ORIGINAL ARTICLE

Antimicrobial Activity of *Lepidium sativum* Ethanolic Extract Against *Madurella.mycetomatis*

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Abstract

Mycetoma is a chronic subcutaneous infection caused by fungi (Eumycetes) or bacteria from the phylum Actinomycetes. This condition, characterized by granulomatous inflammation and the formation of grains containing aggregates of the causative organisms, can extend to the underlying bone, causing severe complications. The grains, discharged through multiple sinuses, are diagnostic of the condition. Treatment of eumycetoma is particularly challenging, requiring a combination of antifungal therapy and surgical debridement. Azoles, including imidazoles (e.g., Ketoconazole) and triazoles (e.g., Itraconazole), are the primary antifungal agents used, although their efficacy varies. This study aims to isolate and identify *Madurella mycetomatis* from specimens using both mycological and molecular techniques, followed by an evaluation of the antimicrobial activity of extracts from the plant *Lepidium sativum* against the isolated pathogen. Black grains were obtained from suspected specimens of eumycetoma, and mycological examination confirmed the presence of broad fungal hyphae and chlamydospores embedded in a brown cement-like substance. The isolate was confirmed as *Madurella mycetomatis* using PCR. Plant extracts were prepared from *Lepidium sativum* seeds using petroleum ether, chloroform, and ethanol. The separation of constituents of the ethanolic extract of the *Lepidium sativum* by using the BAW system of thin layer chromatography gave thirteen components. The Rf was measured for each constituent. The ethanolic extract showed significant antifungal activity against *Madurella mycetomatis*, with a minimum inhibitory concentration (MIC) of 6.25 mg/ml, while extracts from petroleum ether and chloroform showed no activity. Thin-layer chromatography was used to separate the components of the ethanolic extract, revealing thirteen distinct components. The study demonstrates the potential antifungal efficacy of *Lepidium sativum* against *Madurella mycetomatis*, although the higher MIC compared to standard antifungal drugs like Ketoconazole suggests a need for further isolation and purification of the active compounds. This paper aims to investigate for the first time the antifungal properties of *Lepidium sativum* against *Madurella mycetomatis*, providing a foundation for future studies.

Keywords

Madurella mycetomatis, Lepidium sativum, thin layer chromatography, BAW system, Agar dilution method, Ethanolic extract

INTRODUCTION

Mycetoma is a long-lasting subcutaneous infection that can be caused by either actinomycetes bacteria or fungi[1]. This infection triggers a granulomatous inflammatory reaction in the deep layers of the dermis and subcutaneous tissue, which may spread to the underlying bone. Mycetoma is characterized by the formation of grains containing aggregates of the causative organisms that may be discharged onto the skin surface through multiple sinuses[2]. Mycetoma can arise from bacteria classified within the phylum Actinomycetes or from fungi termed Eumycetoma[3,4]. Over 20 different species of fungi and bacteria are known to be causative agents of mycetoma. Table 1 below categorizes these bacterial and fungal species based on the characteristic colors of discharge observed from infected wounds[5].

The treatment of eumycetoma is challenging[6]. Anti-fungal therapy has to be combined with debridement (surgical removal of infected tissue) when possible. Various antifungal drugs have been improved for the treatment of eumycetoma, mainly the azole group[7]. Azoles are synthetic compounds of anti-fungal agents which include: imidazole and triazole[8]. These differ with respect to their chemical structures, the imidazole consists of Ketoconazole, Miconazole, and Clotrimazole, while the second group includes Itraconazole, Fluconazole, and new Voriconazole[9–11].

Table 1. Bacterial and fungal species causing mycetoma[5]

Mycetoma species	Grains Discharged Colour's
Actinomadura pelletieri	Red discharge
Acremonium strictum Actinomadura madurae Aspergillus nidulans Noetestudina rosatii Phaeoacremonium krajdenii Pseudallescheria boydii	White or Yellow discharge
Aspergillus terreus Curvularia lunata Cladophialophora bantiana Exophiala jeanselmei Leptosphaeria senegalensis Madurella grisea Madurella mycetomatis Pyrenochaeta romeroi	Black discharge
Nocardia asteroides Nocardia brasiliensis	Yellow discharge
Streptomcyes spp (Streptomcyes Somaliensis	Yellow or red discharge

Cress (*Lepidium sativum*), often called garden cress to differentiate it from other plants with similar names, is a fast-growing, edible herb[12]. The name "cress" comes from the old Germanic word *cresso*, meaning sharp or spicy. This herb is closely related to watercress and mustard, sharing their distinctive peppery and tangy taste and aroma. In various regions, it is also referred to as mustard and cress, garden pepper cress, pepperwort, peppergrass, or poor man's pepper[13,14].

Lepidium sativum has long been used in traditional Indian medicine to treat various ailments[15]. A cold infusion of its seeds is commonly used to alleviate hiccups, while the seeds themselves are beneficial in cases of chronic liver and spleen enlargement and serve as a carminative adjunct to purgatives. When bruised and mixed with lime juice, the seeds are applied topically to relieve inflammatory and rheumatic pains. The seeds possess a variety of properties, including bitter, thermogenic, depurative, rubefacient, galactagogue, emmenagogue, tonic, aphrodisiac, and diuretic[16]. They are also useful as poultices for sprains and are applied in the treatment of leprosy, skin diseases, dysentery, diarrhea, splenomegaly, and asthma[17].

A seed powder mixed with fine sugar is an effective remedy for indigestion, diarrhea, and dysentery[18]. Another household preparation made from seeds, butter, and sugar is commonly used as a restorative for general debility[18,19]. To relieve flatulence and boost milk secretion in postpartum women, seeds are boiled in milk to form a soft, thin mass, to which sugar or jaggery is added to create a confection. This mixture is also beneficial for treating sexual debility, leucorrhea, and lower back pain caused by rheumatism[20].

The possible mechanism of action of quinoxaline derivatives likely involves multiple pathways that target bacterial cell structures and metabolic processes. Quinoxalines are known to disrupt bacterial DNA and generate reactive oxygen sepsis (ROS)[21,22].

This paper, to the best of our knowledge, is the first to aim at the isolation and identification of *Madurella mycetomatis* from specimens utilizing both mycological techniques (direct examination and culture methods) and molecular techniques (PCR). Following the successful isolation, plant materials will

be extracted using petroleum ether, chloroform, and ethanol as solvents. The study will then evaluate the activity of antimicrobial of these plant extracts against the isolated *Madurella mycetomatis*.

MATERIALS AND METHODS

MADURELLA MYCETOMATIS ISOLATION

Specimens Collection

Cultures of *Madurella mycetomatis* were prepared from black grains from five specimens with eumycetoma attending to the Mycology Lab, "National Health Laboratory". The black grains were collected in sterile normal saline using sterile swabs, and the infection was confirmed by mycological examination and molecular technique (PCR).

Mycological Examination

Collected grains from the specimens were washed with normal saline mixed with 20% Chloromphenicol several times to remove the bacterial contamination, and then The grains were examined microscopically in wet preparation using 20% KOH.

Then the grains were cultured on ordinary blood agar and were incubated at 37°C. The resulting growth was cultured onto slopes of Sabouraud's dextrose agar containing 0.1% Chloromphenicol and incubated at 37°C for 2-3 weeks. Lastly, for more mycologically confirmation, the needle mount technique was used for each culture isolate.

Molecular PCR Examination

The isolates were identified specifically as *Madurella mycetomatis* using a specific primer with PCR technique[21].

DNA Extractions and Purification (CTAB DNA Protocol)

DNA was extracted from all isolates using CTAB (hexa decyltrimethyl ammonium bromide, Sigma, U.S.A). The isolates were subcultured on Sabouraud's agar and incubated for 10 days at 37°C. About 1cm of the mycelia was scraped and ground to a fine powder with liquid nitrogen in a sterile mortar and pestle. The powdered mycelia were transferred to a propylene tube, and 4ml of CTAB lysis buffer (2% CTAB, 100 mM Tris-HCL, 10mM EDTA, 0.7 M NaCl) was added, which has been pre-heated at 65°C. The mycelia were dispersed gently using a pipette, and immediately, 40 µl of 2-mercaptoethanol was added. The tubes were incubated at 65°C for 30 minutes and inverted every 10 minutes to ensure adequate mixing. After incubation, 4 ml of chloroform/isoamyl alcohol (24 ml chloroform: 1 ml isoamyl alcohol V/V) was added to each tube. The tubes were shaken for 20 minutes, and different phases were separated by cold centrifugation at 9500 rpm for 20 minutes. The aqueous phase was removed to new tubes, and an equal volume of chloroform/isoamyl alcohol was added again. The centrifugation step was repeated, and the aqueous phase was separated, and 0.5 volume isopropanol was added to it. The tubes were left on ice for 5 minutes and the DNA was precipitated by cold centrifugation at 9500 rpm for 20 minutes. The pellets were washed with 70% ethanol and centrifuged. The pellets were left to dry at room temperature by inverting the tubes on tissue paper for 30 minutes. Finally, the pellets were re-suspended into 100µl Tris EDTA (TE) buffer (10mM Tris-HCL, 1mM EDTA). The extracted DNA was stored at -20°C pending usage for PCR.

DNA Quantification (Gene Quant pro, England)

In a separate micro centrifuge tube (1.5 ml), 10 µl DNA was mixed with 90 µl H_2O. The combining mixture was vortexed and left to stand for 10 min at room temperature to ensure complete diffusion of DNA throughout the solution. This represents 1/10 dilution. Total genomic DNA concentration was determined using a Gene Quant spectrophotometer (Amersham) according to the manufacturer's recommendation. The absorbance was measured at 260 nm, and the purity of the samples was further assessed by calculating the 260/230 and 260/280 ratios.

PCR Test

PCR was carried out using two *Madurella mycetomatis* species-specific primers, 26.1 A and 28.3 A (Alpha DNA, Montreal, Canada, USA).

The sequences of the two primers were:

- [5'-AATGAGTTGGGCTTTAACGG-3']
- [3'TCCCTGTGATGTGATGGCCCT-5'] respectively.

The reaction volumes of the PCR test were 50µl per sample in 0.5 ml thin-walled PCR tubes. The PCR mixture consisted of 5µl of 10X reaction buffer (500mM KCl, 100 mM Tris-HCl, 0.1% Triton™X-100) (Vivantis, Malaysia), 5µl of 10mM dNTP mix (Vivantis, Malaysia),

3µl of 25mM MgCl2, 3µl from each two primers (100 picomol each), 0.3 µl of thermo-stable DNA polymerase (Vivantis, Malaysia), and 5 µl of template DNA(using telsar mimi-v/PCR). Then the PCR mixtures were completed to 50 µl with double distilled water. The PCR program consisted of 40 cycles on a PCR thermocycler (Bio-Rad). Initial denaturation was done by holding the tubes at 94°C for 4 minutes. Each cycle consisted of denaturation at 94°C for 1 minute, annealing of primers at 58°C for 1 minute, and enzymatic extension at 72°C for 1 minute. The PCR products were examined by electrophoresis in 1% a garose gel stained with ethidium bromide.

Preparation of Crude Extracts of the Plant

Plant material

The *Lepidium sativum* seeds were obtained from the Omdurman market and identified by a taxonomist in the botany department, Faculty of Sciences, Khartoum University. The seeds were cleaned, shade-dried, and powdered by a mechanical grinder.

Extraction Methods

About 1000 gm of *Lepidium sativum* seed was extracted according to Harborne with Petroleum ether, Chloroform, and Ethanol in Soxhlet apparatus for 36 hr, respectively. The extract obtained was filtrated by using filter paper. The extracts were then concentrated under reduced pressure at a controlled temperature of 40-50°C using a rotary evaporator. The crude extracts were stored in a refrigerator at 4°C until further analysis.

In vitro sensitivity test of the isolates of Madurella mycetomatis to the herbal extracts

The sensitivity of the isolates of *Madurella mycetomatis* to the herbal extracts was performed using the agar dilution method. Five hundred milligram herb extracts (petroleum ether, chloroform, and ethanol) were dissolved in 2 ml of DMSO then dissolved in 18 ml of melted Sabouraud's dextrose agar medium at 42˚C. Serial dilutions of this preparation were done by adding 10 ml of this preparation to 10 ml of melted Sabouraud's dextrose agar medium at 42°C and repeating this procedure to other tubes. By doing so, the final concentration obtained was 0.0976mg/ml. Two control media were prepared, one with relevant solvent (DMSO) and the other without solvent. The media were left in a slanting position and left to solidify. The fungus was inoculated by taking a small portion of the *Madurella mycetomatis*, about 2x2 mm, from the periphery of the colonies and then inoculated on the agar surface. After inoculation, cultures were incubated at 37˚C for 15 days. The test was read when good growth was seen in the control bottles, and the minimum inhibitory concentrations (MIC) were estimated by the comparison with the controls[22].

Thin layer chromatography technique

The technique was used to separate the plant constituent by using different solvent systems. The procedure was carried out on aluminum foil coated with a thin layer of silica gel as the stationary phase. A small drop of the sample solution was applied to the plate, approximately 1.5 centimeters from the bottom, using a capillary. The solvent was allowed to fully evaporate to avoid interference with the sample's interaction with the mobile phase in the subsequent step. This process was repeated to ensure sufficient analyte was present at the starting spot for a visible result. Then, the plate was inserted into a screw-top jar containing a small amount of an appropriate solvent (mobile phase) to a depth of less than 1 centimeter. When the solvent had risen to near the top of the plate via capillary action, the plate was removed and dried, and the spots were visualized by projecting ultraviolet light onto the sheet. Chemical processes can also be used to visualize spots; a mixture of sulfuric acid and vanillin was used to clear the reaction, then the thin layer chromatography plate was incubated in a hot air oven at 110˚C for a few to clear the reaction[23]. To obtain clear separation of the components, different solvent systems were used: ethyl acetate + chloroform (8:2), chloroform + petroleum ether (7:3), chloroform + ethanol (7:3), ethanol + chloroform (1:1), chloroform + ethanol (1:1), and the BAW system (n-butanol + acetic acid + water, 4:1:5, with two layers used).

RESULTS

MYCOLOGICAL EXAMINATION

All five specimens showed positive results in the direct microscopic examination of the grains. Broad fungal hyphae and chlamydospores embedded in a brown cement-like substance were observed. All strains were cultured from black grains on blood agar, and Sabouraud's dextrose agar gave positive cultures. On blood agar, the isolates produced white to grey mycelia. On the Sabouraud's dextrose agar, the fungus produced brown pigments, and the colonies themselves were brown, buff to yellow, or grey in colour. Colonies were

flat; some developed raised centers. They were velvety or short cottony. Needle mount of all isolates grown on Sabouraud's dextrose agar revealed broad segmented, hyphae with terminally and itercallary chlamydospores.

MOLECULAR PCR EXAMINATION

Fungal DNA samples extracted with the DNA extraction CTAB method gave positive PCR results with one sample (No 4) compatible with *Madurella mycetomatis*. The isolate showed an amplicon, which estimated roughly to be 420 bp according to the DNA ladder based on the PCR product size. The isolates were identified as *Madurella mycetomatis* (Figure 1)

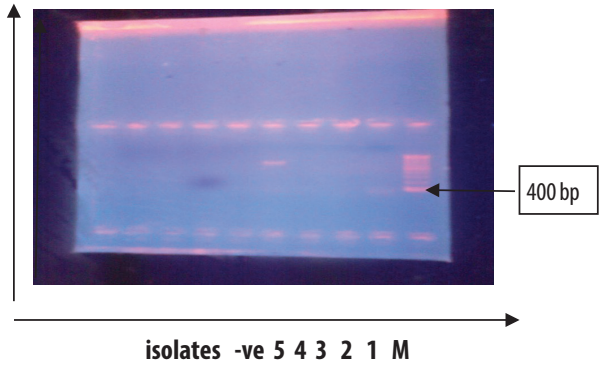

Figure 1. PCR amplification of *Madurella mycetomatis* isolates using primer 26.1A and 28.3A·

Lanes 1 to 5 show *Madurella mycetomatis* isolates; lane labeled M contains 50 b.p.DNA lader; the arrow indicates 400 b.p, and lane label (-ve) contains negative control.

Lane labeled 4 contains a DNA sample that gave a positive result compatible with *Madurella mycetomatis*. The lanes labeled 1, 2, 3, 5 contain DNA samples not compatible with *Madurella mycetomatis.*

IN VITRO SENSITIVITY TEST OF MADURELLA MYCETOMATIS TO THE HERBAL EXTRACTS (AGAR DILUTION METHOD)

The *Madurella mycetomatis* isolate was tested against the *Lepidium sativum* extracts (petroleum ether, chloroform, and ethanol). The ethanolic extract of *Lepidium sativum* showed high activity with MIC 6.25mg/ml (Figure 2), while the Petroleum ether and chloroform extracts did not show activity against *Madurella mycetomatis*.

Figure 2. *In vitro* antifungal susceptibility of *Madurella. Mycetomatis on Sabouraud's dextrose agar containing different concentrations* of ehanolic extract showed high activity with MIC 6.25 mg/ml (from left to right 50,25,12.5,6.25,3.12,1.6,0.78, 0.39,0.195,0.0976 mg/ml respectively). The first two tubes from the left control tubes, one for the viability of fungi and the other for the activity of DMSO).

THIN LAYER CHROMATOGRAPHY

Different systems were used to separate the constituent of ethanolic extract of the *Lepidium sativum*. The best system that gave the best separation was the BAW system (Figures 3 and 4). Thirteen components were separated. Some of them were fluorescent with the long UV light; eight of them gave a blue fluorescent color, and one of them gave a faint red colour. The Rf is measured for each constituent (Table 2).

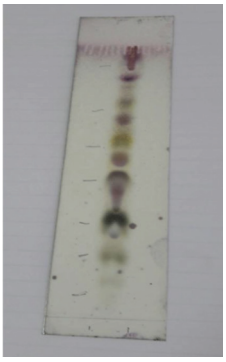

Figure 3. Thin layer chromatography techniques using the BAW system for separation of ehanolic extract components (mixture of sulfuric acid and vanillin was used to clear the components spots).

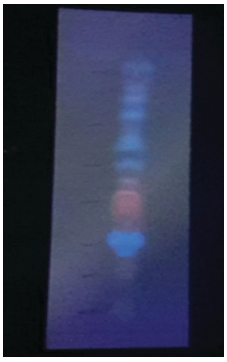

Figure 4. Thin layer chromatography techniques using the BAW system to separate the ehanolic extract components (Long U.V light was used to clear the component's spots)

Table 2. The Rf value of ethanolic extract components by using the BAW system

Component	Rf Value	Observation
1	1.8/16.5 = 0.10	No fluorescence (Long U.V light)
2	3/16.5 =0.18	No fluorescence (Long U.V light)
3	4.2/16.5= 0.25	Fluoresced deep blue colour (Long U.V light)
4	4.7/16.5= 0.28	Fluoresced deep blue colour (Long U.V light)
5	5.3/16.5= 0.32	Faint red (Long U.V light)
6	6.7/16.5= 0.41	Faint red (Long U.V light)
7	7.8/16.5= 0.47	Faint red (Long U.V light)
8	9/16.5= 0.55	Blue colour (Long U.V light)
9	10/16.5= 0.60	Blue colour (Long U.V light)
10	12.7/16.5= 0.69	Blue colour (Long U.V light)
11	12.7/16.5=0.76	Blue colour (Long U.V light)
12	14.5/16.5= 0.87	Blue colour (Long U.V light)
13	15.5/16.5= 0.93	Blue colour (Long U.V light)

DISCUSSION

In this study, for the first time, we showed that the ethanolic extract of *Lepidium sativum* plant showed high activity against *Madurella mycetomatis* with MIC 6.25 mg/ml. The research affirms the antifungal efficacy of the ethanol-based extract originating from this specific plant, a conclusion supported by the discoveries made by Solomon et al., who studied the anti-fungal activity of Lepidium sativum against three types of fungi (Aspergillus niger, Fusarium oxysporum, and *Fusarium solani*)[24]. Furthermore, this outcome aligns with the findings reported by another study, wherein they investigated the antifungal effectiveness of the ethanol-based extract against nine distinct fungal strains (*Aspergillus parasiticus, Aspergillus niger, Yersinia aldovae, Candida albicans, Aspergillus effusus, Fusarium solani, Macrophomina phaseolina, Saccharomyces cerevisiae* and *Trichophyton rubrum*)[25]. Other findings are also in agreement with the finding of[26], who tested the antifungal activity of ethanolic extract of Lepidium sativum seeds against Fusarium equiseta, Aspergillus flavus, and *Alternaria* alternate. A similar finding was obtained in another study that studied *in vitro* antifungal and antibacterial activity of ethanolic extracts of *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds, and *Terminalia chebula* fruits against nine fungal strains (*Aspergillus parasiticus, Aspergillus niger, Yersinia aldovae, Candida albicans, Aspergillus effusus, Fusarium solani, Macrophomina phaseolina, Saccharomyces cerevisiae,* and *Trichophyton rubrum*)[25].

The ethanolic extract of *Lepidium sativum* exhibited antifungal activity against Madurella mycetomatis with MIC 6.25mg/ml, while the MIC for anti-fungal drugs (Ketoconazole and Itraconazole) was 0.16 µg/ml and 1.25µg/ml, respectively. The high MIC of *Lepidium sativum* and low MIC of Ketoconazole are most probably due to the purity of these drugs since the active ingredient of this plant needs further isolation and purification from the ethanolic extract mixture.

The higher MIC observed for *Lepidium sativum* extract, relative to the lower MIC of ketoconazole, can likely be attributed to the purity of the compounds involved. While ketoconazole is a purified and highly concentrated pharmaceutical compound, the ethanolic extract of *Lepidium sativum* is a mixture that contains a variety of bioactive compounds, with the active ingredient likely present in lower concentrations. Further isolation and purification of this active component from the extract could enhance its antifungal potency, potentially reducing the MIC and making it more comparable to the pharmaceutical standards. This highlights the need for additional research to refine and concentrate the active constituents within *Lepidium sativum* to fully realize its antifungal potential.

In alternative medicine, this plant is used topically for the treatment of mycetoma, which may give the possibility to synthesize topical treatment of mycetoma to reduce the risks of the side effects of drugs that are given orally or systemically.

Studies have demonstrated that *Lepidium sativum* exhibits significant anti-inflammatory and immunomodulatory properties. Specifically, research has shown a marked reduction in the levels of key inflammatory markers, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1

(IL-1). Additionally, the production of nitric oxide (NO) was notably decreased, alongside a reduction in the expression of inducible nitric oxide synthase (iNOS), and heme oxygenase-1 (HO-1). These findings suggest that *Lepidium sativum* not only attenuates the inflammatory response by downregulating these pro-inflammatory mediators, but also modulates the immune response, further supporting its potential therapeutic applications in managing inflammation-related conditions. The ability of *Lepidium sativum* to influence such a broad range of inflammatory markers underscores its potential as a natural anti-inflammatory agent with promising implications for future research and clinical use[27]. Recent studies highlight the potential of plant-based synthesis of silver nanoparticles (AgNP). They explored the antimicrobial effects of *Lepidium sativum* (curly garden cress) combined with AgNPs. This combination enhanced antibacterial activity, particularly against ESKAPE pathogens. The LS-AgNP bio-composites showed pH, time, and concentration-dependent antimicrobial actions, confirming their potential as broad-spectrum disinfectants and wound care agents[28]. Two extracts from *Lepidium sativum* L. seeds were analyzed for their phenolic content, as well as their antioxidant and antibacterial properties. The seeds were extracted using 80% ethanol with ultrasonic assistance and distilled water with microwave assistance. The total phenolic and flavonoid contents were measured using Folin-Ciocalteu reagent and AlCl3, respectively. The extracts showed significant antioxidant activity, achieving 54.66% in the β-carotene/linoleic bleaching assay, and demonstrated antibacterial effects, with a 20 mm inhibition zone against *Salmonella Enteritidis*. These findings suggest that the extracts could serve as natural preservatives in the food and pharmaceutical industries, providing a potential alternative to synthetic antioxidants like TBHQ. The results from this study may contribute to the development of natural antioxidants and bioactive agents for enhancing human health[29]. In another study, researchers found that *Lepidium sativum* extracts exhibit both antioxidant and antibacterial activities. Methanol extracts, in particular, showed significant antioxidant properties, while both ethyl acetate and methanol extracts demonstrated antibacterial activity against *Rhodococcus equi*. The antioxidant and antimicrobial effects are likely due to the presence of flavonoids and tannins, as confirmed by chemical tests. These findings suggest that *Lepidium sativum* could be a valuable source of natural antioxidant and antibacterial agents, making it suitable for medical and nutraceutical applications. This research provides scientific validation for the traditional use of these plant extracts in homemade remedies and highlights their potential in treating microbial-induced conditions[30]. Further studies could pave the way for their development as safe alternatives to synthetic antimicrobial drugs. This includes in vivo studies such as rat models to assess the *in vivo* efficacy. Moreover, pharmacokinetics and pharmacodynamics are important for drug development; thus, these experiments are vital to implement.

One of the primary limitations of this study is the use of only a few isolates of *mycetoma*-causing organisms. Due to the rarity of mycetoma, obtaining a larger number of isolates is inherently challenging. This limited sample size may affect the generalizability of the findings, as it may not fully represent the diverse genetic and phenotypic variations present in different strains of *mycetoma* pathogens. Consequently, the outcomes observed in this study, including susceptibility profiles and potential synergistic effects of drug combinations, might differ when tested against a broader range of isolates.

CONCLUSION

In this study, we have demonstrated for the first time that the ethanolic extract of *Lepidium sativum* exhibits significant antifungal activity against *Madurella mycetomatis*, with a minimum inhibitory concentration (MIC) of 6.25 mg/mL. While this MIC is higher compared to the much lower MICs of standard antifungal drugs like ketoconazole (0.16 µg/mL) and itraconazole (1.25 µg/mL), this difference is likely due to the mixed nature of the plant extract, which contains various bioactive compounds. The BAW system gave the best result among the systems that were used for the separation of exanolic extract components of *lepidium sativum* plant (Thirteen components). These findings suggest that further isolation and purification of the active components from *Lepidium sativum* could enhance its antifungal potency, bringing it closer to the efficacy of pharmaceutical drugs. This research not only validates the potential of *Lepidium sativum* as a natural antifungal agent, but also opens the door for developing topical treatments for mycetoma, reducing the side effects associated with systemic antifungal drugs.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest that is related to this study and this article.

DISCLOSURE

The authors did not receive any form of commercial support, including compensation or financial assistance, for this case report. Additionally, the authors have no financial interest in any of the products, devices, or drugs mentioned in this article.

ETHICAL APPROVAL

Not applicable.

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