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Profiling of Antifungal Activities from the Leaf Extract of Selected Apiaceae Family Plants against *Aspergillus fumigates*

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Abstract

Many ethnic plants are used as a source of traditional medicine to cure a variety of illnesses in both humans and animals. Developing secondary metabolites in plants with antifungal characteristics, offer alternative medications for reasonably priced and safe treatments. In the present study, methanolic, ethanolic, hexane and ethyl acetate leaves extracts of fifteen Apiaceae family plants 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. Ethanol and methanol extracts of the plants were more effective than the hexane and ethyl acetate extracts against *A. fumigates*. Extracts of *Cuminum cyminum, Pastinca sativa, Carum carvi, Dacus carota, Centella asiatica, Anthriscus cerefolium, Trachyspermum ammi, Pimpenella anisum* and *Apium graveolens* showed relatively low inhibition effects between 3.5 to 8.5 mm. The MIC value of extracts was determined ranging between 0.8 to 0.43 µg/ml. The extract of *Petroselinum crispum*, *Foeniculum vulgare*, *Ferula assaefoetida*, *Bunium persicum, Anethum graveolens* and *Coriander sativum* could be considered as potential source of antifungal compounds for treating diseases in humans. Conclude remarks that these six extracts show astonishing fungicidal properties that can be used to discover drugs of very high potential.

Keywords

Antifungal, *Aspergillus fumigatus*, Ethnobotanical, Fungicidal, Medicine

1. Introduction

Natural products of plant origin are now receiving more attention as a result of

new drug discoveries than synthetic model and compounds [1]. Due to extensive study and the discovery of numerous drugs with both natural and chemical bases, the field of medicine has advanced significantly. Development of new and more affordable drugs from their natural habitat is greatly aided by the antifungal components found in herbal medicines [2] [3] [4].

The Apiaceae family, which has 3780 species in 434 genera, is one of the most important groups of plants (**Figure 1**). Plants are used for distinctive purposes such as pharmaceuticals, nutrition, beverages, species, cosmetics, fragrances and industrial uses. A few plants from this family are utilized as home remedies in ethno medicine to treat a number of conditions affecting the digestive, endocrine, respiratory and reproductive systems [5]. Secondary metabolites from plants that have intricate molecular structures are thought to be responsible for the antibacterial and antifungal activities. Alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glycosides, terpenes and phenolic chemicals are some of these secondary metabolites [6].

One of the most common types of airborne saprophytic fungi is *Aspergillus fumigates.* Numerous conidia of this fungus are regularly inhaled by people and animals. The only infections seen in immunocompetent hosts are aspergilloma and allergic bronchopulmonary aspergillosis, is the most prevalent etiologic agent, being responsible for 90% of all human infections [7]. Plant extracts with antifungal properties were used for controlling phytopathogens at laboratory, field level and greenhouse [8]. The antifungal effects of many plants extracts have recently been discovered by numerous researchers [9] [10] [11]. In antifungal bioassays, methanolic, ethanolic, hexane and ethyl acetate extracts of plants are preferred over aqueous extracts because this solvent can prevent contamination during plant material extraction. Additionally, fractionation using a series of organic solvents with different polarities is a very helpful technique to separate active compounds [12]. Due to this, the current research work focused on an antifungal activity of several leaf extracts.

2. Materials and Methods

2.1. Plant Material

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

2.2. Drying and Grinding of Plant

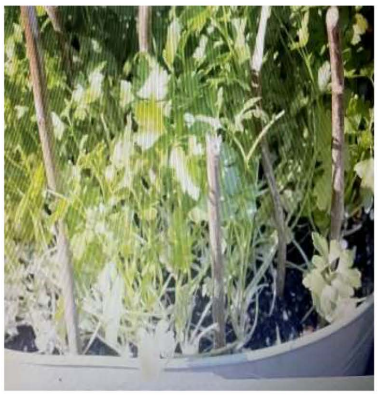
The plants were cleaned and then chopped in to little bits with scissors and knives. They were kept for drying in a room without any exposure to light for about two weeks. After the plants have dried fully, make sure the powder is uniform in size and that the surface area is increased for improved extraction (**Figure 2**). To keep materials dry until extraction, they were kept in tightly closed plastic containers.

***Antheum graveolens* L.**

***Trachyspermum ammi* L.**

***Foeniculum vulgare* Mill.**

***Coriander sativum* L.**



***Petroselinum crispum* L.**

***Daucus carota* L.**

***Pastinca sativa* L.**

Ferula assaefoetida

***Carum carvi* L.**

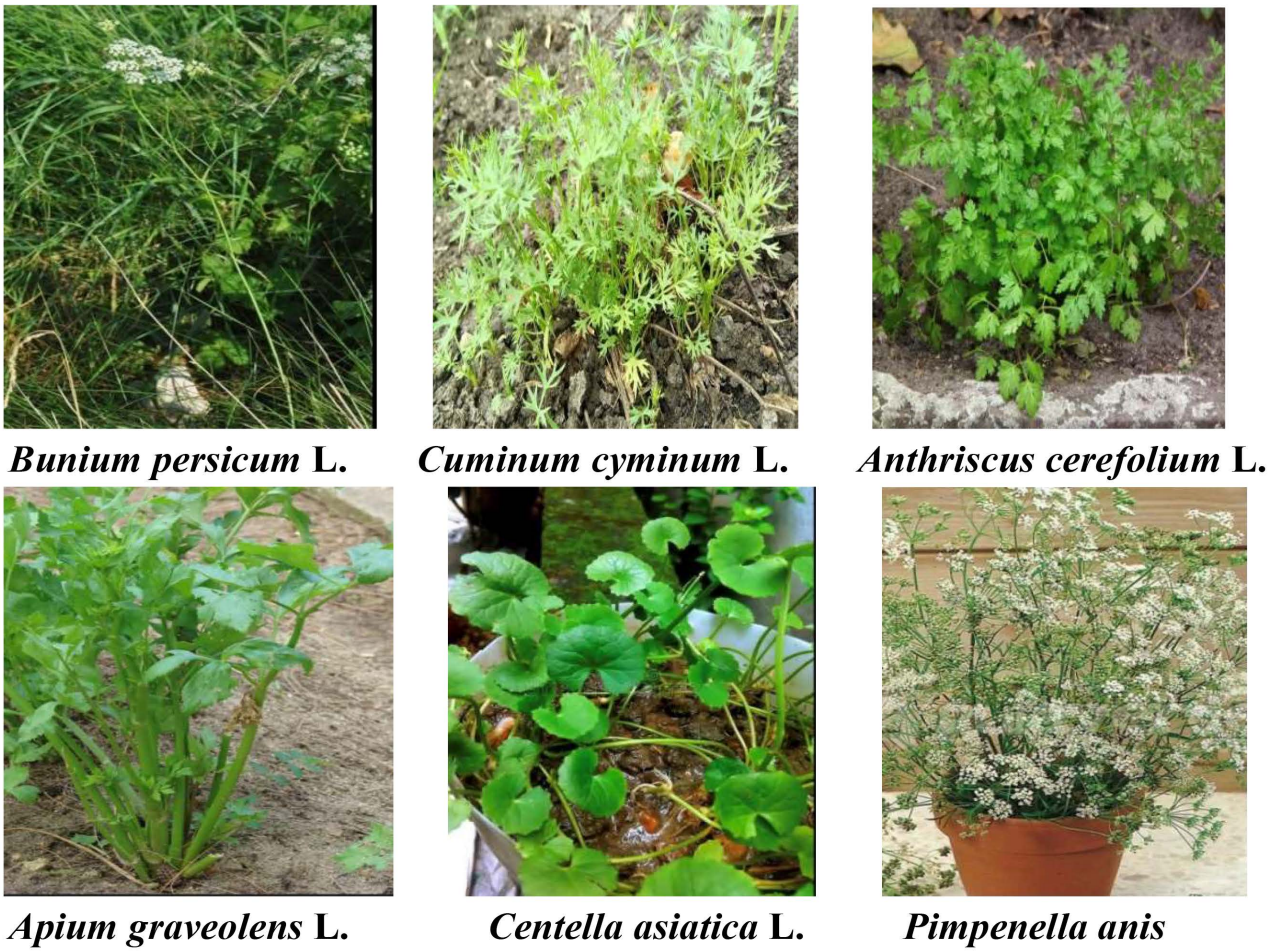


Figure 1. Fifteen plants leaves of Apiaceae family.

2.3. 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 was 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 [13].

2.4. Antifungal Activity

2.4.1. Fungal Strain

A. fumigatus were acquired from Chandigarh's Microbial Type Culture Collection and Gene Bank. The fungus suspension was kept −40˚C in 20% glycerol (**Figure 3**).

2.4.2. Dtermination of Antifungal Activity

Extracts for their ability to inhibit the growth of fungus were done by the agar

Figure 2. (a) Leave dry in room temperature (b) powdered of leave.

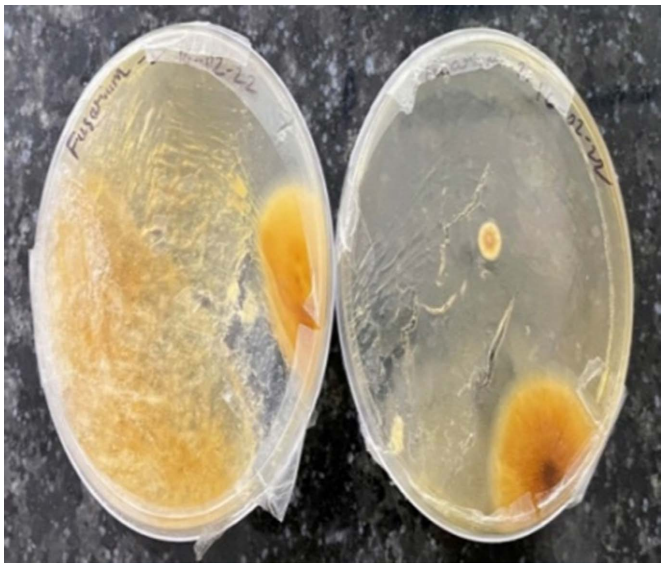


Figure 3. *Aspergillus fumigatus* culture.

well diffusion method [13] [14]. The Potato Dextrose Agar (PDA) medium by Himedia final volume 1 L of DW, autoclave for 15 minutes. Fungus isolates, *A. 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 whse diameter was measured in millimeters and noted [14].

2.4.3. 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 [15].

2.4.4. 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 100 µl 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 [15]. Fluconazole concentrations 10 ranged from 0.03 to 1 g/ml. At 30˚C, the mirocplates were incubated without being stirred. Readings were taken after incubation for 48 hours [16].

2.5. Statistical Analysis

The effect of ethanol, methanol, hexane and ethyl acetate extracts on the Apiaceae family plants evaluated by one way analysis of variance (ANOVA).

3. Results

The antifungal acctivity of several Apiaceae leaves extracts (*Petroselinum crispum, Dacus carots, Foeniculum vulagre, Pimpenella anisum, Apium graveolens, Pastinaca sative, Carum carvi, Bunium persicum, Coriander sativum, Centella asiatica, Cuminum cyminum, Anethum graveolens, Ferula assaefoetida, Anthriscus cerefolium, Trachyspermum ammi*) was investigated in this study in contrast to a microbial strain (**Table 1**). 15 plant extracts were tested against different organic solvents (methanol, ethanol, hexane and ethyl acetate) for antifungal activities based on the diameter of the zone of inhibition (mm) and minimum inhibitory concentration values (MIC). 10% from fiftten plants showed invitro antifungal activity with inhibition values of over 50%. The species with the pronounced antifungal activitiy were ethanol extract of *Pertroselinum crispum* (19.3 ± 0.5), *Ferula assaefoetida* (11.6 ± 0.6), *Foeniculum vulgare* (11.3 ± 0.5), *Bunium persicum* (10.7 ± 0.6), *Coriander sativum* (10.6 ± 1.1) as well as the methanol extracts of *Coriander sativum* (12 ± 1.7) and *Foeniculum vulgare* (12.6 ± 0.5). Hexane and ethyl acetate extracts did not exhibit antifungal acitivity, only *Dacus carota*, *Centella asiatica* and *Apium graveolens* extracts is active (**Figure 4**).

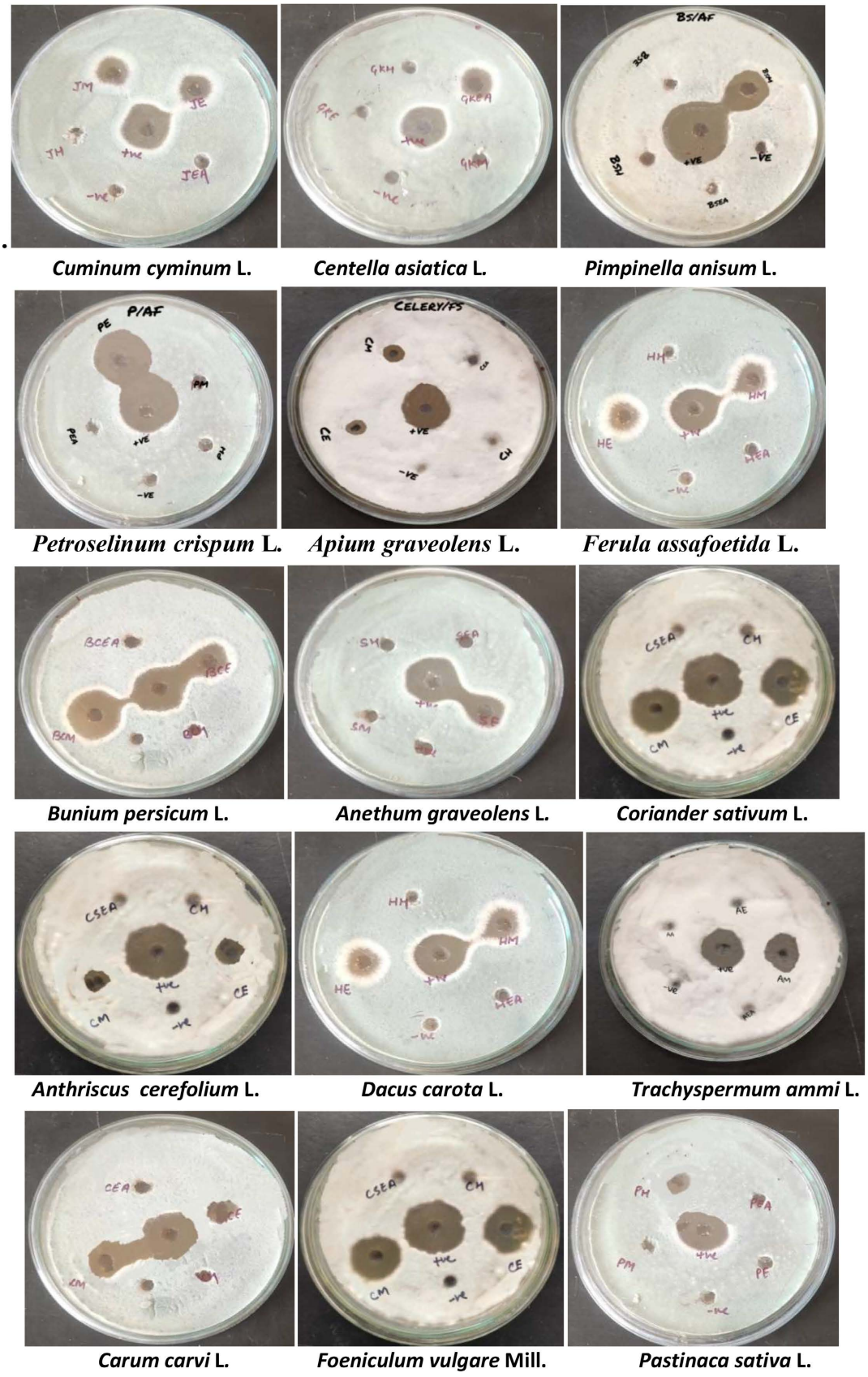


Figure 4. Antifungal activity of fifteen plant extracts against fungus *A. fumigatus.*

Table 1. Antifungal activity of fifften plant extract of Apiaceae family plants against *Aspergillus fumigates.*

Plant samples	Zone of inhibition in mm			
	Methanol	Ethanol	Hexane	Ethylacetate
Anethum graveolens L.	ND	9.6 ± 0.5	ND	ND
Carum carvi L.	8.3 ± 0.5	6.3 ± 0.5	ND	ND
Ferula assaefoetida L.	9.3 ± 1.1	11.6 ± 0.6	ND	ND
Pimpenella anisum L.	5.6 ± 1.1	ND	ND	ND
Foeniculum vulgare Mill.	12.6 ± 0.5	11.3 ± 0.5	ND	ND
Dacus carota L.	8.8 ± 0.3	ND	9.4 ± 0.5	ND
Petroselinum crisapum L.	ND	19.3 ± 0.5	ND	ND
Bunium persicum L.	10.6 ± 1.1	10.7 ± 0.6	ND	ND
Centella asiatica L.	3.5 ± 0.5	ND	ND	6.8 ± 0.9
Coriander sativum L.	12 ± 1.7	10.6 ± 1.1	ND	ND
Apium graveolens L.	9 ± 1.7	ND	ND	7.6 ± 1.5
Cuminum cyminum L.	9.3 ± 0.5	7.3 ± 1.1	ND	ND
Pastinca sativa L.	6.6 ± 0.5	ND	ND	ND
Anthriscus cerefolium L.	5.3 ± 1.5	8.3 ± 1.1	ND	ND
Trachyspermum ammi L.	7.8 ± 1.1	ND	ND	ND
Positive control	20.6 ± 1.1			
Negative control	-			

M—Methanol, E—Ethanol, H—Hexane, EA—Ethylacetate, ND: not detected; values are means of three replicates ± standard deviation.

Table 2. Minimum inhibitory concentration (MIC) of extract from plant species against *A. fumigates.*

Plant samples	Minimum Inhibition Concentration (µg/ml)			
	Methanol	Ethanol	Hexane	Ethyl acetate
Anethum graveolens L.	-	0.12 ± 0.01	-	-
Carum carvi L.	0.12 ± 0.01	0.11 ± 0.02	-	-
Ferula assaefoetida L.	0.32 ± 0.02	0.38 ± 0.02	-	-
Pimpenella anisum L.	0.12 ± 0.01	-	-	-
Foeniculum vulgare Mill.	0.17 ± 0.02	14.5 ± 0.7	-	-
Dacus carota L.	0.12 ± 0.01	-	16.5 ± 2.1	-

Continued

Petroselinum crisapum L.	-	0.43 ± 0.01	-	-
Bunium persicum L.	0.15 ± 0.01	0.16 ± 0.01	-	-
Centella asiatica L.	0.8 ± 0.1	-	-	0.11 ± 0.02
Coriander sativum L.	0.38 ± 0.02	0.20 ± 0.02	-	-
Apium graveolens L.	0.15 ± 0.02	-	-	0.14 ± 0.01
Cuminum cyminum L.	0.14 ± 0.02	0.13 ± 0.02	-	-
Pastinca sativa L.	0.12 ± 0.01	-	-	-
Anthriscus cerefolium L.	0.11 ± 0.02	0.12 ± 0.01	-	-
Trachyspermum ammi L.	0.15 ± 0.01	-	-	-

M—Methanol, E—Ethanol, H—Hexane, EA—Ethylacetate, -: No activity; values are means of three replicates ± standard deviation.

Each of the fifteen plants, MIC for methanol, ethanol, hexane and ethyl acetate were established. The MIC value ranged from 0.8 to 0.43 μg/ml, with *Centella asiatica, Carum carvi and Anthriscus cerefolium* extracts having the lowest MIC and *Petroselinum crispum*, *Ferula assaefoetida*, *Coriander sativum* and *Foeniculum vulgare* extracts having the highest MIC (Table 2). Index data were evaluated and compared to the activity of standard fluconazole for fungal strain. Overall evaluation suggests that some extracts have potential as potent antifungal drug sources against fungus *A. fumigates*.

4. Discussion

The aim of the current investigation was to evaluate the antifungal activity of fifteen plants from Apiaceae family against *Aspergillus fumigatus*. The selection of these mediicnal plants was based on either traditional usages or studies that have previously shown using different extracts with antifungal characteristics. Aspergillus fumigatus is a typical fungus that can infect both humans and animals [17]. According to recent studies, *A. fumigatus* is one of the species with airborne conidia. Conidia released into the atmosphere to reach lung alveoli, person will inhale at least a few *A. fumigatus* conidia per day and get fungal infections [18]. It is most common etiologic agent being responsible for 90% of human infectons. However, these infections are difficult to manage and treat because they are resistant to the majority of drugs for fungi [19]. This demonstrates the value of these plants in the potential management of illnesses brought on by *Aspergillus spp*. [20]. However, no studied have examined the effects of these plants on *A. fumigatus.* According to the result obtained the methanol and ethanol extracts are quite similar.

The ethanol extracts of *P. crispum* was the most active compared to other extracts because flavonoids are one of the substances that have antifungal activity

and are found in plant extract [21] [22]. The flavonoids can break down protein bonds into their basic structure on the cell membrane of *A. fumigatus* which results in the lysis of the fungal cell membrane and allow the flavonoids to enter the nucleus cell. The growth of *A. fumigatus* can be inhibited by the presence of flavonoids on the cell nucleus [23]. The effectiveness of this plant against *A. fumigatus* hasn't been investigated though. The methanol extract of *F. vulgare* (12.6 mm) had a suspectible antifungal activity because estragole, limonene and fenchone, which have significant growth inhibitory properties, are present in higher levels in *F. vulgare* [24] [25].

Ethanolic extracts of *F. assaefoetida* had shown activities against several fungal spp. Due to the presence of a variety of phytoconstituents, ethanol, methanol, and ethyl acetate extract has strong antimicrobial action and may be a source of novel antibiotic compounds [26] [27]. Research on the antimicrobial activities of the plant's essential oils has revealed that coriander is a medicinal herb with strong antibacterial activity [28]. It has been found that coriander extract has reduced antibacterial impact on bacteria because these bacteria have cell wall polysaccharides, which inhibit active chemicals from reaching their cytoplasmic membrane [29]. When compared to bacteria, fungi are more sensitive to plant essences [29]. *B. persicum* showed activities against methanol and ethanol extracts because the major component of *B. persicum*,is essential oil and cuminaldehyde, which gives the plant its antifungal properties against phytopathogenic fungus [30]. Findings demonstrated that the MIC concentration of EO and cuminaldehyde totally inhibited sporulation and spore germination. *Aspergillus tamarii, A. flavus, A. parasiticus, A. ochraceus*, all had their spore germination inhibited by caraway extract, which showed the strongest inhibitory impact [31] [14]. As a result of their ability to kill the fungus, EO and a component of cuminaldehyde were able to suppress the anthracnose infection. When compared to synthetic fungicides like carbendazim and mancozeb, the MIC values for EO and its primary constituents were significantly lower. In the present study, *Dacus carota* extracts was active against methanol and hexane with the MIC value ranging from 0.12 to 16.5 µg/ml but it did not show the zone of inhibition against ethanol and ethylacetate extracts. This suggests that active substances would not diffuse easily in hydrophillic agar matrices and might instead be hydrophobic compounds. Cumin was very efficient against *A. fumigatus*. When applied to soild medium, it fully inhibits the mycelial growth of all fungi. Anise had a range of effects on fungi, from total suppression of growth in the case of susceptible isolates to little or no growth inhibition in the case of resistant isolates. The most effective inhibitor was *P. anisum*, with MICs of 40 µl/ml for Penicillium sp. and *A. niger* and 60 µl/ml for *P. chrysogenum* [31]. The extracts of Apium graveolens active against methanol and ethyl acetate because the presence of D limonene and D carvonein in *A. graveolens* extracts have exhibited strong antifungal activity against *Aspergillus spp*. [32].

Studied that the growth of *Aspergillus spp*. was inhibited by 69% in PDA

plates containing 2% powdered leaves of *T. ammi* compared to control plates after the fifth day of inoculation. This demonstrated that *Aspergillus spp*. is significantly reduced by 2% *T. ammi* leaves powder. 2% ground leaves powder of *T. ammi* reduces the risk of *Aspergillus spp.* by 69%. The active plants discovered in this study could be used in further studies to find possible treatments for infections caused by this organism that are more efficient and cheap for the local people [33]. To isolate and identify the active compounds from these extracts that could lead in the development of new and effective antifungal drugs. In the present study, we have shown that a number of plants that have historically been used to treat human diseases caused by fungi effective against *Aspergillus fumigatus.* Therefore, Aspergillus infection in human and animals may be manged with the help of these plants. The usages of these plants by local traditional healers in the treatment of fungal disorders are also supported by this study. All of the Aspergillus examined were most effectively inhibited by the leaves of *P. crispum* when it was extracted with ethanol. The active plants discovered in this study may be employed in additional research to find possible treatments for infections caused by this organism that are more efficient and cheap for the local people.

5. Conclusion

The findings of this study demonstrated that the screened medicinal plants have antifungal activity against *A. fumigatus*. The methanolic and ethanolic extracts of *Petroselinum crispum*, *Foeniculum vulgare*, *Ferula assaefoetida*, *Bunium persicum* and *Coriander sativum* show effective antifungal activity that can suppress the growth of fungus. These species show antifungal activity that was nearly equal to the commercial fungicide which is used as a positive control. This could help in the identification of novel chemical classes of antibiotics and antifungal that could act as selective agents for the maintenance of human health and give biochemical tools for the research of fungus related infectious diseases.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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