**Research Article**

# Antifungal potential of different parts of *Olea europaea* and *Olea cuspidata* growing in Azad Jammu and Kashmir

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## Abstract

Development of more effective and less toxic antifungal agents is required for the treatment of fungal diseases of plants and human being. The medicinal plants and their various extracts have been used as medicines against infectious diseases and found as potent against crops as well as human pathogenic fungal stains. In this study, the antifungal activity of *O. europaea* (cultivated olive) leaves, seeds and *O. cuspidata* (wild olive) leaves, roots bark, stem bark and seeds were evaluated against a range of human and crop pathogenic fungal species by using well diffusion assay. The extracts of investigated parts of *O. europaea* and *O. cuspidata* obtained with organic solvents were found to be effective against some of tested fungal strains particularly extracts of *O. cuspidata* parts proved to be more potent. The ethylacetate extracts of *O. cuspidata* leaves and seeds were found to be effective against fungal pathogens as compared to *O. europaea* extracts. Nystatin was also used as positive control and respective solvent as negative control. This study demonstrated that the use of wild olive leaves extracts as medicines may reduce the risk of fungal infections, particularly in situations where lengthy usage of synthetic fungicidal inspire growth of opportunistic contagions.

**Keyword:** Fungicidal; Wild Olive; Cultivated Olive; Organic Solvents; HPLC

## Introduction

Extracts of olive leaves has been used in traditional medicine for the treatment of malaria and fever [1]. In animals the olive leaf extracts found to be useful particularly in lowering the blood pressure [2] and increases blood flow in coronary arteries, relieves arrhythmia and prevents intestinal muscle spasms [3]. Furthermore, olive leaf extract and its phenolic compounds such as oleuropein, tyrosol, hydroxytyrosol, caffeic acid, gallic acid and luteolin have antimicrobial activity against viruses, retroviruses, bacteria, yeasts, fungi, molds and other parasites [4-6]. The growth of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* was inhibited by the phenolic compounds isolated from olive fruit [7, 8]. Gourama and Bullerman [9] evaluated the oleuropein *Aspergillus parasiticus*'s aflatoxin and found that oleuropein used mold growth but inhibited the production of aflatoxins. Markin *et al.* [10] also evaluated the antimicrobial potential of olive leaf water extract and found potent against tested yeast and bacterial strains. Moreover, Sousa *et al.* [11] screen out phenolic compound against a rang of G+ve, G-ve and fungi (*Candida albicans* and *Cryptococcus neoformans*) and conferred a prominent activity. The fungi i.e. *Tricophyton mentagrophytes*, *Microsporum canis* and *Candida* spp. were sensitive from some aldehydes isolated from fruit of olive as conferred good antifungal activity [12]. Many of the problems caused by yeasts results from the much higher attention paid to bacteria and moulds which are more significant in terms of public health. Yeasts play a central role in the spoilage of foods and beverages, mainly those with high acidity and reduced water activity [13]. Ismail *et al.* [14] reported that *Kloeckera apiculata*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida* spp. are responsible for the spoilage of foods that have been processed and packaged according to the normal standards of good manufacturing practice. In addition, yeasts are used during biological control following harvest (*Candida oleophila*, *Metschnikowia fructicola*, etc.).

The objectives of this study were to investigate antifungal activity of organic solvents extracts from wild and cultivated olive parts which has been utilized as traditional folk medicine and to evaluate the potential usage of olive parts extracts as natural preservative.

### Materials and Methods

The seeds, leaves, stem and roots bark of wild (*Olea cuspidata* Wall. Cat.) while seeds and leaves of cultivated (*Olea europaea* L) were collected from Kotli, Azad Jammu and Kashmir. All parts of wild and cultivated olive were dried carefully under shade then homogenized to powdered material and stored at 4 °C for further analysis.

### Fungal strains Used

The fungal strains i.e. *Aspergillus flavus*, *Alternaria alternata*, *Fusarium moniliform*, *Mauginiella scaettae*, *Trichothecium roseum*, *Magnaporthe grisea*, *Botrytis cinerea* and yeast i.e. *saccharomyces cerevisiae* were used in the present study.

### Extraction of Potential Bioactive Compounds

Extraction is very important first step in the investigation of medicinal plants because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. Keeping in view the crucial nature of chemical compound extraction procedure, for the present study the homogenized plant parts, already stored at 4 °C were weighed and laid into separate flasks. The 250 grams of each crushed plant portion was macerated separately in 400 ml ethylacetate, acetone, chloroform and methanol for ten days at room temperature (25 ± 2 °C). The solvent extracted material was filtered by using

whatmman filter paper in separate sanitized flasks. These extracts of plants portions were dried on rotary evaporator at low temperature (60 °C) and reduced pressure [15].

**Culture Medium**

For fungal growth PDA (Potato dextrose agar) was used as culture medium. The PDA was mixed and dissolved in distilled water. The solution was sterilized in an autoclave at 121 ºC (with 15 lb/sq-inch pressure) for 15 minutes. The pH of the medium was maintained at 5.4.

**Determination of Antifungal Activities**

In past research on antifungal activity of medicinal plants has been encountering several problems because of the diversity of the criteria and techniques employed for testing. The lipophilic properties of some extracts such as oil make it very difficult to use an aqueous media in the study of antimicrobial activity. Furthermore, different microorganisms have varying degree of sensitivity to different antimicrobial agents **[16]**. Among the several methods which were employed in the plant research to determine their antimicrobial activities, in-vitro antifungal activities were assessed by using the well diffusion method **[17]**.

**Preparation of Inoculum**

Fungal cultures which were 72 hours old, used as inoculums for test. The slants of PDA were prepared to maintain the fungal culture by following agar slant culture technique **[18]**. The slants of PDA (potato dextrose agar) were prepared in test tubes and were streaked with fungus. Each test tube was labeled with the name of fungus present in it and incubated for 72 hours at 25ºC.

**Preparation of Extract Dilution and Suspension**

The dried ethyl acetate, acetone, chloroform and methanol extracts were then dissolved in their respective solvents. Test tubes were sterilized in an autoclave at 121ºC for 15 minutes. 10 ml of distilled water was taken in each test tube. Then a loop of yeast or fungus was inoculated in distilled water in test tube under aseptic condition.

**Preparation of Plates**

The petri dishes were sterilized in an oven at 200 ºC for 2 hours. The sterilized petri dishes were also labeled with the yeast and fungal names. The petri dishes were labeled for leaves, seeds, stem bark, roots bark and solvent used. 1ml of inoculum was transferred from the test tube into corresponding previously labeled petri dishes, with the help of micropipette. The sterilized potato dextrose agar medium was poured in the petri plates containing fungal suspension then allowed to solidify at room temperature.

**Antifungal Assay by Well Diffusion Method**

The ethyl acetate, acetone, chloroform and methanol extracts of *O. cuspidata* leaves, seeds, stem bark, roots bark while *O. europaea* leaves and seeds were assessed for antifungal activity by using agar well diffusion method **[17]**. The well was made with sterile cork borer of 6.0 mm diameter. The 72 hours old cultures of fungi, grown on potato dextrose agar (PDA) medium were used as inoculum. 1 ml of dilution of each fungal strain was transferred into labeled petri plate and then molten PDA was poured in it by pour plate procedure. After solidification of medium, the appropriate wells were made then 10 µl of crude extract laid into these labeled wells. Eight different solid-phase fractions, 100 percent $H_2O$ and 5, 10, 15, 20, 30, 40 and 100 percent methanol were also tested in same way that were fractionated form original methanol extracts of leaves by using solid phase extraction procedure. The same volume (10 µl) of nystatin with concentration 100 mg/ml was used as positive control and respective solvent as negative control. The preparative

HPLC fraction of 100 percent $H_2O$, 5 and 100 percent methanol were also used to evaluate the antifungal potential of each fraction. All the experiments were carried out in triplicates. All these plates were incubated at 25 ºC for 72 hours. After the incubation period, all the plates were examined for the presence of zones of inhibition as a possession of antifungal activity. The zone of inhibition was taken by measuring the diameter of the zone in millimeters (mm) and was tabulated in tables.

**Statistical Analysis**

All values were expressed as means ± standard error of means (S.E.M). The data for each microorganism were analyzed by using one way analysis of variance (ANOVA) technique and means were compared by using LSD at 5 percent (0.05) probability level **[19]**.

**Results and Discussion**

Fruits and oil from cultivated olive trees were broadly used in diet and drugs. The information about chemical constituents with medicinal possessions has been conformed from the widely available fruits, leaves, and oil of *O. europaea*. It has been revealed through the investigation that the wellbeing effects of olive oil such as postponement of aging must be credited to all its metabolite constituents and not to a single compound. They act through the decrease of the risk factor important to high blood pressure, numerous types of cancer, to the alteration of immunity and inflammatory **[20]**. Leaves of olive have been used for the treatment of wounds, fever, diabetes, gout, arterioscleroses, and hypertension since ancient times **[21]**. Olive leaf extract has conferred as useful for lowering blood pressure in humans **[22]** but recently, it has been revealed that leaf extracts of *O. europaea* have the predictable antimicrobial activity **[23]**. The studies about the therapeutic potential, quality of oil, metabolite constituent and antimicrobial potential of *O. cuspidata* (wild olive) was ignored all over the world because it has fruit of small size, contain small quantity of extractable oil and samples are difficult to collect from their original habitat often inaccessible or remote.

The antifungal activity of *O. europaea* L. leaves, seeds and *O. cuspidata* Wall. leaves, roots bark, stem bark and seeds were evaluated by well diffusion method against *Aspergillus flavus, Alternaria alternata, Fusarium moniliform, Mauginiella scaettae, Trichothecium roseum, Magnaporthe grisea, Botrytis cinerea* and yeast i.e. *saccharomyces cerevisiae*.

The antifungal potential of organic solvent extracts of investigated parts of *O. europaea* and *O. cuspidata* were tabulated in tables and also graphical representations of results shown in figures. The methanol extracts of *O. europaea* leaves and leaves, roots barks, stem bark and seeds of *O. cuspidata* were exhibiting some activity against *Mauginiella scaettae* (8.7 mm) and *Magnaporthe grisea* (14.3 mm), while all other fungi i.e. *Aspergillus flavus, Alternaria alternata, Fusarium moniliform, Trichothecium roseum, Saccharomyces cerevisiae* and *Botrytis cinerea* were resistant to this extract (Table. 1 and Fig. 1E).

The antifungal properties of *O. cuspidata* leaves were shown in Table, 3.11 and graphical presentation of results was recorded in Figure 3.6E. The methanol extract of *O. cuspidata* leaves revealed significant activity against *Alternaria alternata* (10. 70 mm), *Mauginiella scaettae* (24.70 mm) and *Magnaporthe grisea* (27.70 mm) while no activity was observed against other strains of fungi tested. The methanol extract of roots bark was also proven to be effective only against *Magnaporthe grisea* (18.30 mm) while all other fungi were resistant to this extract (Table 1, Fig 1A). The antifungal prospective of methanolic extract of stem bark of *O. cuspidata*

exhibited affective against *Magnaporthe grisea* (16.30 mm) whereas all other fungi verified were unaffected by the extract (Table 1, Fig.1A).

The ethylacetate crude extracts of *O. europaea* leaves, seeds and *O. cuspidata* leaves, roots bark, stem barks and seeds were also tested against fungi which conferred variable results against these pathogens. The *O. europaea* leaves revealed some activity with zones of inhibition, 14.30 mm and 11.30 mm against *Fusarium moniliform* and *Magnaporthe grisea* whereas *O. cuspidata* leaves showed zones of inhibition against *Alternaria alternata* (11.30 mm), *Mauginiella scaettae* (9.3mm) and *Magnaporthe grisea* (14.0 mm). The *O. europaea* seeds extracts was observed affective against *Aspergillus flavus* (10.0 mm), *Alternaria alternata* (20.7 mm), *Mauginiella scaettae* (19.70 mm), *Trichothecium roseum* (15.7 mm), *Magnaporthe grisea* (25.7 mm) and *Botrytis cinerea* (19.30 mm) whereas *O. cuspidata* seeds exhibited potential against *Aspergillus flavus* (15.7 mm), *Alternaria alternata* (24.3 mm), *Fusarium moniliform* (24.3 mm), *Mauginiella scaettae* (30.7 mm), *Trichothecium roseum* (19.3 mm), *Saccharomyces cerevisiae* (15 mm), *Magnaporthe grisea* (35.7 mm) and *Botrytis cinerea* (25.3 mm). The ethylacetate crude extracts of *O. cuspidata* leaves and seeds were proved to be highly effective against these pathogens as compared to *O. europaea* extracts of the same parts which revealing less activity only against some fungal strains (Table 1, Fig.1B). The antifungal activity of ethylacetate extracts of roots bark and stem barks of *O. cuspidata* were exposed with mean zones of inhibition against *Magnaporthe grisea* i.e. 17.7 mm and 14 mm respectively. Stem bark extract also showed activity against *Mauginiella scaettae* (10.7 mm).

The acetone crude extracts of *O. europaea* leaves, seeds and *O. cuspidata* leaves, roots bark, stem barks and seeds were also verified against fungi which were active against some strains. The *O. europaea* leaves revealed some activity with zones of inhibition, 11.30 mm against *Magnaporthe grisea* whereas *O. cuspidata* leaves showed zones of inhibition against *Alternaria alternata* (10 mm) and *Magnaporthe grisea* (17.7 mm). The *O. europaea* seeds extracts was observed inactive against all pathogens tested whereas *O. cuspidata* seeds exhibited potential against *Aspergillus flavus* (12.7 mm), *Alternaria alternata* (15.7 mm), *Mauginiella scaettae* (14.3 mm), *Saccharomyces cerevisiae* (10.3 mm), *Magnaporthe grisea* (13.3 mm) (Table 1, Fig.1C). These results are comparable with results of nystatin used as control except *Mauginiella scaettae* and *Magnaporthe grisea* which were resistant to this antibiotic but susceptible to the cure extracts used.

There is an incessant and crucial requirement to discover novel bioactive component particularly from plant sources with diverse chemical structures and new mechanisms of action to combat with infectious ailments. Additionally, the expansion of synthetic antimicrobials resistance in current healing procedure is a world concern **[24]**. Medicinal plants encompass a substantial number of different bioactive constituents. It has been demonstrated that bioactive components isolated from plants are very effective against microorganisms specifically human and plant pathogens **[25, 26]**.

Most of the wild floras of Azad Jammu and Kashmir are rich in medicinal and aromatic properties. They are important sources of bioactive molecules, with application for the production of medicines and cosmetics **[27]**. In this study, the olives parts extracted with ethylacetate and methanol as compared to other solvents extracts proved to be

significantly effectual against the fungal strains tested. Ethylacetate extracts exhibiting potential against pathogenic fungi. The *O. cuspidata* parts exhibited more antifungal potential against tested pathogens. This is because the polar solvent as compare to other solvents, may allows extracting all the phenolic compounds from the olives **[28]**. The current results revealed that the activity is mostly engrossed with ethylacetate and methanol extracts, indicating that the possible antifungal compounds were in the polar extracts. It is also evident that higher plants comprehend methanol soluble chemicals with substantial antimicrobial potential **[29]**. The polar solvents extracts conferred more potential activity against pathogenic microorganisms **[30]**. Buwa and Staden **[31]** concluded that the ethylacetate extracts were more effective against fungi compared with methanol extracts.

Traditional healers usually used water as solvent to prepare plant extracts. Though, in present study it was found that ethylacetate and methanol extracts exhibit more reliable antifungal activity and this assumption is also shared by other investigators **[32]**.

Olive leaves may be useful in situations where lengthy usage of synthetic antibiotics inspire growth of opportunistic contagions **[33]**, being especially effective against *Staphylococcus*, *Bacillus*, *Klebsiella* and *Pseudomonas*, four genera of bacteria which posture a chief resistance problem **[34]**. The antifungal properties of *O. cuspidata* leaves, stem and root barks extracts were tested for the first time in present work subsequently previous studies available in literature were focused exclusively on *O. europaea* fruits and leaves **[35-37]**. The ethylacetate and methanol extracts investigated in present study conferred a strong activity against disastrous pathogenic fungal strain (Table 1). *O. cuspidata* parts extracts prove to be good with antifungal potential while the nystatin an antifungal commercially available medicine was inactive against these pathogens (Fig.1 A-E). The information presented in literature concerning antimicrobial potential of phenolic compounds particularly obtained from cultivated olive seeds, predominantly oleuropein and hydroxytyrosol **[38, 39]**. Investigations of Markin *et al.* **[10]** and Pereira *et al.* **[23**] about antimicrobial activity of *O. europaea* leaves revealed the higher minimum inhibitory concentrations for the inhibition of fungal growth than the present investigation. In the present study methanolic extracts of leaves, seeds of cultivated olive and seeds, leaves, roots bark but not stem bark exhibited potent antifungal activity. Further, extracts from wild olive appeared to be particularly potent. The solid phase and HPLC fractions of *Olea cuspidata* leaves particularly 100 water, 5 and 100 percent methanol fractions exhibited potential antifungal activity against *Magnaporthe grisea* (Fig. 2, 3). According to Regasini *et al.* **[40]** and Helmerhorst *et al.* **[41]**, the deaths from opportunistic fungal diseases is above fifty percent and only narrow spectrum antifungal medicines are available, therefore it is a need to isolate new antifungal drugs. On the bases of present investigation it is clear that olive species are best source for the antifungal medicines as they exhibiting excellent inhibitory against human as well as crop pathogens tested particularly wild olive. These results are in agreement with the previous studies **[42, 43]**. Moreover, in current study different extracts of wild and cultivated olives parts were investigated for their potential against eight fungi, which are known to cause reduction in the yield and quality of crops. As all other fungi, they are extremely responsive to environmental pressures and exhibit a capacity to adapt and

**Table 1. Antifungal activities of different extracts of *O. cuspidata* and *O. europaea***

| S.No. | Name of Plant | Part Used | Solvent | Mean Zone of Inhibition (mm) ± Standard Error Mean (SEM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ***Asperg*** | ***Altern*** | ***Fusari*** | ***Maugi*** | ***Tricho*** | ***Sacch*** | ***Magna*** | ***Botry*** |
| 1 | *O. europea* | L | Ethyl acetate | 0.0±0.00 | 0.0±0.00 | 14. 3±0.33 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 11.3±0.33 | 0.0±0.00 |
| 2 | *O. europea* | L | Acetone | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 11.3±0.33 | 0.0±0.00 |
| 3 | *O. europea* | L | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 4 | *O. europea* | L | Methanol | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 8.7±0.3 | 0.0±0.00 | 0.0±0.00 | 14.3±0.33 | 0.0±0.00 |
| 5 | *O. europea* | S | Ethyl acetate | 10.0±0.00 | 20.7±0.3 | 0.0±0.00 | 19.7±0.33 | 15.7±0.3 | 0.0±0.00 | 25.7±0.33 | 19.3±0.33 |
| 6 | *O. europea* | S | Acetone | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 7 | *O. europea* | S | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 8 | *O. europea* | S | Methanol | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 10.3±0.33 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 11.3±0.3 |
| 9 | *O. cuspidata* | L | Ethyl acetate | 0.0±0.00 | 11.3±0.33 | 0.0±0.00 | 9.3±0.3 | 0.0±0.00 | 0.0±0.00 | 14.0±0.6 | 0.0±0.00 |
| 10 | *O. cuspidata* | L | Acetone | 0.0±0.00 | 10.0±0.3 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 17.7±0.3 | 0.0±0.00 |
| 11 | *O. cuspidata* | L | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 12 | *O. cuspidata* | L | Methanol | 0.0±0.00 | 10.7±0.33 | 0.0±0.00 | 24.7±0.33 | 0.0±0.00 | 0.0±0.00 | 27.7±0.33 | 0.0±0.00 |
| 13 | *O. cuspidata* | S | Ethyl acetate | 15.7±0.33 | 24.3±0.3 | 24.3±0.33 | 30.7±0.33 | 19.3±0.3 | 15.0±0.6 | 35.7±0.3 | 25.3±0.33 |
| 14 | *O. cuspidata* | S | Acetone | 12.7±0.3 | 15.7±0.33 | 0.0±0.00 | 14.3±0.3 | 0.0±0.00 | 10.3±0.33 | 13.3±0.33 | 0.0±0.00 |
| 15 | *O. cuspidata* | S | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 16 | *O. cuspidata* | S | Methanol | 0.0±0.00 | 9.3±0.33 | 0.0±0.00 | 11.3±0.33 | 0.0±0.00 | 10.7±0.3 | 19.7±0.3 | 0.0±0.00 |
| 17 | *O. cuspidata* | SB | Ethyl acetate | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 10.7±0.3 | 0.0±0.00 | 0.0±0.00 | 17.7±0.3 | 0.0±0.00 |
| 18 | *O. cuspidata* | SB | Acetone | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 18.0±0.00 | 0.0±0.00 |
| 19 | *O. cuspidata* | SB | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 20 | *O. cuspidata* | SB | Methanol | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 16.0±0.3 | 0.0±0.00 |
| 21 | *O. cuspidata* | RB | Ethyl acetate | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 14.0±0.33 | 0.0±0.00 |
| 22 | *O. cuspidata* | RB | Acetone | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 19.7±0.3 | 0.0±0.00 |
| 23 | *O. cuspidata* | RB | Chloroform | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 |
| 24 | *O. cuspidata* | RB | Methanol | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 0.0±0.00 | 18.3±0.33 | 0.0±0.00 |
| 25 | Nystatin | 1500U/10µl | | 30.7±0.3 | 30.0±0.00 | 30.0±0.00 | 0.00±0.00 | 20.0±0.00 | 30.7±0.3 | 0.0±0.00 | 35.3±0.33 |

**Key:** ***Asperg*** *= Aspergillus flavus,* ***Altern*** *= Alternaria alternata,* ***Fusari*** *= Fusarium moniliform,* ***Maugi*** *= Mauginiella scaettae,* ***Tricho*** *= Trichothecium roseum,* ***Sacch*** *= Saccharomyces cerevisiae,* ***Magna*** *= Magnaporthe grisea,* ***Botry*** *= Botrytis cinerea*
**L**= Leaves **S**= Seeds **SB**= Stem Bark **RB**= Roots Bark

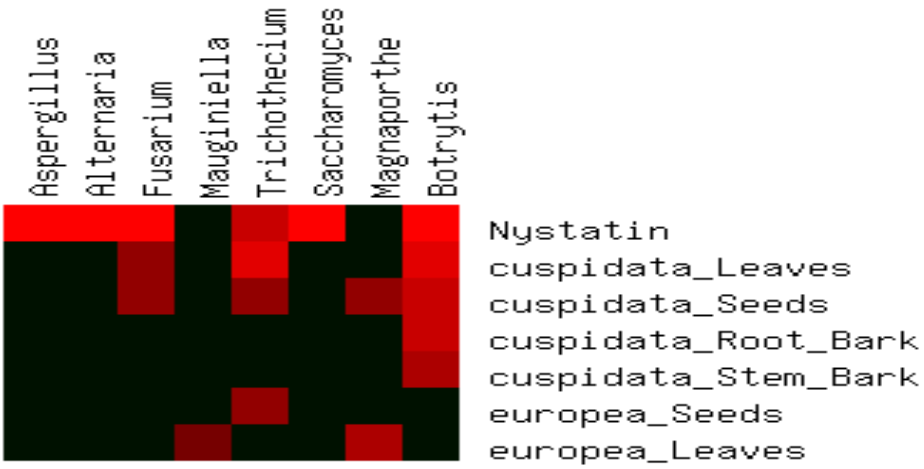


**Figure 1 (A): Antifungal activity of methanol extracts of all parts**

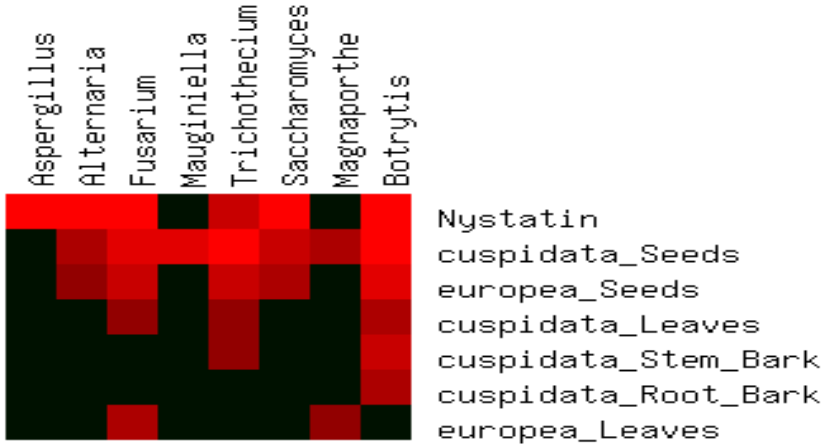


**Figure 1 (B): Antifungal activities of ethylacetate extracts of all parts**

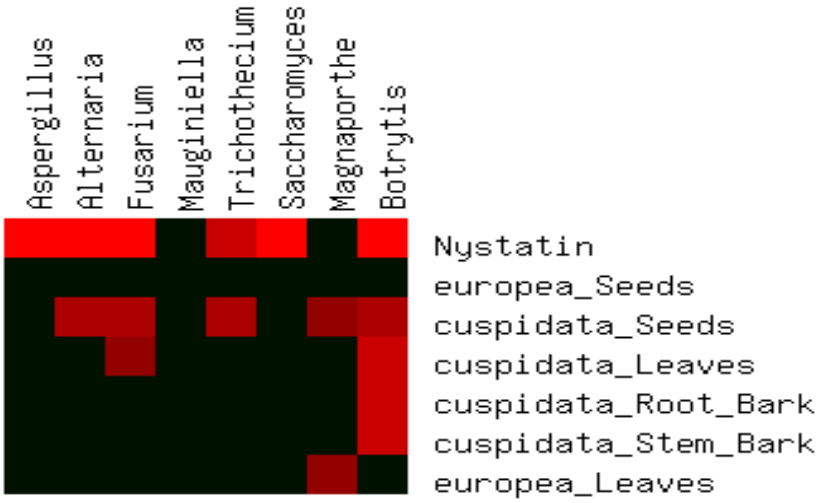


**Figure 1 (C): Antifungal activities of acetone extracts of all parts**

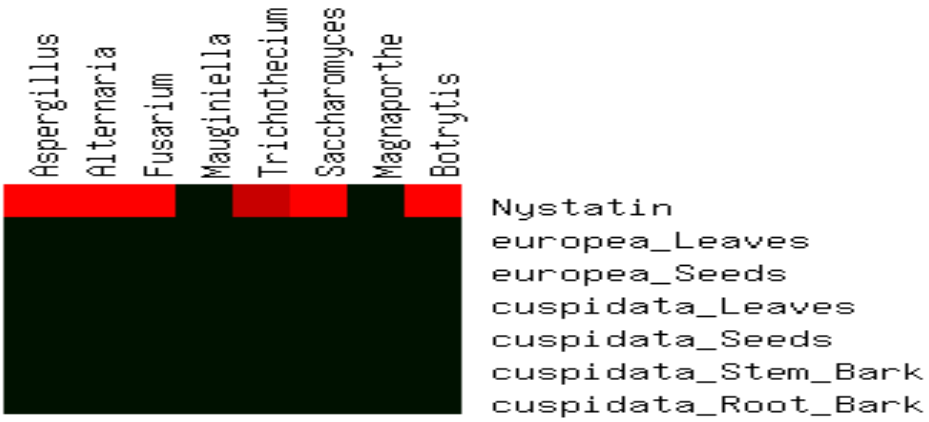


**Figure 1 (D): Antifungal activities of chloroform extracts of all parts**

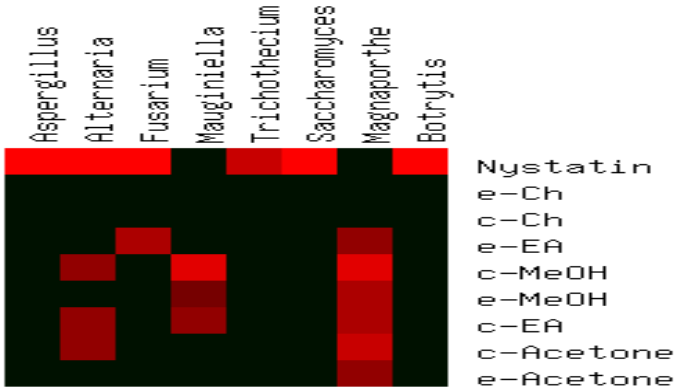


**Figure 1 (E): Antifungal activities of leaves of *O. europaea***

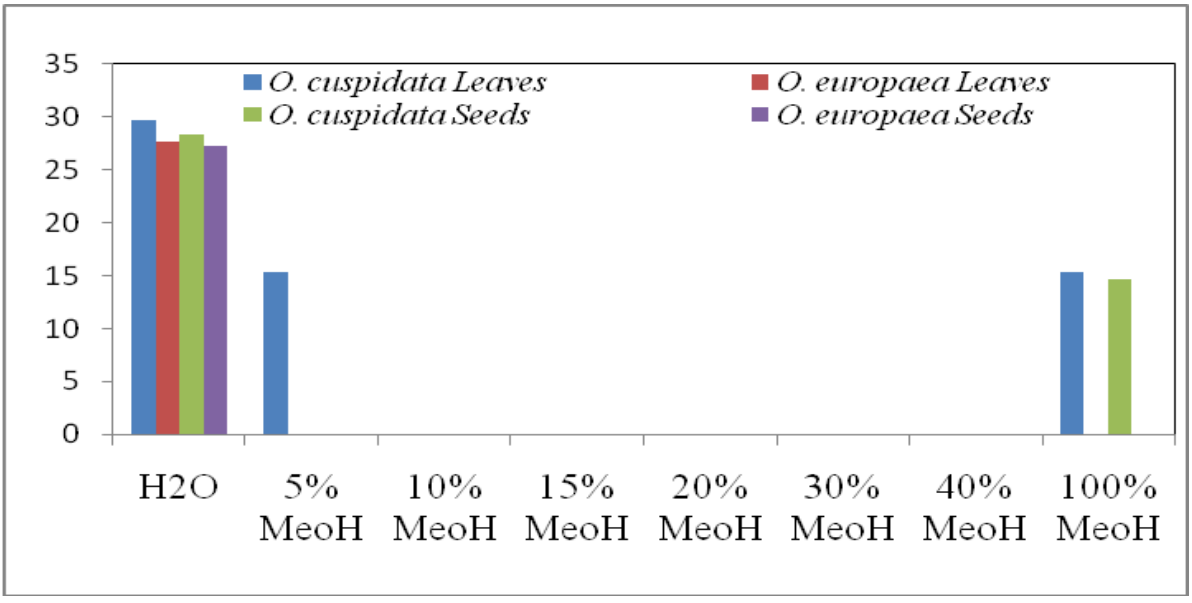


**Figure-2. Effect of solid phase extracts against *Magnaporthe grisea***

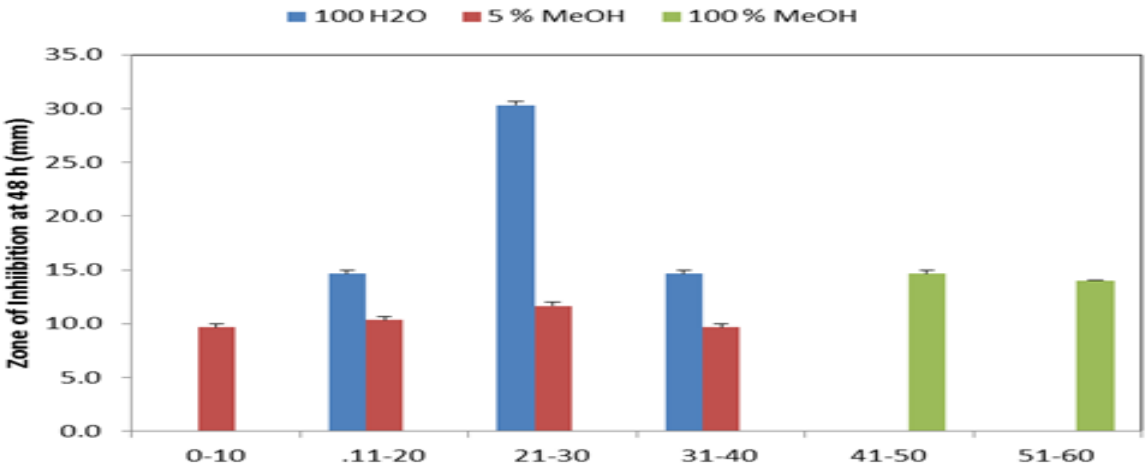


**Figure-3. Effect of preparative HPLC fractions against *Magnaporthe grisea***

to colonize a variety of ecological niches. *Alternaria alternata* is an attendant to tomatoes, potatoes, paprika, *Botrytis cinerea* attacks grapes, berry-fruits, some vegetables too, while *Fusarium culmorum* causes a degradation of cereal grains. The latter is also known for its production of mycotoxine. Extracts of wild olive parts showed a potential antifungal activity against *Aspergillus flavus, Alternaria alternata, Mauginiella scaettae, Saccharomyces cerevisiae* and *Magnaporthe grisea* (Table 1, Fig.1 A-E).

In the present investigations the crude extracts, solid phase and HPLC fractions were used instead of individual phenolic compound due to reasons that the antimicrobial properties of phenolic components are recognized **[25, 44]** and extracts may be more advantageous as compare to single isolated chemical, since a bioactive single compound can alter its possessions in the presence of other constituents found in extracts **[24]**. The synergistic effects of phytochemicals in vegetables and fruits are responsible for their potent bioactive activities and the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods **[45]**. This explains why no individual antifungal can replace the mixture of natural phytochemicals to attain the health benefits.

The *Olea cuspidata* leaves proved to be highly effective against tested fungi as compared to *Olea europaea*. The wild olive extracts are efficiently inhibiting or delaying the growth rate of microorganisms. This study is in line with the previous study of Bisignano *et al.* **[38]**. Antifungal activities observed observed in current study are also in line with the former investigations that reported in literature **[10, 33, 34]**.

**Conclusion**

On the bases of present investigation it is concluded that olive species are best source for the antifungal medicines as they exhibiting excellent inhibitory effect against human as well as crop pathogenic fungi tested. In the present study methanolic extracts of leaves, seeds of cultivated olive and seeds, leaves, roots bark but not stem bark exhibited substantial antifungal activity. Further, extracts from wild olive appeared to be particularly potent. This study demonstrate that the use of wild olive leaves extracts as medicines may reduce the risk of fungal infections, particularly in situations where lengthy usage of synthetic fungicidal inspire growth of opportunistic

contagions. The use of extracts is suggested to achieve benefits due to the additive and synergistic effects of phytochemicals present in whole extract.

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