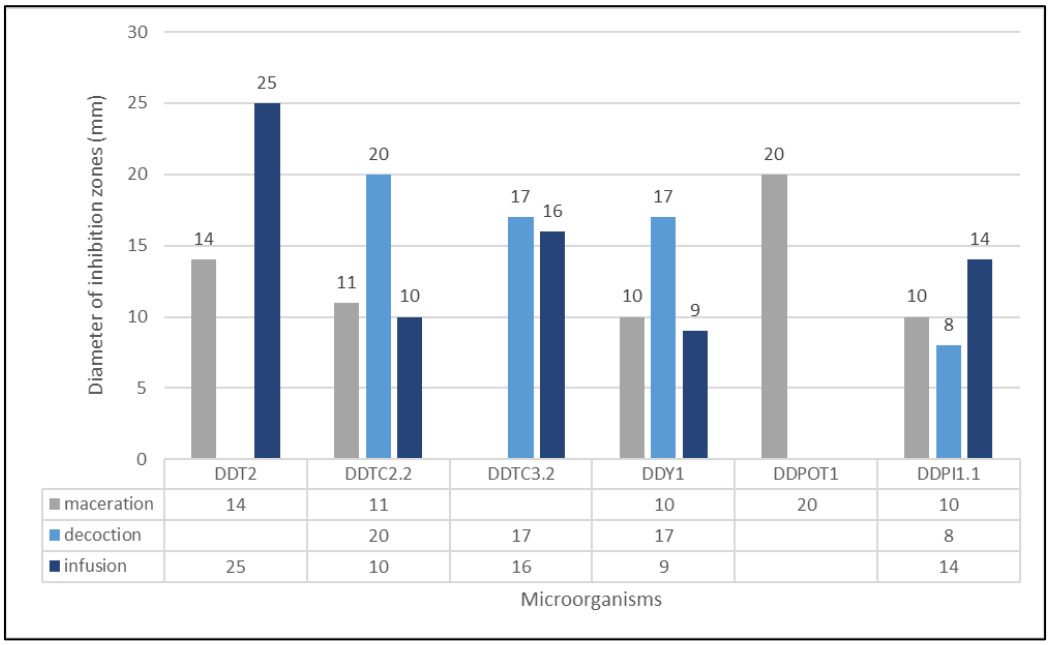


Figure 13: Diagram of antifungal activity of *Laurus nobilis* against spoilage fungi.

The antifungal activity test after 24 hours showed that the infusion and maceration extracts exhibited activity against *Rhizopus* sp. (DDT2), as this effect appeared as an inhibition zone with a diameter of 25mm and 14mm, respectively **(Figure 13)**. However, this inhibition was temporary as the inhibition zones became smaller in diameter and the fungus spread throughout the entire dish. While the inhibition zone shown by the decoction extract is almost negligible.

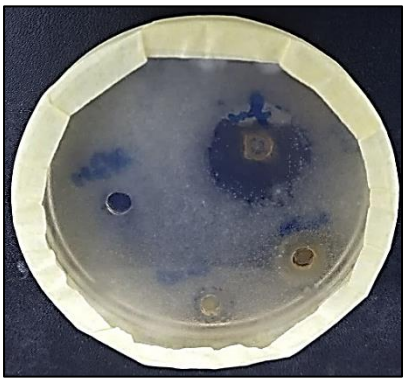


Figure 14: the antifungal activity against *Rhizopus* sp. (DDT2) **(original).**

Regarding (DDTC2.2), the results of the antifungal activity test after 24 hours showed varying effects for all extracts, where inhibition zones appeared around all wells containing inhibitory extracts, and their diameters were 11mm for the maceration extract, 20mm for the decoction, and 10mm for the infusion.

The results of the antifungal activity test against (DDTC3.2) showed a complete absence of inhibition zones around the well of the maceration extract, while inhibition zones appeared around the wells of the decoction and infusion extracts, with diameters of 17mm and 16mm, respectively **(Figure 14)**. Over time, the diameters of the inhibition zones became smaller as the fungus grew around the wells.

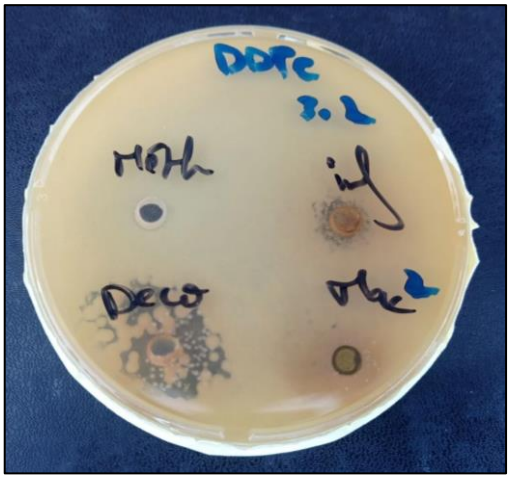


Figure 15: The antifungal activity against *Penicillium* sp. (DDTC3.2) **(Original)**.

Concerning the antifungal activity against DDY1, inhibition zones were observed around all wells with different diameters. The maceration extract had a diameter of 10mm, while the decoction had a diameter of 17mm, indicating a stronger effect. The infusion had a lesser effect with an inhibition zone diameter of 9mm. Over time, the inhibition zones became smaller due to fungal growth.

For *Fusarium* sp. (DDPOT1), the maceration extract is the only one that showed an effect, with an inhibition zone of 20mm in diameter. This effect remained stable for a long time. Meanwhile, the other extracts didn't affect this fungus **(Figure 15)**.

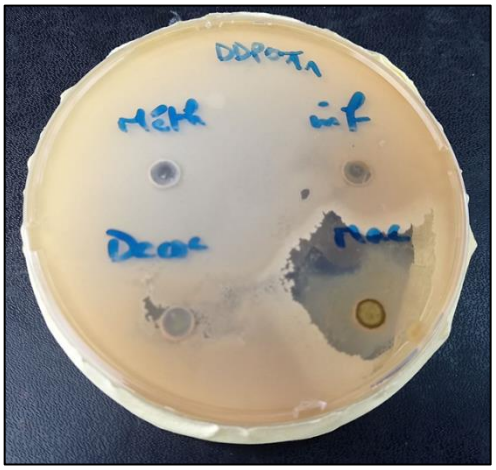


Figure 16: The antifungal activity test on *Fusarium* sp. (DDPOT1) **(Original).**

After 48h, the antifungal activity test against (DDPI1.1) showed different inhibitory effects of laurel extracts; the infusion extract exhibited an inhibition zone of 14mm. The maceration extract showed an inhibition zone of 10mm. An inhibition zone was also observed around the well of the decoction extract with a diameter of 8mm.

III.1.4 Determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC showed variable antifungal activity depending on the extract and fungal species **(Figure 16)**. The infusion extract managed to inhibit the growth of *Fusarium* sp. at 25mg/mL, whereas maceration and decoction required 50mg/mL to inhibit fungal growth. Both infusion and maceration extracts inhibited *Aspergillus niger* at 25mg/mL, while decoction extract was effective only at 50mg/mL. Regarding *Rhizopus* sp., infusion and maceration extracts were active at 12.5mg/mL, whereas decoction extract permitted slight regrowth at 25mg/mL, indicating a higher MIC. For *Penicillium* sp. (DDTC2.2), infusion extract was inhibitory at 12.5mg/mL, decoction extract at 25mg/mL, while maceration extract was effective at all tested concentrations, indicating a MIC ≤ 3.125mg/mL. *Penicillium* sp. (DDTC3.2) was completely inhibited by both infusion and decoction extracts at all concentrations tested (≤ 3.125 mg/mL), and by maceration extract at 12.5mg/mL. Finally, *Penicillium* sp. (DDY1) showed sensitivity to infusion extract at 12.5mg/mL, and to both maceration and decoction extracts at 25mg/mL.

Each tube was carefully compared with positive and negative controls to confirm fungal presence or inhibition based on turbidity**.**

Table V: MIC values of the different extracts against fungal strains

Fungal strain	**Infusion MIC (mg/mL)**	**Maceration MIC (mg/mL)**	**Decoction MIC (mg/mL)**
Fusarium sp.	25	50	50
Aspergillus niger	25	25	50
Rhizopus sp.	12.5	12.5	50
Penicillium sp. (DDTC2.2)	12.5	≤3.125	25
Penicillium sp. (DDTC3.2)	≤3.125	12.5	≤3.125
Penicillium sp. (DDY1)	12.5	25	25

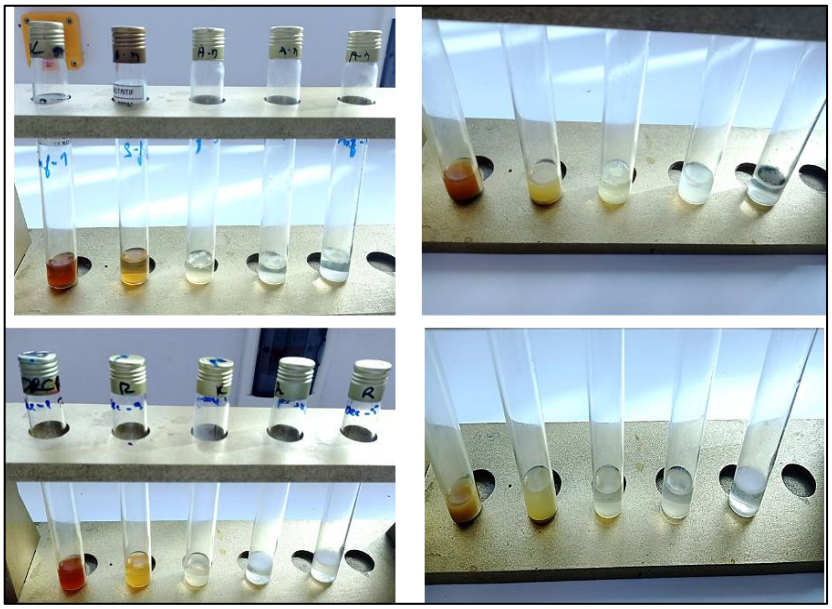


Figure 17: Minimum Inhibitory Concentration test results **(Original).**

III.1.5 Determination of Minimum Fungicidal Concentration (MFC)

The MFC analysis highlighted different levels of fungicidal potency among the extracts. Fungicidal concentration against *Fusarium* sp. for all the three extracts was 50mg/mL. A regrowth was observed for *Aspergillus niger* even at the highest infusion extract concentration, indicating no fungicidal activity, whereas maceration and decoction extracts were fungicidal at 25mg/mL. *Rhizopus* sp. did not regrow with any extract that had been re-cultured from the clear tubes, suggesting that fungicidal action starts from 12.5 mg/mL with maceration and infusion extracts, and is higher (25mg/mL) with decoction extract. All of the tested concentrations of

the maceration extract were fungicidal against *Penicillium* sp. (DDTC2.2), so its MFC was 3.125mg/mL, while the infusion extract was fungicidal at 12.5 mg/mL, and the decoction one at 25 mg/mL. For *Penicillium* sp. (DDTC3.2), some regrowth was observed with infusion and decoction extracts at 6.25 mg/mL and at 12.5 mg/mL and lower concentrations, which determines their MFC values in order 12.5 mg/mL and 25 mg/mL, respectively, while maceration MFC was indicated at 12.5 mg/mL. Finally, *Penicillium* sp. (DDY1) was eliminated by both infusion (12.5 mg/mL) and decoction (25 mg/mL) extracts, while maceration allowed regrowth at 25 mg/mL, indicating that its MFC was at 50 mg/mL.

Table VI: MFC values of the different extracts against fungal strains

Fungal strain	**Infusion MFC (mg/mL)**	**Maceration MFC (mg/mL)**	**Decoction MFC (mg/mL)**
Fusarium sp.	50	50	50
Aspergillus niger	/	25	25
Rhizopus sp.	12.5	12.5	25
Penicillium sp. (DDTC2.2)	12.5	≤3.125	25
Penicillium sp. (DDTC3.2)	12.5	12.5	25
Penicillium sp. (DDY1)	12.5	50	25

III.1.6 Determination of Phenolic Compounds

This test aims to detect the content of polyphenolic and flavonoid compounds present in this plant through reactions that depend on the phenomenon of coloration by specific reagents (Folin-Ciocalteu and Quercetin). The results obtained for the dosage of polyphenolic compounds are expressed in µg of gallic acid equivalent per mg of extract (µg EQ/mgE) and µg of quercetin equivalent per mg of extract (µgEQ/mgE) for the flavonoid compounds.

III.1.6.1 Total polyphenol content

Based on the calibration curve of gallic acid and the equation (Y=0.0074x-0.0215, R^2=9827), we found that each mg of the extract contains 55.58 µg of gallic acid equivalent.

Table VII: Absorbance of the extract and Polyphenols content.

Abs of the extract	Polyphenols content
0,801	55.58 µgEQ/mgE

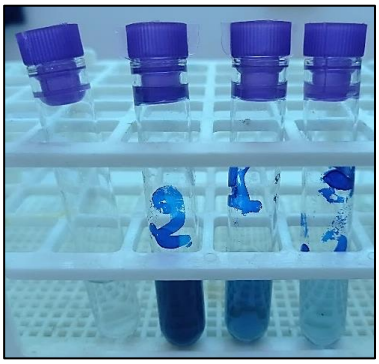


Figure 18: Polyphenol assay results (original)

III.1.6.2 Flavonoid content

The extract's absorbance was 0.155, which corresponds to a flavonoid content of 18.04 µg of quercetin equivalent per mg of extract. This indicates that the extract contains a good percentage of flavonoid compounds.

Table VIII: Absorbance of the extract and flavonoid content.

Abs of the extract	flavonoid content
0,155	18,04 µgEQ/mgE

III-2. Discussion

The study we conducted aims to evaluate the effect of laurel plant extracts on food spoilage fungi, and to determine the polyphenol and flavonoid content. This is within the framework of discovering the important properties of *Laurus nobilis* to utilize it in preserving food from spoilage and relying on it instead of chemical preservatives.

Among the three extraction methods, maceration had the strongest antifungal effect, in both the diffusion assay and MIC/MFC tests. It maintained high fungicidal effect across most tested fungal species at different concentrations, down to 3.125 mg/mL against *Penicillium* sp. (DDTC2.2), and showed a stable inhibition zone of 20mm against *Fusarium* sp. The maceration extract doesn’t involve heat, so heat-sensitive compounds such as essential oils and flavonoids

stay preserved and intact **(Pandey et Tripathi, 2014; Verep *et al*., 2023)**. These compounds are known to disrupt fungal cell membranes, inhibit enzyme activity, and interfere with spore germination **(Mohamed et Al-Azawi, 2022; Silveira *et al*., 2022)**, which likely explains their strong effect.

On the other hand, the infusion method was less effective against the tested fungal strains. While it initially produced notable inhibition zones such as 25mm against *Rhizopus* sp. and 16mm against *Penicillium* sp. (DDTC3.2), these effects often diminished over time, as observed with *Rhizopus* sp., where the inhibition zone shrank and the fungus recolonized the plate. Similar to that in MIC/MFC tests, it was able to inhibit the growth of certain fungi such as *Rhizopus* sp and *Penicillium* sp. (12.5 mg/mL), but didn't affect stronger and more resistant strains such as *Fusarium* sp. and *Aspergillus niger* on the same level (50 mg/mL). In those cases, fungal strains reappeared after subculturing from clear tubes, confirming a fungistatic rather than fungicidal activity. The possible reason may be due to the partial degradation or evaporation of volatile compounds during the hot water steeping process **(Silveira *et al*., 2022).**

Decoction gave an intermediate result. It produced moderate inhibition zones and was able to affect the majority of strains at high concentrations (50 and 25 mg/mL). However, similarly to infusion, it was less effective compared to maceration extract, most likely because of the thermal breakdown of some antifungal agents due to the heat **(Verep et *al*., 2023).** Still, this method may favor the extraction of water-soluble and heat-stable compounds like phenolic acids and saponins **(Pandey et Tripathi, 2014; Verep *et al*., 2023)**, which might explain its partial effectiveness.

The fungal species tested gave different antifungal susceptibility. *Rhizopus* sp. showed high sensitivity to the extracts, especially to maceration and infusion. This sensitivity is probably due to the fungus's relatively thin cell wall and high metabolic rate **(Meussen *et al*., 2012; Farooq, 2023),** which could make it more prone to damage from antifungal compounds. In contrast, a different study that used the essential oil of the same plant revealed that *Rhizopus* sp. was extremely resistant **(Belasli et *al.*, 2020)**, suggesting that *Laurus nobilis* contains active antifungal compounds against *Rhizopus sp*. in the polar or hydrophilic fractions, which are more effectively extracted by maceration and infusion methods.

However, both *Aspergillus niger* and *Fusarium* sp. have a great resistance against *Laurus nobilis* compounds, which corroborate with the results of Belasli et *al.*, (2020). All extracts effect were temporary against *Aspergillus niger*. Only maceration extract showed measurable antifungal activity (20mm inhibition zone, which remained stable over time). Still,

another study proved that the same species was strongly inhibited by the essential oil of *Laurus nobilis* flowers **(Mssillou et *al.*, 2020)**, highlighting the importance of choosing the right plant part; Against *Fusarium* sp., neither infusion or decoction created any visible inhibition zone, and gave very limited results in MIC/MFC tests. These fungi are characterized by their robust spore structures and thick cell walls rich in chitin and glucans **(Schoffelmeer et *al.*, 1999; Brauer et *al.*, 2023; Qu et *al.*, 2024)**, which can prevent antifungal substances from penetrating. In addition, *Fusarium* species are known to produce mycotoxins **(Ponts, 2015; Qu et *al.*, 2024)** and use defensive mechanisms such as active efflux pumps and stress response pathways **(Ponts, 2015),** which may need higher concentrations of bioactive compounds for effective control.

The three *Penicillium* strains showed different sensitivity to laurel extracts. *Penicillium* sp. (DDTC2.2) was the most sensitive one to all three extracts, with no signs of regrowth even at lower concentrations, however, *Penicillium* sp. (DDTC3.2) resisted both infusion and maceration extracts up to 12.5mg/mL and decoction up to 25mg/mL. Whereas *Penicillium* sp. (DDY1), although it showed no regrowth with infusion and decoction extracts, was not eliminated by maceration extract. These variations may reflect strain-specific genetic characteristics that impact cell wall permeability or metabolic resistance **(Kanashiro et *al.*, 2020; Tannous et *al.*, 2020; Fierro et *al.*, 2022)**.

Similar studies have reported that *Laurus nobilis* has high antifungal activities against *Candida* spp., *Aspergillus solani, and Cryptococcus* spp. **(Fernandez-andrade et *al.*, 2016; Bayar et *al.*, 2018)**, *Alternaria alternata* **(Rizwana et *al.*, 2019)** and high antibacterial activity against *Saccharomyces cerevisiae* **(Fidan et *al.*, 2019**); it even gave a better effect against some microorganisms than tetracycline antibiotics **(Fidan et *al.*, 2019)**.

The antifungal activity of *Laurus nobilis* cannot be attributed to a single compound, as it has a complex mixture of major and minor constituents that act synergistically. Previous studies proved that even the minor compounds contribute significantly to its antifungal activity. This complicity in composition reduces the likelihood of resistance development, unlike synthetic preservatives **(Belasli et *al.*, 2020).** Many researchers tried to understand the mechanism of action of laurel extracts and essential oil, however, it is still unclear, and only suggestions and hypotheses have been made **(Mssillou et *al.*, 2020)**. The chemical composition of laurel varies with genotype, plant age, plant part, geographic origin, environmental conditions, extraction methods, etc., which all influence its bioactivity **(Fidan et *al.*, 2019; Belasli et *al.*, 2020).** Studies have shown that the higher the level of phenolic compounds, the

stronger the antifungal effects **(Fernandez-andrade et *al.*, 2016)**. Moreover, its effectiveness in food preservation depends on the food matrix, fungal load. Moisture and storage conditions **(Belasli et *al.*, 2020).**

The results of the total polyphenol and flavonoid dosage showed polyphenol content of 55.58 µgEQ/mgE, and the flavonoid content of 18,04 µgEQ/mgE. The content of our plant in polyphenols and flavonoids is higher compared to the quantities obtained by Kivrak et *al.* (2017), where the polyphenol content was found to be 25.32±0.10 (µg PEs/mg extract), and the flavonoid content was 8.60±0.12(µg QEs/mg extract)., Altın et *al.* (2025) study revealed polyphenols and flavonoids content of 22.72 and 57.36 (µg GAE/ QE mg extract), respectively. Mohamed & Al-Azawi (2022) found that the polyphenol content in the methanolic extract of laurel is about 28.60 ± 0.12 (mg/g). Taroq et *al*. (2018) found that the polyphenol content in the ethanolic extract is about 284.11±3.71 mg GAE/g. The difference between the values we obtained in our experiment and the previously mentioned studies is due to several reasons. Phenolic compounds in laurel leaves, including polyphenols and flavonoids, undergo several quantitative and qualitative variations. This is attributed to various factors such as the extraction method, the harvesting area, and the solvent used for extraction **(Khodja et *al*. 2023).**

Conclusion

This research aimed to evaluate the effectiveness of *Laurus nobilis* leaf extracts prepared by infusion, decoction, and maceration as natural antifungal agents against food spoilage fungi. With dangerous foodborne illnesses affecting millions of people every year due to microbial contaminations, the need for more natural and safer alternatives to synthetic preservatives has increased. Furthermore, there is a growing demand from public for chemical-free ways to preserve food to the importance of such research.

The results from various tests (antifungal activity, MIC and MFC) showed that the extraction method plays a critical role in how effective *Laurus nobilis* is. Out of the three methods, maceration gave the best results. It showed strong fungicidal activity against a range of spoilage fungi such as *penicillium* sp. (DDTC 2.2) with MFC of 3.125 mg/mL, *Penicillium* sp. (DDTC3.2) and *Rhizopus* sp. at 12.5 mg/mL, likely because it preserves heat-sensitive compounds like essential oils and flavonoids, which play a crucial role in disrupting fungal growth.

On the other hand, decoction and infusion weren't as effective, pointing to the need to carefully select extraction techniques to maximize bioactivity. Also, the different results between minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) further emphasizes the complexity of antifungal activity, as some extracts showed a fungistatic effect rather than a fungicidal one, for example, infusion extract inhibited *Aspergillus niger* at a MIC of 25 mg/mL, but regrowth was observed at the same concentration in MFC testing, indicating a fungistatic rather than fungicidal effect. This insight is critical for developing practical applications that ensure complete elimination of spoilage fungi in food systems.

These results indicated that *Laurus nobilis* extracts (maceration particularly) as a natural antifungal agent that might be used for food preservation due to its high content of phenolic and flavonoid compounds, with total phenolics (55.58 µgEQ/mgE) and flavonoids (18,04 µgEQ/mgE). This alternative meets the increasing consumers demand for chemical-free preservatives, as well as providing eco-friendly solutions for increasing food safety and extending shelf life of food products. They could be used in many forms, including sprays for fresh produce, additives in food packaging materials, or treatments in food storage systems.

The results obtained are promising, however, to apply *Laurus nobilis* extracts in food preservation more researches must be done. The identification and quantification of the particular antifungal compounds, and evaluation of their stability, toxicity and sensory effects.

Additionally, confirming their effectiveness in real food systems, since their performance can be influenced by interactions with fats, proteins, and other components.

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Abstract

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The laurel leaves extracts were obtained using simple conventional methods (maceration, decoction, and infusion). The fungi strains used in this study were isolated from various spoiled foods

Abstract

This work aims to study the antifungal activity of *Laurus nobilis* against food spoilage fungi.

The laurel leaves extracts were obtained using simple conventional methods (maceration, decoction, and infusion). The fungi strains used in this study were isolated from various spoiled foods (tomato, yogurt, potato…). The antifungal activity was realized with the well diffusion method on agar media, then the determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicidal Concentration (MFC) for the three extracts obtained. The total phenolic and flavonoid content of laurel leaves was also determined using maceration extract (hydroethanolic).

The isolated fungi were *Rhizopus* sp*., Penicillium* sp*., Fusarium* sp. and *Aspergillus niger*. The results of antifungal activity of the previous extracts on these fungal strains were very interesting, an inhibition zone diameter of 20mm against *Fusarium* sp., with maceration extract and 25mm against *Rhizopus sp.* with the infusion extract. MIC and MFC test results conducted to minimum inhibitory and fungicidal concentration of 3.125mg/ml against *Penicillium sp*. (DDTC2.2) with the maceration extract.

The results of phenols and flavonoids dosage showed that the Laurel plant we tested contains a considerable and satisfactory amount of total phenolics (55.58 µgEQ/mgE) and flavonoids (18,04 µgEQ/mgE) compared to previous research.

The results obtained showed interesting antifungal activity with strongest antifungal effect of the maceration extract compared to the other extracts.

Résumé

Le présent travail porte sur l'étude de l'activité antifongique de la plante *Laurus nobilis* vis-à-vis de champignons de détérioration d'aliments.

Les extraits des feuilles du laurier ont été obtenus par les méthodes conventionnelles d'extraction (macération, infusion et décoction). Les souches fongiques utilisées dans cette étude ont été isolées à partir d'aliments altérés (tomates, yaourt, pomme de terre…). L'activité antifongique a été réalisée avec la méthode des puits sur gélose, ainsi que la détermination de la Concentration Minimale Inhibitrice (CMI) et de la Concentration Minimale Fungicide (CMF) pour les trois extraits obtenus. Le contenu en polyphénols et flavonoïdes a été dosé pour l'extrait de macération (hydro éthanolique).

Les champignons isolés étaient *Rhizopus sp., Penicillium sp., Fusarium sp*. Et *Aspergillus niger*. Les résultats de l'activité antifongique des extraits précédents sur ces souches fongiques étaient très intéressants, avec un diamètre de zone d'inhibition de 20mm contre *Fusarium* sp. avec l'extrait de macération et 25 mm vis-à-vis de *Rhizopus* sp. avec l'extrait d'infusion. Les tests de la CMI et de la CMF ont abouti à la concentration minimale inhibitrice et fongicide de jusqu'à 3,125 mg/ml vis à vis *Penicillium sp*. (DDTC2.2). avec l'extrait de macération. Les résultats du dosage des composes phénoliques et flavonoïdes ont montré que la plante du laurier contient une quantité considérable de polyphénols totaux de (55.58 µgEQ/mgE) et de flavonoïdes totaux de (18,04 µgEQ/mgE).

Les résultats obtenus ont montré une activité antifongique interessante avec un effet antifongique plus intéressant de l'extrait de macération (hydro éthanolique) par rapport aux autres extraits.

ملخص

يهدف هذا العمل إلى دراسة النشاط المضاد للفطريات لنبات الغار (*Laurus nobilis*) ضد فطريات فساد الطعام.

تم الحصول على مستخلصات أوراق الغار باستخدام طرق تقليدية بسيطة (النقع، الغلي، والنقع في الماء). عُزلَت سلالات الفطريات المستخدمة في هذه الدراسة من أطعمة فاسدة متنوعة (طماطم، زبادي، بطاطس...). تم تحقيق النشاط المضاد للفطريات باستخدام طريقة الانتشار في الأجار، ثم تم تحديد التركيز المثبط الأدنى (MIC) والتركيز القاتل الأدنى (MFC) للمستخلصات الثلاثة. تم أيضًا تحديد المحتوى الكلي للفينولات والفلافونويدات في أوراق الغار باستخدام مستخلص النقع (الهيدروإيثانولي).

الفطريات المعزولة كانت *Rhizopus sp.* ، *Penicillium sp.*، *Fusarium sp.* و *Aspergillus niger.* كانت نتائج النشاط المضاد للفطريات للمستخلصات السابقة على هذه السلالات الفطرية مثيرة جدًا للاهتمام، على سبيل المثال، منطقة التثبيط لمستخلص النقع بقطر 20 مم ضدFusarium sp ، ومناطق التثبيط لمستخلص النقع بقطر 25 مم ضد *Rhizopus sp.* تم إجراء اختبارات MIC و MFC لتحديد الحد الأدنى من التركيز المثبط والقاتل للفطريات، حيث أظهر مستخلص النقع أكبر تأثير بتركيز 3.125 ملغ/مل ضد *Penicillium sp.* (DDTC2.2).كان المنقوع أقل فعالية، حيث لم يكن له أي تأثير على *Fusarium sp.* و *Aspergillus niger* حتى عند أعلى تركيز 50 ملغ/مل.

أظهرت نتائج جرعة الفينولات والفلافونويدات أن نبات الغار الذي اختبرناه يحتوي على كمية كبيرة ومرضية من الفينولات الكلية (55.58 ميكروغرام مكافئ/ملغ من الاستخلاص) والفلافونويدات (18.04 ميكروغرام مكافئ/ملغ من الاستخلاص) مقارنةً بالأبحاث السابقة.

أظهرت النتائج التي تم الحصول عليها أن مستخلص النقع كان له أقوى تأثير مضاد للفطريات مقارنةً بالمستخلصات الأخرى.