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Isolation and Characterization of Potent Antifungal Compounds from *Bunium pericum* Seeds against *Aspergillus fumigates*

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Abstract

Bunium persicum is a plant belonging to Apiaceae family. It possesses medicinal and antifungal properties, which suggest it has great potential for use in medicinal and food industries. In the present study, ethanolic seed extracts of the *Bunium persicum* were taken on the premise of their ethno botanical uses. The antifungal activity was assessed against significant fungal strain; *Aspergillus fumigates* by measuring minimum inhibitory concentration (MIC) and Zone of inhibition compared with standard drug fluconazole. The analysis of bioactive compound found in plant extracts using chromatographic method like TLC and LC-MS. Ethanolic extracts of seed is more effective against *A. fumigates* (18.6mm). The lowest concentrations of antimicrobial agents (0.3125 µ/ml) causing almost complete inhibition of growth in seeds of *Bunium persicum* (0.73 µg/ml). TLC studies of ethanolic extract of *Bunium persicum* showed two spots revealed the presence of carbonyl compound, lipid as it gives indigo, blue and orange colour of the spots. After identification four compounds were isolated from seeds of *Bunium persicum* are Dodecanol, 6-7-Dihydro-2-methyl-5H-cyclopents pyrazine, Lanosteroyl oleate, Methylselenic acid. These findings not only contribute to our understanding of the intricate chemistry of botanical extracts but further exploration of these compounds and their biological activities holds promise for unlocking new therapeutic and functional properties in natural products.

***Key words*:** *Bunium persicum, Aspergillus fumigates,* Antifungal activity, Apiaceae, Ethanolic extracts

Plant-based natural products are an abundant source of compounds with remarkable chemical and functional diversity and play a major role in drug development. To isolate the secondary metabolites from natural resources, extensive study has been conducted worldwide [10]. Plants produce a wide range of bioactive compounds, such as alkaloids, flavonoids, terpenoids, and phenolics, which have shown promising antimicrobial properties. Despite the potential, isolating these compounds remains a challenging and labor-intensive process. The use of plant products for the prevention and treatment of various microbial infestations has received a lot of attention recently. However, isolating compounds continues to be a difficult and enormous effort. Traditionally, a number of bioassays are used to check for the existence of such compounds in plant extracts before isolating them [23]. These bioassays help identify extracts with promising antimicrobial properties, saving time and resources in the drug discovery process.

Bunium persicum, is a plant of Apiaceae family also known as wild caraway. It is a medicinal spice that is widely utilized as a flavor enhancer and preservative in various food systems. Its antimicrobial and antioxidant properties make it an excellent natural preservative and health-promoting ingredient. Ongoing research and development efforts are aimed at fully harnessing its potential in food preservation and medicine. Numerous phytochemicals, including cuminaldehyde, terpinene-7-al, terpinene, cymene, and pinene, among others, are present in it. These phytochemicals have been shown to have a variety of therapeutic benefits, including antioxidant, antimicrobial, anti-inflammatory, lipid/glucose lowering activity, and anti-carcinogenic properties [3]. It is said to possess an essential oil that is up to 7% rich in monoterpene aldehydes; cuminaldehyde, p-mentha-1,3-dien-7-al, and p-mentha-1,4-dien-7-al are its primary constituents [22]. *Aspergillus fumigates* is one of the most widespread species of airborne saprophytic fungi. Both humans and animals frequently inhale large number conidia of this fungus. It plays a major role in the decomposition of organic matter but can also be a significant pathogen, particularly in immunocompromised individuals. Ninety percent of human infections are caused by allergic bronchopulmonary aspergillosis, the most common etiologic agent, and aspergilloma are the only infections observed in immunocompetent hosts [17]. Aspergillus fumigatus is a prevalent and potentially dangerous fungal pathogen due to its ability to produce airborne conidia that are easily inhaled by humans and animals. While it is a critical decomposer in the environment, it poses significant health risks, especially to immunocompromised individuals. Understanding its pathogenic mechanisms and implementing effective preventive and therapeutic strategies are essential in managing the infections it causes. To manage phytopathogens in labs, fields, and greenhouses, plant extracts with anti-fungal qualities were employed. This study aimed to isolate and characterize the antifungal compounds from seeds of *Bnnium persicum* by using

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thin layer chromatography (TLC) and liquid chromatography - mass spectrometry (LC-MS).

MATERIALS AND METHODS

Plant material

The Lucknow local market was visited to obtain the plant seeds used in this investigation. With the assistance of Central Institute of Medicine and Aromatic plants, Lucknow, and other literature survey comparisons, the plant seeds were recognized and verified.

Drying and grinding of plant

The plant seeds were kept for drying in a room without any exposure to light for about two weeks. After the seeds have dried fully, make sure the powder is uniform in size and that the surface area is increased for improved extraction. To keep materials, dry until extraction, they were kept in tightly closed plastic containers.

Organic solvent extraction

30 g of finely ground, uniform-size powder from the plant sample is kept in a thimble, and then thimble is placed in the thimble chamber of the Soxhlet. In the bottom of the Soxhlet, 300 ml of ethanol, methanol, hexane, ethyl acetate solvents were used for extraction. By adding water inflow and outflow, the upper portion was equipped with a condenser. The solvent was heated to a moderate 40 °C temperature over a Mantox heater. 48 hours were spent continuing the process until solvent drops left no traces after evaporating. The fraction was stored for further analysis of biological activities [20].

Antifungal activity

Fungal strain

Aspergillus fumigatus were acquired from Chandigarh's Microbial Type Culture Collection and Gene Bank. The fungus suspension was kept -40 °C in 20% glycerol.

Dtermination of antifungal activity

Extracts for their ability to inhibit the growth of fungus were done by the agar well diffusion method [6-8]. The Potato Dextrose Agar (PDA) medium by Himedia final volume 1L of DW, autoclave for 15 minutes. Fungus isolates, *Aspergillus fumigatus* on PDA media. For this, 100 µl of the culture broth. After incubation spreading, sterile microtips were used to pierce wells into the media plates, subsequently filled with 20 µl extract. The samples were allowed to diffuse into the media and incubated for 48 hrs at 27 °C (Fungus). Two well one of the positive controls, filled with fluconazole and the negative control pure solvent in which samples were prepared. After 5-7 days of incubation, the plates were examined for the zone of inhibition, a clear area surrounding the well whose diameter was measured in millimeters and noted [8].

Preparation of fungal inoculum

The fungus strain were grown in slant of PDA. The sporulated fungus were taken out of the agar slant and suspended in sterile water to create inocula. Conidia were successfully suspended, and the concentration was checked using a serial dilution plate count and a hemacytometer cell counting chamber. Conidia suspensions rapidaly votexed and adjusted by adding sterile distilled water to a concentration of 105 CFU/ml to create final suspensions, these fungus suspensions were diluted 1:5. When combined with antifungal solution, these condidal suspensions had a final concentration of 104 CFU/ml [9].

Broth microdilution method

To achieve the requisite 10 final concentrations, sterile distilled water was used to dilute the drug stock solutions. The concentrations of these 10 medications dilutions were doubled by diluting them with 1:5. Aliquots of 100µl of the drug dilution were inoculated. Well, the growth control was made up of each row. Except for the fluconazole plates, which were kept at 4°C for a maximum of one month, all the microplates were kept at 20 °C until they were utilised. To bring the drug dilutions to the final concentrations for the susceptibility testing, 100 µl of the diluted inoculum suspensions were added to each well. Fluconazole concentrations 10 ranged from 0.03 to 1g/ml. At 30 °C, the mirocplates were incubated without being stirred. Readings were taken after incubation for 48 hours [10].

Separation of bioactive substances

Thin layer chromatography

Thin layer chromtography typically results in similar elution patterns for column chromatography. All of the fractions were subjected to thin layer chromatography using the one-way ascending approach on TLC pre coated plates (silica gel). Each sample is evenly applied to the plates using capillary tubes. Using solvent systems including chloroform: ethyl acetate: methanol: water (15:8:4:1), the plates were processes in a chromatographic tank. The plates were dried and observed under normal daylight and ultraviolet light (366 nm) [15].

Struture determination of the bioactive compounds

Liquid chromatography - Mass spectrometry (LC-MS)

Intensity Solo 2 RP -18, 2.0 µm, 100×2.1 mm column was the column that was used. The sample was dissolved in methanol for the LC-MS preparation, and the vial was then placed in the autosampler. For the sample, columns were kept at 35 °C respectively. The following settings were used for negative mode operation of the mass spectrometer: collision cell energy, 200 °C dry heater, 5.0 eV; nebulizer gas (N_2), -3.5 kV of ion spray voltage, 8.0 L/min dry gas (N_2), 2.0 bars; and 500 V plate offset. On the HPC mode, the internal calibration procedure is performed using a solution of 0.5 ml 1N NaOH solution, 50 ml water, 250 µL formic acid, 750 µL acetic acid and 250 ml iPrOH. Using Data Analysis software 4.4™, the raw data samples from the LC-MS analysis were processed to extract their mass spectrum characteristics. Confirming fragment ions is done using the auto MS/MS mode [9].

RESULTS AND DISCUSSION

The results for antifungal screening of extracts obtained from seeds of *Bunium persicum* against *Aspergillus fumigates* representing in (Table 1). The seed with the zone of inhibition was examined in ethanol extract of *Bunium persicum* L. against *Aspergillus fumigates* (18.6 ± 1.7 mm), whereas, positive control was noted 20.3 mm in (Fig 1). The lowest concentrations of antimicrobial agents causing almost complete inhibition of growth in seeds of *Bunium persicum* L (Table 2). The concentration of seed extract was prepared in ranged of 0.3 to 10µg/ml against *Aspergillus fumigates.* The ethanol extract of *Bunium persicum* L. seed exhibited the highest minimum inhibitory concentration (MIC) 0.73µg/ml at lower concentration of 0.3125µg/ml against *A. fumigates* in (Fig 2).

Table 1 Antifungal activity of ethanol extracts of *Bunium persicum* seeds against *Aspergillus fumigates*

Plant sample (seed)	Zone of inhibition (mm)
Bunium persicum	18.6 ± 1.7
Positive control (Flucanazole)	20.3 ± 0.5

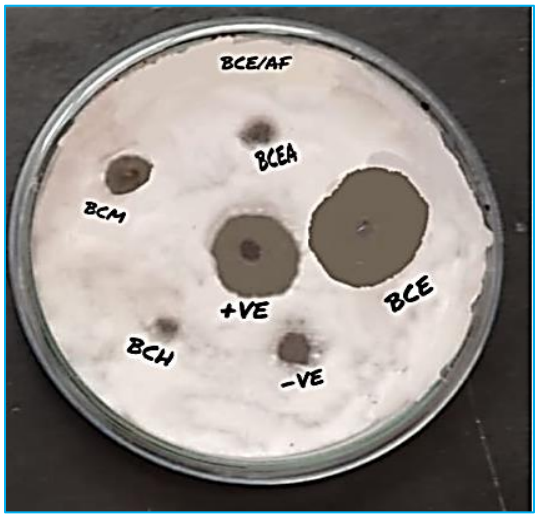


Fig 1 Antifungal activity of *Bunium persicum* seed

Fig 2 MIC of *Bunium persicum* seed

Table 2 MIC value of seed extract of *Bunium persicum*

Plant sample (seed)	Concentrations (µg/ml)					
	10	5	2.5	1.25	0.625	0.3125
Bunium persicum	0.14±0.01	0.28±0.01	0.42±0.01	0.57±0.01	0.63±0.01	0.73±0.01

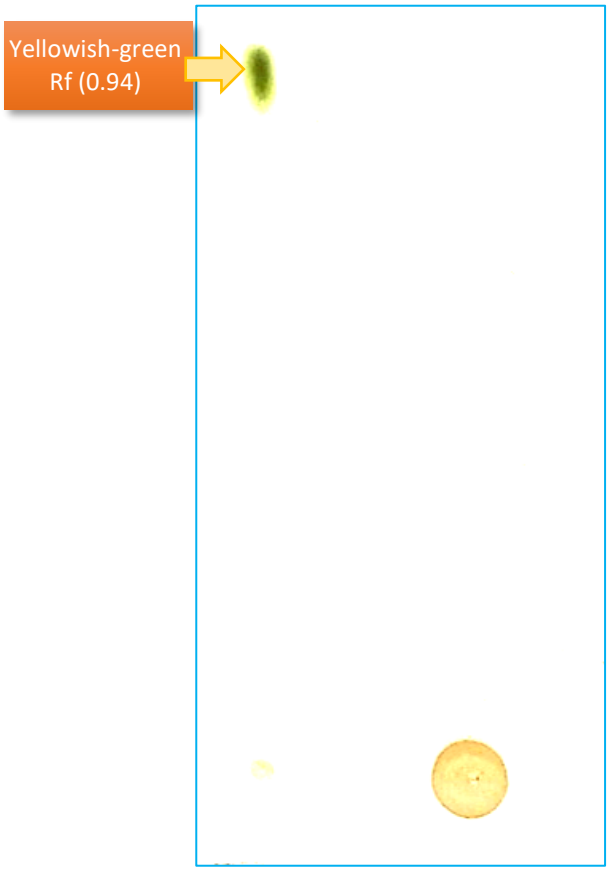
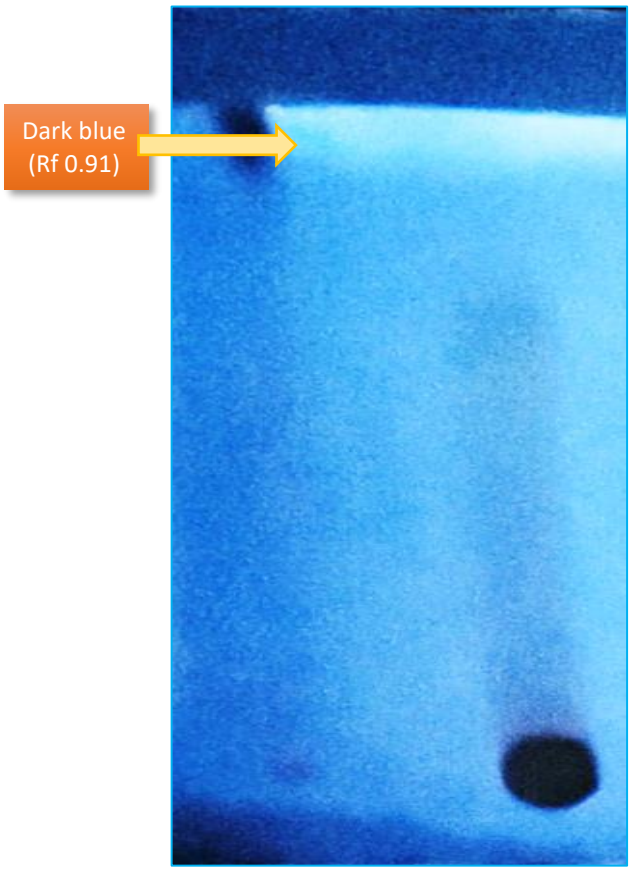


Fig 3 (a) TLC plate in day light



(b) TLC plate in UV light

The seed extract of *Bunium persicum* when run in solvent system chloroform: ethyl acetate: methanol: water (15:8:4:1) showed two spots at different R_f values. In (Fig 3) the TLC plate shows one spot visible in day light and one spot visible under UV light. Under daylight conditions, the *Bunium persicum* L. (E) extract revealed the presence of "carbonyl compounds" (Rf value is 0.94), which give yellowish-green colour of the spot.

When exposed to UV light demonstrate the presence of "lipids" (Rf value is 0.91), as it gives indigo, blue and orange colour of the spots.

Table 3 Rf value of seed extract of *Bunium persicum* L.

Plant sample	Color		Retention factor =	Solvent
	In UV	Without UV		Sample
Bunicum persicum L. (E)	Dark blue	Yellowish-green	0.91	0.94

Many compounds have been identified which have already been mentioned were isolated from the ethanol extract of *Bunium persicum* L. seed. Out of which there are four compounds which probably have not been given in the paper yet identified by a combination of TLC and LC-MS. The isolated compounds are:

Table 4 LC-MS analysis of ethanol extract of *Bunium persicum* L.

Exact mass	Measured mass	Compounds	Retention time	Product ion
400.6	400.4	1-Dodecanol	1.78	187.2, 186.2, 169.1
198.4	198.1	6-7-Dihydro-2-methyl-5H-cyclopenta pyrazine	4.63	135.0, 134.1, 157.0
365.2	365.3	Lanosteroyl oleate	7.99	655.6, 673.6, 691.6
172.7	172.9	Methylselenic acid	3.39	127.9, 128.9, 134.9

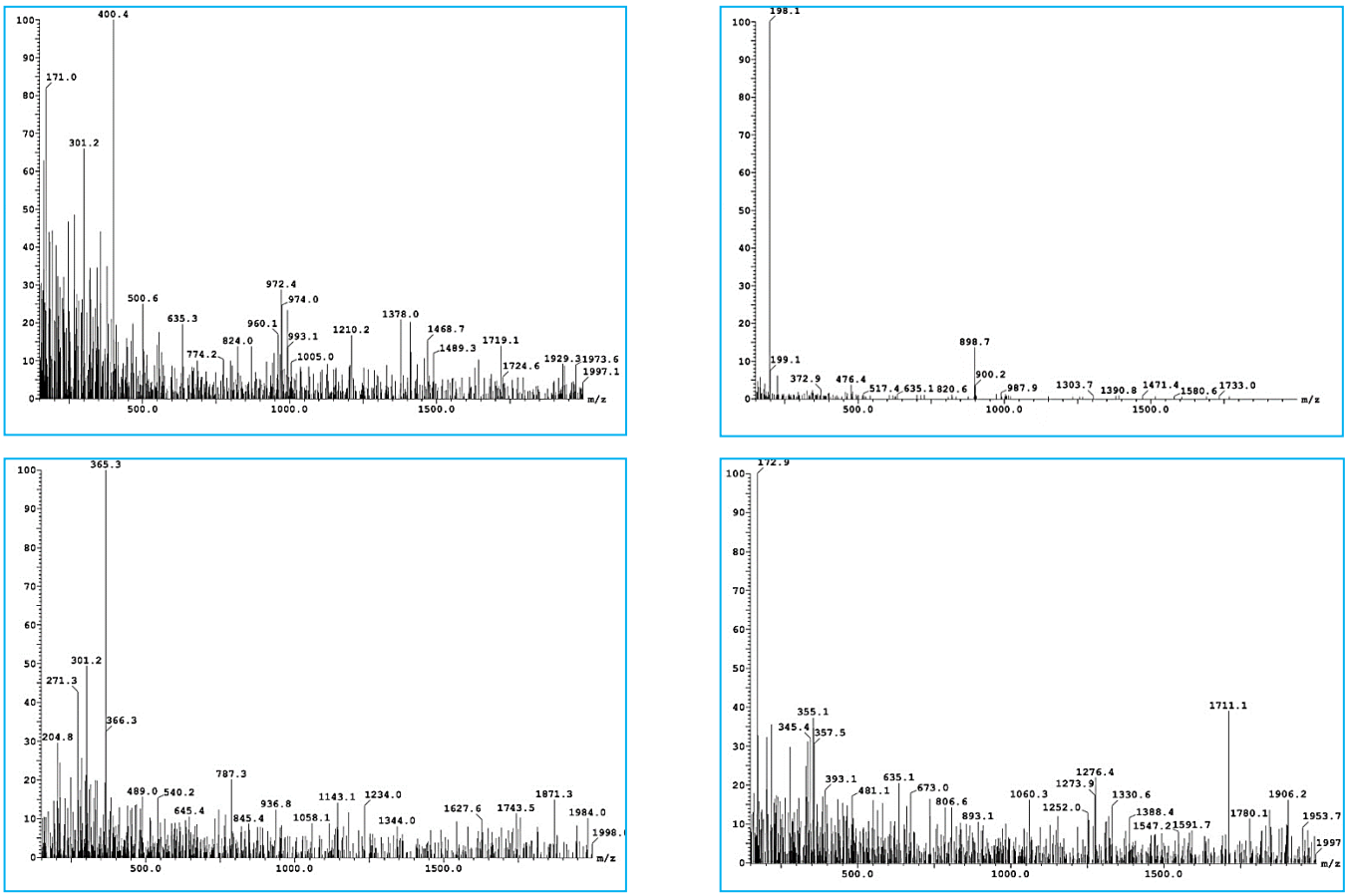


Fig 4 LC-MS spectra of *Bunium persicum* L. seeds

The aim of the present study was to isolate and characterize potent antifungal compounds from seed of *Bunium persicum* against *Aspergillus fumigatus*. These medicinal plants were chosen based on their historical applications or research that demonstrated the use of extracts with antifungal properties. *Aspergillus fumigates* is a common fungus that can affect both people and animals [13]. Recent research indicates that *A. fumigates* is one of the species whose conidia are airborne. A person will inhale at least few conidia each day and develop a fungal infection. Ninety percent of human infections are caused by this most prevalent etiologic agent [14]. Because these infections are resistant to most fungal infections, they are challenging to control and cure.

It was clear from an analysis of the literature that there was a standardized procedure for extracting compounds from plants. Soxhlet method was used in this study, because it is quick and efficient at extracting compounds. Since, ethanol is widely used and has the capacity to dissolve a variety of compounds; it was selected for use in the extraction process. Water is a universal solvent, but it wasn't effective at extracting non-polar compounds and didn't work well at high temperature. It was shown that ethanolic extract of seed have significant antifungal activity against *A. fumigates*. In particular, *Bunium persicum* had a notable inhibitory effect against *A. fumigates* with a zone of inhibition 18.6 ± 1.7 mm. Essential oil components as γ-terpinene, cuminaldehyde, and β-pinene, which have been shown to have strong natural inhibitory effects against *Aspergillus* species, are likely responsible for this increased activity [2], [7], [12]. Findings demonstrated that the MIC concentration of EO and cuminaldehyde totally inhibited sporulation and spore germination.

The process of extract screening revealed the presence of a range of antifungal compounds, including lipids, alkaloids, tannins, flavonoids, and phenols. These components are essential to the pharmacological effects of plants. In solvent systems, their different retention factors (Rf values) are acting

as polarity indicators. Comparing these Rf values across different solvents is necessary to determine which solvent system is best for column chromatography [1], [5]. According to Sharifi-far *et al*. (2010), phenolics and carbonyl compounds are present in ethanol extract of *B. persicum* seeds. The extract of *B. persicum* included phenolic acids and flavonoids, among other elements that are included in these phenolic compounds. Analgesic, anti-inflammatory, antioxidant and anti-ulcerogenic are few of the many biological functions associated with flavonoids. Conversely, certain phenolic compounds, such as p-coumaric acid and caffic acid support anti-tyrosinase and antioxidant activities [16]. These results highlight the medicinal potential of phenolic and flavonoid compounds that were isolated from *Bunium persicum* L.

The LC-MS chromatogram presented in Fig 4 shows the chemical retention periods and intensities for the seed extract. The identification of the compound was based on structural features and molecular masses. 1-Dodecanol, the first component to be eluted, had a mass of 400.4 m/z at a retention time (RT) of 1.78 minutes. Kazek *et al*. (2021) state that dodecanol is used in the manufacturing of surfactants, lubricants, medicines, monolithic polymers, and food additives. It functions as a precursor to 1-bromododecane, which increases the lipophilicity of organic molecules, and use as a common emollient in cosmetics.

6-7-Dihydro-2-methyl-5H-cyclopents pyrazine was found to elute in 4.63 min and have a mass of 198.1 m/z (Fig 16). According to Rychen *et al*. [19], it possesses anti-inflammatory, anti-angiogenic, antioxidant, and anti-cancer effects in specific cell lines, suggesting that it may have potential uses in the treatment of neurological conditions. The compounds lanosteroyl oleate (365.3 m/z) and D-Glycero D-mannoheptose (714.2 m/z) were found to elute at 7.99 and 34.84 min, respectively. These compounds may be used as ingredients in over-the-counter eye medications to prevent cataracts [24].

In *Bunium persicum* seeds, methylselenic acid was found at 3.39 minutes, having a mass of 172.9 m/z. According to Qi *et al*. (2012), it can improve paclitaxel's effectiveness while treating triple-negative breast cancer. Overall, these results point to the variety of antifungal compounds found in seeds of *Bunium persicum*, which indicate their possible therapeutic uses. However, additional study is necessary to clarify their modes of action and therapeutic effectiveness.

CONCLUSION

The finding of this study demonstrated that the *Bunium persicum* have antifungal activity against *Aspergillus fumigates.* The ethanolic extract of *Bunium persicum* seeds was found to be most effective against fungal activity. The isolation of active compounds from the leaves of *Bunium persicum* using thin layer chromatography, and LC-MS yielded four compounds. The main antifungal compounds found in plants include flavonoids, terpenoids, lipids, and carbonyl compounds. These compounds may have anticancer, anti-inflammatory, and antifungal properties that support the health of the world's population. Because of this, it is extremely important to isolate and characterize the antifungal compounds from *Bunium persicum.*

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